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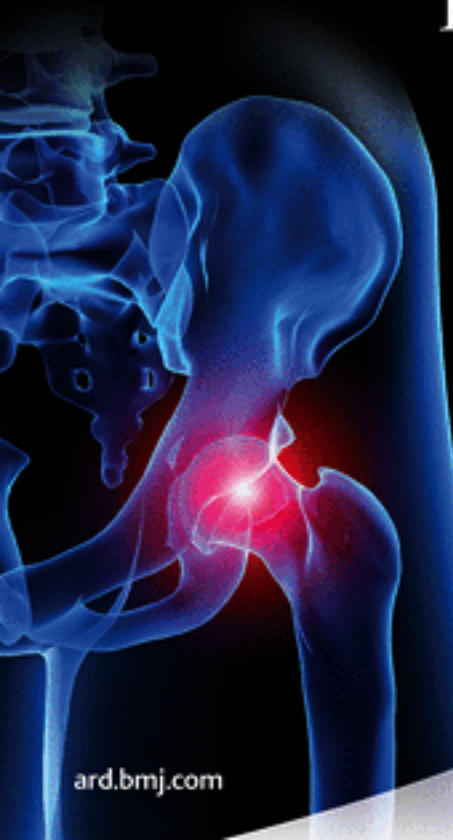

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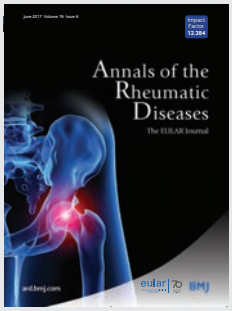

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EULAR recommendations for disease management: guidance not guidelines

David S Pisetsky

The past two decades have witnessed remarkable advances in the treatment of inflammatory arthritis that have made remission in previously untreatable conditions a realistic goal for many patients. These advances derive from new insights into disease mechanisms; the advent of the biologics and other new therapies; the development of robust measures of disease activity; coherent treatment strategies to guide therapy (ie, treat to target or T2T); and a sufficient supply of rheumatologists to implement the new approaches.[1]

The advances in rheumatology have been unprecedented and, arguably, the treatment of inflammatory arthritis has progressed faster and further than that of any other serious chronic disease in all of medicine. Indeed, a patient with rheumatoid arthritis (RA) today can lead an essentially normal life, whereas, a few decades ago, such a patient would have a restricted existence and be easily recognised by the appearance of grave illness, wasting and deformity.

Along with better outcomes have come challenges in the utilisation of the current armamentarium of disease modifying antirheumatic drugs or DMARDs (table 1). These challenges are, of course, welcome since they signify progress. In the face of literally hundreds to thousands of ways to treat arthritis and the continuing influx of novel agents, they also demand guidance for both providers and patients to establish a treatment plan, recognising potential risks, benefits and costs. The publication of three articles on European League Against Rheumatism (EULAR) recommendations for the treatment of early inflammatory arthritis, RA and axial spondyloarthritis (axSpA) is therefore an important event and provides a much needed perspective and framework for the delivery of best care.[2–4]

Each article represents a remarkable effort by panels of rheumatologists, health professionals and patient representatives to encompass an ever-expanding literature and provide overarching principles as well as specific recommendations. The work of the panels followed guidelines established by EULAR[5] and involved systematic literature reviews (SLRs) which are published separately.[6–12] The methodology is state of the art and meticulous, reflecting expertise of skilled methodologists and exceptional work of fellows and medical librarians to construct the SLRs. As described in the articles, deliberations of panel members were fair and democratic, with balloting on recommendations conducted until there was agreement.

The discussions in these articles are detailed and thoughtful as the authors explain their reasoning and choice of words. 'Wordsmithing' is sometimes denigrated as a seemingly pedantic exercise. Wordsmithing, however, is a serious undertaking to clarify thinking and enhance communication. Thus, in the RA recommendations, the wording on the approach to therapy when the first attempt does not reach the treatment target has been revised from 'change to another csDMARD strategy should be considered' to 'other csDMARDs should be considered.' This difference is important and many examples of such word choices illustrate the care devoted to their selection. Among these is the use of the word recommendation rather than guideline.

Of the recommendations, those on the management of early arthritis and RA address a central element of rheumatology. Given the size of the published literature, the data underpinning the recommendations are extensive and provide a solid evidence base. In many respects, the recommendations are consistent with the current practice in which T2T approaches are widely followed.[13] These recommendations contain few surprises or controversial elements. Perhaps the major changes relate to the position of triple therapy in the hierarchy of therapy and the role of glucocorticoids.

As is the case of many treatment strategies for RA, the role of glucocorticoids remains uncertain despite almost 70 years of their use. Unquestionably, glucocorticoids are potent anti-inflammatory agents. For a person with active disease, symptom relief is essential and glucocorticoids can achieve that goal for many patients. Such symptom relief can improve quality of life almost immediately, give hope and solidify a relationship with a rheumatologist. Furthermore, a period of glucocorticoids can provide an umbrella until the onset of action of a DMARD.

The recommendations on RA management state that 'Short-term glucocorticoids should be considered when initiating or changing csDMARDs…but should be tapered as rapidly as clinically feasible.' Such an approach can spare the long-term toxicity of high-dose glucocorticoids but the rapidity of tapering and clinical feasibility are often unclear. Review of clinical trials indicates that a significant number of patients with chronic RA remain on low-dose glucocorticoids (5–7.5 mg prednisone or equivalent), suggesting that short term can be months to years and clinical feasibility may not readily occur. Glucocorticoids may provide bridge therapy and can be included in therapy in a number of different dose protocols, including daily oral administration as well as intravenous pulses, but, in the real world, the bridge can be very long especially as these agents have benefits on radiographs.[14–20]

Another issue relates to the recommendation that 'Treatment should be aimed at reaching a target of sustained remission or low disease activity.' While composite measures (eg, Disease Activity Score 28 (DAS28), Clinical Disease Activity Index and Simple Disease Activity Index) are valuable, the difference between a state of low disease activity and moderate disease activity can be as small as one or two tender joints, a small elevation in the C-reactive protein (CRP) value or a heightened patient global assessment

Table 1 Disease-modifying antirheumatic drugs (DMARDs)

Class	Example
Conventional synthetic DMARD	Methotrexate, lefunomide
Targeted synthetic DMARD	Tofacitinib
Biological DMARD	
Biological originator	Infliximab
Biosimilar	Infliximab-dyyb

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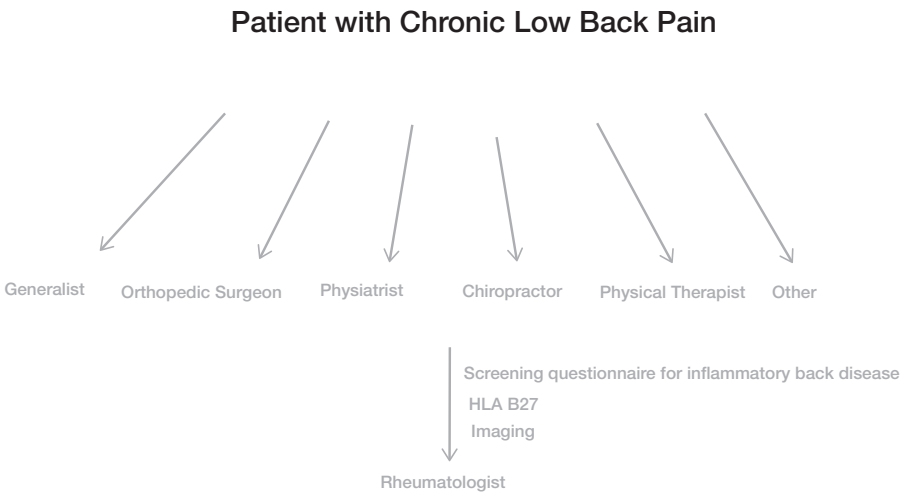

Figure 1 The flow of patients with chronic low back pain to the rheumatologist. Currently, patients with chronic low back pain can seek care from a wide variety of specialists as well as generalists. While the rheumatologist could perform the initial evaluation, in most healthcare settings, that circumstance would be unusual. Because of the large number of patients with chronic low back pain, a screening strategy is needed to assure referral for those with a likelihood for axial spondyloarthritis (axSpA). It is therefore essential that primary providers be aware of the concept of axSpA and screen patients with inflammatory back pain appropriately so that rheumatologists can handle a burgeoning flow of patients for evaluation.

on a 'bad day.' For a patient, a decrease in the DAS28 from 6 to 4 can represent extraordinary improvement and, even if the disease activity rates as moderate, there can be reluctance to switch therapy especially as the benefits of any new agent are unknown and flare is possible. Such concerns can represent an important patient factor in recommendations on T2T.[21] Furthermore, in the 2016 recommendations, patient factors are considered in the context of the Overarching Principle B, with further discussion in the text related to Recommendation 2.[4]

In contrast to the arthritis recommendations, those for axSpA raise more weighty questions reflecting the differences in these conditions. The management of RA derives from a well-established narrative in which inflammation leads to joint destruction, deformity and impaired quality of life. Biomarkers (ie, anticyclic citrullinated peptide and rheumatoid factor) aid in patient diagnosis while imaging can show damage by erosions on plain X-rays. The situation with axSpA is different since diagnosis can be tricky; radiographic findings may be scant; and diagnostic biomarkers, beyond HLA B-27, are generally lacking.[22–25]

In the face of these conundrums, the recommendations on axSpA (a combined effort of EULAR and the Assessment of SpondyloArthritis international Society) are therefore timely. A central challenge in formulating recommendations for axSpA relates to disease definition. In the past, a diagnosis of ankylosing spondylitis was based on the presence of sacroiliitis (SI) by plain X-rays in a patient with inflammatory back pain. Inflammatory back pain entails insidious onset, worsening in the morning, improvement with activity and a lack of improvement with rest. The difficulty with this construct relates to inherent problems with SI joint radiography and, indeed, the absence of any X-ray evidence of SI disease in some patients with inflammatory back disease.[26]

The more recent conceptualisation of axSpA disease takes a different direction and posits that axSpA can be either radiographic or non-radiographic.[27 28] Those with findings of SI can be termed ankylosing spondylitis; those without such findings are termed non-radiographic axSpA or nr-axSpA. Patients with nr-axSpA may have MRI findings but such imaging is expensive and often not available; levels of inflammatory markers such as CRP are often not elevated. As a result, the diagnosis of nr-axSpA can be uncertain unless there is a 'classic' inflammatory back pain and other findings (eg, psoriasis, uveitis) that support the diagnosis by an experienced rheumatologist.

An important issue in managing patients with axSpA concerns their care before diagnosis. Patients with inflammatory back pain can see orthopaedists, physiatrists, chiropractors and physical therapists among others and years may pass before the diagnosis of axSpA is made or even considered. As shown in studies on screening strategies to identify patients with axSpA, many patients with chronic low back pain may have unrecognised axSpA.[29–32] Thus, the recommendations for management of axSpA are relevant only for patients who have a diagnosis but it is very likely that many patients with axSpA never get a diagnosis.

Treatment of RA and axSpA also differs in the impact of biological therapy on radiographic outcome. While the effects of DMARDs on erosions are clear, studies have not definitely shown that agents such as tumour necrosis factor (TNF) blockers can change the development of syndesmophytes likely because the process is slow. Interestingly, non-steroidal anti-inflammatory drugs may affect radiographic progression.[33–38] In the absence of radiographic evidence of disease modification, the evaluation of efficacy of therapy is based substantially on patient-reported outcomes of pain, stiffness and function. This situation may have contributed to the decision of the Food and Drug Administration to withhold approval for the use of TNF blockers for nr-axSpA.

Cost is now an important consideration in the formulation of treatment recommendations.[39] The treatment of RA involves both new and old agents. The older agents (ie, conventional synthetic DMARDs like methotrexate) are not costly and can produce results comparable to those of the newer, more expensive agents, either targeted synthetic DMARDs or biological DMARDs. Since the older agents can be combined and used in conjunction with low-dose prednisone, a satisfactory treatment programme can be developed at a low cost.[40]

For axSpA, the situation is different. As the recommendations state, in describing the agents for axSpA, 'Some of them are very cheap; others are very expensive;' there is little middle ground. Thus, as stated in the recommendations, 'For the first time, cost considerations received a prominent place in the axSpA recommendations.' Given the lack of more strong evidence for the effects of treatment on radiographic progression, the cost–benefit calculation for the new agents will be a subject of inquiry and, likely, debate. Furthermore, the introduction of biosimilars should yield cost savings in the administration of biological agents for axSpA as well as RA; the extent of these savings will likely vary by country and the magnitude of cost reduction. As long as the safety and efficacy of the biosimilars are comparable to the reference products, the cost savings can allow more widespread treatment with biological agents and a decrease in health disparities related to economic differences among countries.[41 42]

The authors of these three sets of recommendations have performed a valuable service for the field, facilitating best practice, providing guidance that is actionable and highlighting areas for future research. Thus, as inferred in the recommendations on axSpA, the rheumatologist should be facile in using measures such as Ankylosing Spondylitis Disease Activity Score and Bath Ankylosing Spondylitis Activity Index and the nuances of treating a disease where patient-reported outcomes predominate.[43] Training, especially at the fellowship level, may be necessary to gain those skills. Furthermore, rheumatologists must develop an interdisciplinary approach to collaborate with other providers to identify among the large population of patients with low back pain, the minority of patients who have an axSpA. The logistical challenges for such an undertaking are large but, to improve the care of patients with axSpA, correct diagnosis is essential. Figure 1 illustrates the flow of patients to the rheumatologist and the importance of screening strategies.

Each article concludes with a research agenda, addressing such issues as induction strategies, remission and drug tapering. Such agendas are only likely to increase as new products achieve regulatory approval. Despite their importance, many of the proposed research questions will never be fully answered as the field constantly shifts and expands. The list of research questions, however, is a sign of vitality and dynamism in rheumatology and it was not so long ago that a central issue in the field concerned whether impacting the course of serious inflammatory arthritis was at all possible. Clearly, much is now possible and the research agendas exemplify the achievements of today as well as the hopes for tomorrow.

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Mitochondrial DNA haplogroups and ageing mechanisms in osteoarthritis

Ana M Valdes,[1,2] Mary B Goldring[3,4]

MITOCHONDRIAL DNA AND AGEING

Osteoarthritis (OA) is the most common form of arthritis affecting more than 12% of people over the age of 60.[1] Although late-onset articular cartilage degeneration is common and age is one of the most important risk factors for the disease, the relationship between old age and OA is not fully understood.[2] In the past it was believed that the link with age was due to 'wear and tear' of articular cartilage by continuous mechanical stress; we now know, however, that OA involves an active response to injury comprising remodelling of articular cartilage and subchondral bone, in addition to synovial inflammation and damage to other joint structures such as ligaments and menisci.[3]

Biological ageing is a complex process and it is now widely accepted that ageing starts with molecular damage, leading to cell, tissue and, ultimately, organ dysfunction.[4] Extensive evidence from animal models and in vitro studies indicates that mitochondria contribute to specific aspects of the ageing process, including cellular senescence, chronic inflammation and the age-dependent decline in stem cell activity.[5]

Perhaps the best known and most long-standing hypothesis to explain ageing is the free radical theory that proposes a central role for the mitochondrion as the principal source of intracellular reactive oxygen species (ROS) leading to mitochondrial DNA (mtDNA) mutations.[4 5] Somatic (acquired) mtDNA mutations and their association with the decline in mitochondrial function during ageing are well described, but these observations do not necessarily imply a causal relationship between mitochondrial dysfunction and human ageing. The maternally inherited mtDNA sequences encode the key proteins involved in energy production, although the relevance of high sequence variability of mtDNA had been considered of little functional relevance. Latorre-Pellicer and coauthors showed recently that transferring mtDNA from a mouse strain to the nuclear DNA (nDNA) background of another strain results in huge differences in insulin signalling, obesity and longevity throughout the life of the mouse.[6] The two mtDNA sequences differ in genetic variants that confer 12 amino acid substitutions and 12 changes in RNA molecules involved in mitochondrial protein synthesis; this level of variation is enough to result in striking differences in the ROS generation, insulin signalling, obesity and cell-senescence-related parameters such as telomere shortening and mitochondrial dysfunction. Showing the direct relevance of mtDNA in human ageing and in age-related diseases, such as OA, is a big challenge and one which is, at least in part, addressed in this issue.[7]

mtDNA in OA

Over the past 10 years, the group led by Francisco Blanco and Ignacio Rego-Perez has shown that differences in mtDNA haplogroups correspond to variations in the prevalence and progression of cartilage loss in large joint OA.[8] In a series of studies from Spanish OA cases and controls, the evidence has accumulated for an association between OA prevalence and the J haplogroup[9 10] (table 1). However, two studies in samples from the UK have failed to find an association with the J haplotype,[9–11] whereas evidence of association of the T haplotype with lower disease risk was found in a small UK cohort[9] (table 1).

The mtDNA haplogroups J and T share the same phylogenetic origin and a set of common uncoupling mitochondrial polymorphisms.[12] These uncoupling polymorphisms confer different metabolic characteristics compared with other mitochondrial lineages, particularly the most common and highly efficient mtDNA haplogroup H.[13]

The jury is still out regarding the role of mtDNA T and J haplogroups with regard to genetic susceptibility in populations with large joint OA, particularly when compared with the evidence accumulated for nuclear genetic variants identified from genome wide association studies (GWAS) or otherwise.[14] To date, eight variants associated with knee OA have been reported with significance of $p<1\times10^{-7}$ and 11 variants with hip OA in Caucasians. At least three other variants have been reported at high significance levels in Asians (see ref. 14 for details).

On the other hand, with the exception of variants mapping to *GDF5* and *FTO* genes, the mechanisms underlying the risk conferred by variants linked to knee OA are yet to be unveiled.[14] Importantly, as of today, very few efforts have been made to identify genetic risk factors contributing to risk of progression or incidence of disease.

The mtDNA haplotypes T, J and the JT cluster, on the other hand, are significantly associated in populations from the USA, the Netherlands and Spain with radiographic incidence and progression of the disease[7 15] (table 1). Fernandez-Moreno and coauthors report that the mtDNA haplogroup J, the same haplogroup associated with lower OA prevalence, lower disease progression and lower cartilage loss, is also associated with a significantly lower risk of incident knee OA in a population of 3124 individuals from two prospective cohorts from the Netherlands and the USA.[7]

FUNCTIONAL ANALYSIS OF MTDNA VARIANTS

From previous studies, it is known that the low OA risk haplogroup J is associated with lower serum levels of markers of collagen type-II degradation and of matrix metalloproteinases, but all of these studies failed to address the key question arising from this large body of evidence: 'What is the functional role of these mtDNA haplogroups?'

To answer this question, Fernandez-Moreno *et al*[7] used cytoplasmic hybrid (cybrid) cell lines. Cybrids incorporate mitochondria from human subjects and perpetuate the mtDNA-encoded components while maintaining the nuclear background of different cybrid lines as constant.[16] Thus, this technique allows investigators to assess the influence of mtDNA variation on cell function. To investigate the role of mtDNA haplogroups, they also created cybrids using osteosarcoma cell lines with the same nuclear background, one of them harbouring the haplogroup J (which protects against OA) and another harbouring the haplogroup H (linked to higher risk of OA).

The cybrids carrying the haplogroup H produced higher ATP levels than those

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Table 1 Selected associations between T, J and TJ cluster mitochondrial DNA and OA prevalence, progression and incidence of OA

Study	Origin	Haplogroup	Trait studied	Total N	Effect (95% CI) p value	Reference
Case–control	Spain	J	OA prevalence	2557 OA, 1339 controls	OR=0.57 (0.46 to 0.71) p<0.00001	10
Case–control	UK	J	OA prevalence	7846 OA, 5402 controls	OR=1.19 (0.72 to 1.95) ns	10
Case–control	UK	T	OA prevalence	453 OA, 280 controls	OR=0.57 (0.35 to 0.940 p<0.027	9
CHECK cohort	The Netherlands	T	OA progression	431 OA	HR=0.645 (0.419 to 0.978) p<0.05	15
OAI	USA	T	OA progression	891 OA	HR=0.50 (0.28 to 0.88) p<0.05	24
Spanish OA cohort	Spain	T	OA progression	281 OA	HR=0.69 (0.38 to 1.28) ns	25
Meta analysis		T	OA progression	1603 OA	HR=0.61 (0.45 to 0.82) p=0.001	15
CHECK cohort	The Netherlands	JT	OA progression	431 OA	HR=0.71 (0.50 to 0.96) p<0.05	15
OAI	USA	JT	OA progression	891 OA	HR=0.81 (0.59 to 1.11) ns.	15
Spanish OA cohort	Spain	JT	OA progression	281 OA	HR=0.80 (0.50 to 1.26) ns	15
Meta analysis		JT		1603 OA	HR=0.77 (0.62 to 0.94) p=0.009	15
CHECK cohort	The Netherlands	J	OA incidence	635	HR=0.73 (0.47 to 1.00) p<0.05	7
OAI	USA	J	OA incidence	2579	HR=0.68 (0.47 to 0.97) p<0.05	7
Meta analysis		J	OA incidence	3214	HR=0.70 (0.54 to 0.91) p=0.006	7

OA, osteoarthritis; OAI, osteoarthritis initiative.

with the haplogroup J, but this higher energetic efficiency was accompanied by higher production of ROS and the proportion of cells that survived in the presence of hydrogen peroxide was almost half the number of cybrids with haplogroup J. In chondrocytes during OA, oxidative stress may act together with inflammatory and/or mechanical stress to accentuate catabolic processes by increasing the levels of ROS relative to antioxidants.[17 18] The increased levels of ROS also contribute to the senescence secretory phenotype, in which the age-related decline in the responses of chondrocytes to anabolic growth factors are related to increased oxidative stress.[19 20] The depletion of antioxidants promotes mitochondrial dysfunction in chondrocytes,[21] which in turn can amplify the stress responses through increased production of nitric oxide and ROS and activation of NF-κB signalling.[21–23]

In the presence of staurosporine, which induces cell apoptosis, the cybrids with the haplogroup H had over 50% more apoptotic cells than the cybrids with the low OA risk haplogroup J.[7] These data, therefore, prove the functional relevance of mtDNA variation linked to risk of OA on cell function and survival and is in agreement with recent work by the same group showing that OA cartilage exhibits signs of early molecular ageing compared with healthy age-matched cartilage.[2]

CLINICAL RELEVANCE

The data accumulated on the role of mtDNA on cell function and on OA risk have potential clinical implications. On the one hand, it may allow investigators in the future to define an 'age-related OA' genetic type (haplogroup H) versus one which is protected from the effects of ageing. This group with lower incidence and progression can be excluded from clinical studies that require rapidly progressing OA populations. At the same time, haplogroup J carriers are not fully protected from OA; therefore, studying risk factors in this haplogroup can help identify a group of individuals where other molecular mechanisms linked to OA, for example, those derived from bone changes or from inflammation, may be stronger predictors for progression. These data also raise the important question of the contribution of interactions between nDNA and mtDNA haplogroups, which have yet to be investigated. Finally, OA is a disease that occurs together with cardiometabolic comorbidities which are known to be influenced by mitochondrial dysfunction. Haplogroup H carriers may therefore be the group of OA sufferers at higher risk of metabolic syndrome and cardiovascular disease and with the most chance to benefit from regenerative therapies targeting early cartilage damage or, at more advanced stages, early joint replacement.

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Diagnostic criteria for cryopyrin-associated periodic syndrome (CAPS)

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ABSTRACT

Cryopyrin-associated periodic syndrome (CAPS) is a rare, heterogeneous disease entity associated with *NLRP3* gene mutations and increased interleukin-1 (IL-1) secretion. Early diagnosis and rapid initiation of IL-1 inhibition prevent organ damage. The aim of the study was to develop and validate diagnostic criteria for CAPS. An innovative process was followed including interdisciplinary team building, item generation: review of CAPS registries, systematic literature review, expert surveys, consensus conferences for item refinement, item reduction and weighting using 1000Minds decision software. Resulting CAPS criteria were tested in large cohorts of CAPS cases and controls using correspondence analysis. Diagnostic models were explored using sensitivity analyses. The international team included 16 experts. Systematic literature and registry review identified 33 CAPS-typical items; the consensus conferences reduced these to 14. 1000Minds exercises ranked variables based on importance for the diagnosis. Correspondence analysis determined variables consistently associated with the diagnosis of CAPS using 284 cases and 837 controls. Seven variables were significantly associated with CAPS ($p<0.001$). The best diagnosis model included: Raised inflammatory markers (C-reactive protein/serum amyloid A) plus ≥two of six CAPS-typical symptoms: urticaria-like rash, cold-triggered episodes, sensorineural hearing loss, musculoskeletal symptoms, chronic aseptic meningitis and skeletal abnormalities. Sensitivity was 81%, specificity 94%. It performed well for all CAPS subtypes and regardless of *NLRP3* mutation. The novel approach integrated traditional methods of evidence synthesis with expert consensus, web-based decision tools and innovative statistical methods and may serve as model for other rare diseases. These criteria will enable a rapid diagnosis for children and adults with CAPS.

INTRODUCTION

Cryopyrin-associated periodic syndrome (CAPS) is a rare, heterogeneous disease entity. It encompasses a spectrum of clinical phenotypes associated with gain-of-function mutations in the *NLRP3* gene encoding cryopyrin, a key regulatory protein, resulting in constitutive increased interleukin-1 (IL-1) secretion.[1 2] While previously considered three distinct clinical diseases including familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS) and chronic infantile neurological, cutaneous and articular syndrome (CINCA)/neonatal-onset multisystem inflammatory disorder (NOMID), the discovery of a common causative gene mutation led to an amalgamation into the entity CAPS.[3] *NLRP3* gain-of-function mutations were shown to result in characteristic, yet diverse clinical symptoms of systemic and organ-specific inflammation and raise of inflammatory markers, most importantly C-reactive protein (CRP), serum amyloid A (SAA) and the neutrophil protein S100A12.[4 5]

CAPS is rare, affecting an estimated one to three in a million children and adults worldwide; no gender or ethnic predilection has been identified.[6] CAPS-related inflammation causes fever, fatigue and organ irritation at the early stages, while long-standing uncontrolled inflammation results in irreversible organ damage. This includes sensorineural hearing loss, amyloidosis, vision loss, skeletal deformities and cognitive disability. Early diagnosis and inflammatory control is critical to prevent irreversible organ damage. In clinical practice, establishing the diagnosis of a rare disease, such as CAPS, is challenging resulting in significant delay to diagnosis.[7] This delay or even complete lack of recognition can be attributed to different factors including limited ability of healthcare providers to recognise and diagnose a rare disease, the involvement of multiple subspecialists in the care of these complex patients with multisystem involvement and their lack of communication. Commonly, the main subspecialty responsible for the care of a patient is determined by the leading organ manifestation, such as hearing loss, urticaria-like skin rash, conjunctivitis or nephritis in a patient with CAPS.

Diagnostic criteria are limited in rare diseases. Their development heavily relies on international collaborative efforts of medical experts. Currently, there are no validated diagnostic criteria available capturing the entire spectrum of CAPS. This entails a significant risk for missing a window of opportunity for the reversal of IL-1-mediated inflammation and prevention of organ damage in CAPS. Therefore, the aim of the study was to develop and validate diagnostic criteria for children and adults with CAPS to enable an early diagnosis and prevent irreversible organ damage secondary to inflammation in CAPS.

METHODS

A rigorous and innovative process was followed including: (a) an interdisciplinary, international expert team building of different paediatric and

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adult CAPS subspecialty experts plus rare diseases methods experts, (b) item generation and refinement: review of CAPS items in actively recruiting registries, systematic literature review, CAPS expert surveys and consensus conferences, (c) item reduction and weighting, (d) diagnostic model building using correspondence analysis and (e) model validation.

Expert team building

The multidisciplinary team had to include international experts in the care of children and adults with CAPS including rheumatologists and other subspecialists. It gathered experts in rare diseases research and methodology from both Europe and North America. Participants were invited based on their clinical and scientific expertise and geographical representation. They remained connected throughout the process including multiple surveys, decision analysis exercises and iterative face-to-face meetings.

Item generation

CAPS items were derived from registries, published literature and expert opinion surveys and meetings. Any sign or symptom or laboratory test used to characterise a CAPS patient or group was considered.

CAPS registry item review: All actively recruiting North American and European autoinflammation registries were reviewed for CAPS diagnosis items including Eurofever (Genoa, Italy), β-confident Canakinumab Registry (Novartis Pharma AG, Basel, Switzerland), Arthritis and Rheumatology Documentation and Information System (ARDIS, Tuebingen, Germany) and AutoInflammatory Disease-Network (AID-NET, Essen, Germany). All included registries have obtained institutional ethics board approval.

Systematic literature review: Published studies were identified through searches of MEDLINE, COCHRANE and EMBASE databases for the period from 1970 to 2013 following the European League Against Rheumatism (EULAR) rules for developing best practices[8] (see online supplementary table S1). Keyword, title and abstract information were used. All synonyms of CAPS, CINCA/NOMID, MWS and FCAS were searched. In addition, a search for 'autoinflammatory diseases' and synonyms was performed; references and reviews were screened for additional articles. The review was performed as previously described.[9] A total of 33 CAPS items were identified combined from the review of the CAPS registries and the systematic literature search.

Item refinement, reduction and weighing

CAPS expert surveys: Using web-based survey methodology, experts were asked to review all items, add additional items, if applicable, and evaluate each item for its relevance in making the diagnosis of CAPS and applicability for CAPS subtypes including FCAS, FCAS/MWS, MWS, MWS/CINCA/NOMID and CINCA/NOMID (see online supplementary table S2). The survey had to be completed and returned by >80% of participants. Items were considered relevant, if there was ≥80% consensus agreement among experts.

CAPS consensus conference Istanbul, Turkey: Survey results were shared. All putative items were discussed and refined using nominal group technique.[10] Refined items were voted on for their relevance for diagnosing CAPS and/or CAPS subtypes. Items were considered relevant, if there was ≥80% consensus agreement among experts.

CAPS consensus meeting in Boston, Massachusetts, USA: CAPS diagnosis items were shared and refined further using nominal group technique. Fourteen final putative CAPS diagnosis items were ranked for their relevance using 1000Minds decision analysis software.[11] Experts were presented pairs of CAPS items and asked to identify the item of higher relevance for diagnosing CAPS (eg, sensorineural hearing loss present and amyloidosis absent or sensorineural hearing loss absent and amyloidosis present, all other manifestations being considered equal). The resulting ranking of CAPS items was computed; correlations between expert decisions were calculated.

Diagnostic model development and validation

Multiple correspondence analyses (MCA) were used to assess the multidimensional relationship between putative CAPS diagnosis items and patient diagnoses. MCA allows the optimal representation of a contingency table in low-dimensional space. Items with close relationship to the diagnosis of CAPS were then tested in multivariable logistic regression models resulting in a proposed diagnostic model.

Model development was guided by statistical significance. CAPS expert guidance was considered the gold standard. The proposed diagnostic model was validated in a large, multicentre cohort of children and adults with CAPS and true CAPS controls including systemic juvenile idiopathic arthritis (JIA), Schnitzler syndrome, familial Mediterranean fever (FMF), unclassified fever syndromes, typical Kawasaki disease (KD) and incomplete KD. MCA computed the χ^2 statistic between each variable of interest and the outcome and transformed this statistic into a Euclidean distance. The quality of the lower-dimensional representation of the data is derived from singular values and is expressed as the percentage of the total inertia that is explained by each dimension. The total inertia of the data table can be regarded as the weighted average of the squared deviations between the subjects' profiles (the subjects' scores proportional to their total score) and the average score profile representing the amount of variation among the subjects' score patterns.[12,13] The model was developed using 1000Minds potentially all pairwise rankings of all possible alternatives (PAPRIKA) methodology and then applied to a validation data set using MCA. The model was then refined and tested as described. For a quantitative interpretation of the correspondence plot results, the χ^2 statistics were transformed into Pearson residuals (a standardised χ^2 statistic). Subanalyses were performed for all CAPS subtypes and evidence of *NLRP3* mutation. Sensitivity analyses were performed and the final diagnostic model was proposed. All analyses were performed using SAS software, V.9.4 (SAS Institute, Cary, North Carolina, USA).

RESULTS

Expert team

The multidisciplinary CAPS team included a total of 16 paediatric (JBK-D, SO, IK-P, HMH, EW-R, BH, TK, MG, FD, LC) and adult (RG-M, HL, NB, AG) subspecialists and methodology experts in rare diseases research (PNT, SMB) and was supported by two fellows (NTH, MO). The team members were selected based on their exceptional expertise in care and research in autoinflammatory diseases and the clinical severity spectrum of CAPS.

Item generation

The systematic literature review identified a total of 1698 unique records; 47 articles were selected for full-text screening, of which 33 were relevant and underwent validity assessment excluding four. The remaining 29 articles included a total of 794 patients with CAPS and generated a total of 33 putative

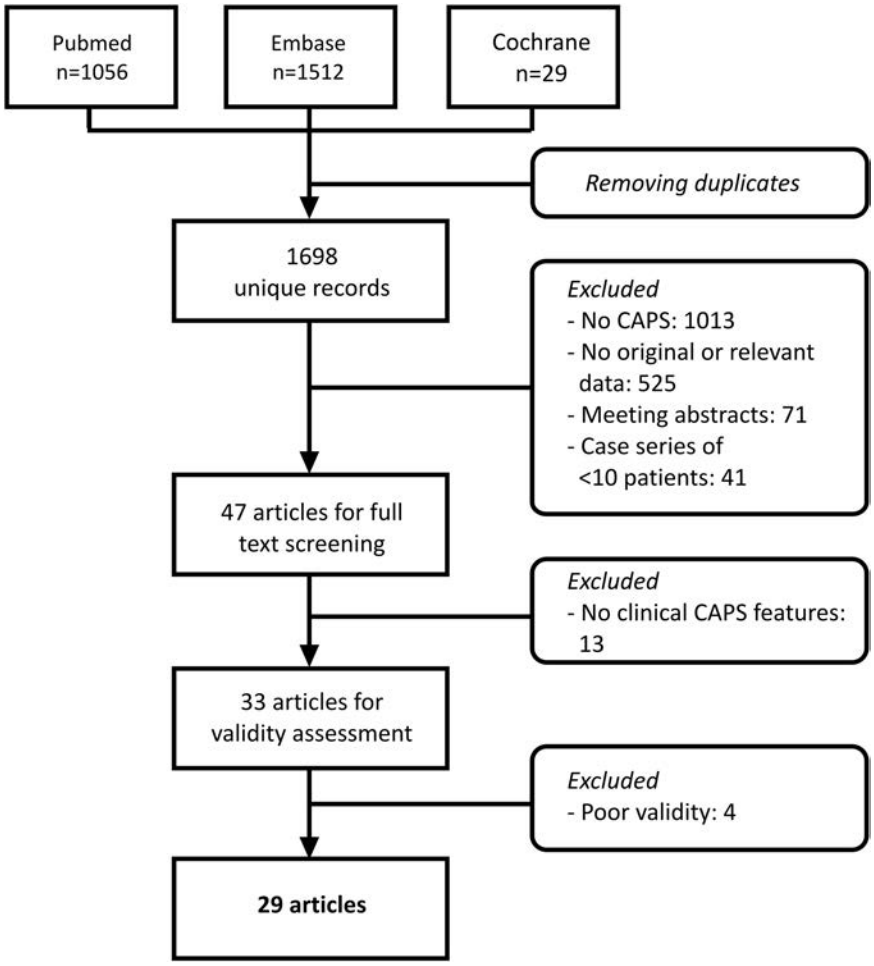

Figure 1 Systematic literature review of putative cryopyrin-associated periodic syndrome (CAPS) items.

CAPS diagnosis items (figure 1). The review of the CAPS registries did not yield any additional putative diagnosis items beyond those identified in the systematic literature review.

Item refinement, reduction and weighing

CAPS expert surveys: Iterative surveys including the 33 putative CAPS diagnosis items were completed and returned by 100% of participants. In addition, seven new items were generated based on responses of CAPS experts.

CAPS consensus conference Istanbul: All 40 items were discussed, refined and grouped into (1) patient-related items including positive family history of CAPS and evidence of *NLRP3* mutation, (2) disease course-related items: symptom onset in infancy, persistent inflammation with/without episodic attacks with worsening symptoms and induction of characteristic symptoms after generalised cold exposure, clinical signs and symptoms of CAPS coupled with laboratory findings of acute phase response, (3) CAPS-typical symptoms: recurrent episodes of systemic symptoms of fever and/or chills/rigors and/or fatigue, diffuse urticaria-like rash (neutrophilic infiltration in skin biopsy will clarify the origin in uncertain cases), recurrent eye inflammation including conjunctivitis with/without other inflammatory ocular findings, sensorineural hearing loss, clinical, laboratory and/or imaging evidence of chronic aseptic meningitis, musculoskeletal signs and symptoms of arthralgia, myalgia, arthritis and/or periarticular swelling, skeletal abnormalities including clubbing and/or frontal bossing and/or epiphyseal bony overgrowth and amyloidosis. A total of 14 CAPS items reached ≥80% agreement among experts.

CAPS consensus conference Boston: Items were reviewed and refined further resulting in the final item list (table 1). All items had achieved ≥80% agreement. Experts then participated in the iterative 1000Minds exercise process resulting in a ranking of items based on their importance for the diagnosis of CAPS. Mean criterion rankings (weighting) were calculated and ranged from 4 to 11. Results demonstrated excellent correlations between experts and for all subtypes.

Diagnostic model development

The unique multicentre, multinational cohort included 284 paediatric and adult patients with FCAS (30), MWS (164) and CINCA/NOMID (90). The CAPS control cohort consisted of 837 children and adults with either systemic JIA (100), Schnitzler syndrome (13), FMF (178), unclassified fever syndromes (93), typical KD (280) or incomplete KD (173). Multiple correspondence analysis was performed (see online supplementary tables S3, S4 and S5) including items from table 1 and identified three distinct entities: CAPS, non-CAPS autoinflammatory diseases and monophasic inflammatory diseases (figure 2). Correspondence analysis was successful in representing the contingency table in low-dimensional space with an overall retention of 78.78% (% total inertia) for a two-dimension solution (see online supplementary table S3) as determined by trace analysis for dependencies (χ^2=2696.6, df=78, p<0.0001) and test for dimensionality (axis inertia >16.7%). The quality of representation of a particular row or column has been provided as contributions to the total χ^2 statistic (see

Table 1 Items and definitions for diagnostic criteria for CAPS

CAPS-typical symptoms	Definition
Amyloidosis	Evidence of organ amyloid deposits eg, kidney
Recurrent episodes of systemic symptoms	Evidence of systemic features like fever and/or chills and/or fatigue and/or rigors
Urticaria-like rash	Histologically characterised by neutrophilic dermatitis
Chronic aseptic meningitis	Evidence of clinical, laboratory and/or imaging evidence of non-infectious inflammation of the meninges
Recurrent eye inflammation	Recurrent non-allergic, non-infectious conjunctivitis with/without other inflammatory ocular manifestations
Sensorineural hearing loss	Evidence of increased hearing thresholds on audiogram
Musculoskeletal signs and symptoms	Evidence of arthralgia, myalgia, arthritis and/or periarticular swelling
Skeletal abnormalities	Evidence of epiphyseal overgrowth, frontal bossing, clubbing and/or growth failure
Patient-related symptoms	
Confirmed *NLRP3* mutation	Genetic confirmation of *NLRP3* mutation
Family history of CAPS	Phenotype and or genetic confirmation of CAPS in other family members
CAPS course variables	
Early disease onset	Age at onset of CAPS-typical symptoms in infancy or early childhood
Episodic disease course characterised by CAPS-typical symptoms with or without persistent inflammation	Disease course with episodes of clinically active CAPS disease
Triggered inflammatory attacks	Triggers: cold or other stress factors
Coupling	
Raised inflammatory markers associated with CAPS-typical symptoms	Evidence of CAPS-typical clinical signs coupled with increased parameters of systemic inflammation eg, CRP or ESR

Final CAPS item list (Consensus conference Boston). All items had achieved ≥80% agreement.
CAPS, cryopyrin-associated periodic syndrome; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

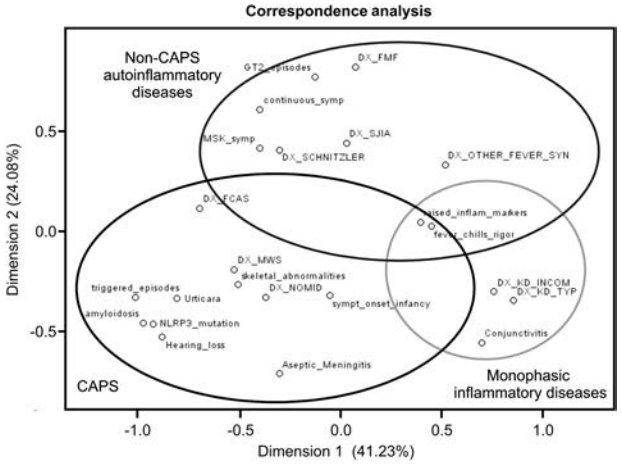

Figure 2 Capturing the diagnostic challenge of autoinflammation—discriminating cryopyrin-associated periodic syndrome (CAPS) from other inflammatory diseases using correspondence analysis. Multiple correspondence analyses (MCA) were used to assess the multidimensional relationship between putative CAPS diagnosis items and patient diagnoses in 284 CAPS cases including familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS) and chronic infantile neurological, cutaneous and articular syndrome/neonatal-onset multisystem inflammatory disorder (CINCA/NOMID) and 837 inflammatory controls. MCA computed the chi-squared statistic between each variable of interest and the outcome and transformed this statistic into a Euclidean distance. CAPS, non-CAPS autoinflammatory diseases and monophasic inflammatory diseases were the three distinct entities identified (see circles). Key variables consistently associated with the diagnosis of CAPS included urticaria-like rash, triggered episodes, sensorineural hearing loss, amyloidosis, musculoskeletal symptoms of arthralgia/arthritis/myalgia, chronic aseptic meningitis and skeletal abnormalities of epiphyseal overgrowth/frontal bossing ($p<0.01$). Raised inflammatory markers (C-reactive protein/serum amyloid A, (CRP/SAA)) and systemic symptoms of fever/chills/rigor were associated with all three entities.

online supplementary table S4) and Pearson's residuals (see online supplementary table S5).

Key variables consistently associated with the diagnosis of CAPS included urticaria-like rash, cold-triggered episodes, sensorineural hearing loss, amyloidosis, musculoskeletal symptoms (arthralgia/arthritis/myalgia), chronic aseptic meningitis and skeletal abnormalities (epiphyseal overgrowth/frontal bossing) ($p<0.001$ for all). Raised inflammatory markers (CRP and/or SAA) and systemic symptoms of inflammation (fever/chills/rigor) were associated with all three entities. In contrast, conjunctivitis was closely associated with monophasic inflammatory diseases, while continuous/persistent symptoms and episodic nature of disease had a closer relationship with non-CAPS autoinflammatory diseases. *NLRP3* mutation was removed as predefined and amyloidosis due to its rarity.

Stepwise logistic regression was used in an attempt to identify variables independently and significantly ($p<0.01$) associated with CAPS. They included urticarial-like rash, cold-triggered episodes, sensorineural hearing loss, musculoskeletal symptoms, chronic aseptic meningitis and skeletal abnormalities. However, the resulting multivariable model was found to be dominated by musculoskeletal symptoms revealing evidence of lack of fit (H-L χ^2 48.2, $p<0.01$). These predictors of interest were therefore considered separately or in combination with each other.

Diagnostic model validation

Different combinations of variables significantly associated with CAPS were tested for their association. Different models were explored. The best CAPS diagnosis criteria model included: raised inflammatory markers (CRP/SAA) plus ≥two of six CAPS-typical signs/symptoms including (1) urticaria-like rash, (2) cold-triggered episodes, (3) sensorineural hearing loss, (4) musculoskeletal symptoms (arthralgia/arthritis/myalgia), (5) chronic aseptic meningitis and (6) skeletal abnormalities (epiphyseal overgrowth/frontal bossing) ($p<0.001$) (figure 3). These

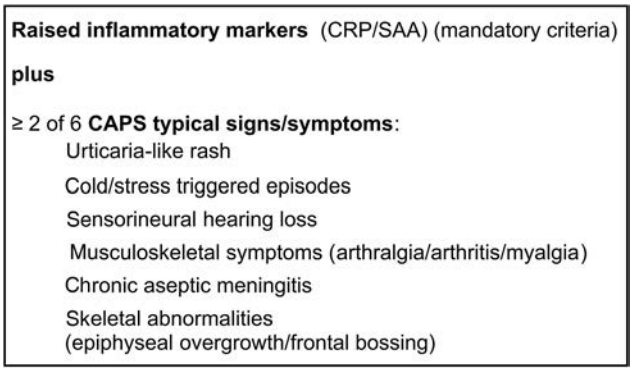

Figure 3 A model for the diagnosis of cryopyrin-associated periodic syndrome (CAPS). The proposed model for diagnosing CAPS including one mandatory criterion, namely raised inflammatory markers, plus at least two of six CAPS-typical symptoms, had a sensitivity of 81% and a specificity of 94%.

last six criteria had similar mean criterion rankings (weighting) ranging between 5 and 9.8 and were pooled together in order to increase ease of use for a clinical setting. The final CAPS diagnosis criteria model had a specificity of 94% and a sensitivity of 81%. It performed equally well for all CAPS subtypes and in subgroups with and without evidence of *NLRP3* mutation ($p<0.001$).

DISCUSSION

Criteria enabling physicians worldwide to make a diagnosis of the rare and heterogeneous autoinflammatory disease CAPS were developed and validated by an international team of experts using an innovative approach that integrated published evidence, registry expertise and expert opinion. It resulted in a comprehensive, well-defined list of putative CAPS diagnosis items capturing both the heterogeneous phenotype and the disease severity spectrum in children and adults with CAPS. The iterative review and refinement strategy using nominal group technique coupled with the 1000Minds decision analysis tool allowed for the development of a CAPS diagnosis model, which contained clinical and laboratory variables only, resulting in excellent generalisability. Most importantly, it did not mandate evidence of a disease-causing *NLRP3* mutation. It performed well in a large validation cohort of more than 1000 patients with CAPS and controls ($p<0.001$) achieving a high sensitivity and specificity.

The CAPS diagnosis criteria development followed an innovative, comprehensive process, which integrated diverse clinical expertise with rare diseases research methodology. The process was iterative; items were refined and strict rules of communication and knowledge gain (nominal group technique) were followed. It used an easy-to-use web-based decision tool, the 1000Minds instrument. This item generation and refinement strategy had successfully been used previously for the development of classification criteria for adult scleroderma.[14] Both the European and North American rheumatology societies promote its application.

The unique next step in this study was the exploration of the relevance of putative diagnosis items using correspondence analyses. This analysis highlighted the principles of the differential diagnostic challenges when diagnosing CAPS and its subtypes and discriminating these from other autoinflammatory and monophasic inflammatory diseases. It depicted both disease-specific variables and those representing the overlap between illnesses. It then permitted the development of a highly specific, sensitive and, most importantly, clinically relevant diagnostic model for CAPS. This approach may serve as a model for other rare diseases.

The proposed criteria are diagnostic criteria for CAPS and its subtypes. The study suggested that the presence of raised inflammatory markers (CRP or SAA) plus at least two of six CAPS-typical signs or symptoms including (1) urticaria-like rash, (2) cold-triggered episodes, (3) sensorineural hearing loss, (4) musculoskeletal symptoms of arthralgia/arthritis/myalgia, (5) chronic aseptic meningitis and (6) skeletal abnormalities of epiphyseal overgrowth/frontal bossing is highly likely to confirm the diagnosis of CAPS. This was confirmed in the presence and absence of a disease-causing *NLRP3* mutation.

There are few diagnostic criteria in inflammatory diseases: The most commonly cited and used criteria are the Jones criteria for acute rheumatic fever[15] and the KD criteria.[16] Both are derived from clinical expert observation. The KD criteria were refined by the American Heart Association in order to capture the entire disease spectrum, even including children with incomplete features using laboratory markers to confirm the diagnosis.[17] The vast majority of criteria for inflammatory diseases are classification criteria; developed within a group of overlapping conditions and aiming to establish well-characterised cohorts for research.[18 19] Recently proposed classification criteria include the paediatric EULAR/Paediatric Rheumatology INternational Trials Organisation (PRINTO)/Paediatric Rheumatology European Society (PRES) criteria for childhood vasculitis,[20] the Eurofever classification criteria for autoinflammatory diseases,[19] the FMF criteria[21] and the paediatric Behcet's disease classification criteria.[22]

In daily practice, criteria that enable a rapid diagnosis in rare diseases are urgently needed, in particular, in autoinflammatory diseases resulting in preventable organ damage. While the vast majority of available and stakeholder endorsed criteria sets are classification criteria—developed to identify homogenous cohorts for research studies[23]—the group of CAPS experts unanimously voted for these criteria being diagnostic criteria emphasising that care providers of children and adults with rare diseases need criteria to enable a rapid and reliable diagnosis.

The proposed CAPS diagnosis criteria are primarily clinical criteria. Clinical criteria are operator dependant and therefore carry the risk of limited sensitivity and specificity, the latter resulting in a risk of 'overdiagnosing' CAPS. This risk has to be considered prior to initiating therapies as the liability continues to be with the treating physician. The proposed CAPS criteria were developed by a group of CAPS experts and validated in a large cohort of inflammatory conditions. They will likely best perform when considered by inflammation experts in the context of a suspected systemic inflammatory disease. They have not been validated in potential non-inflammatory mimics, infectious or malignant conditions mimicking of CAPS. Robustness to each of the criteria can be added by symptom diaries such as the auto-inflammatory diseases activity index (AIDAI) questionnaire, pictures of clinical signs to be reviewed at clinic visits, serial blood tests for CAPS-typical inflammatory markers or skin biopsies, when suspecting a neutrophilic dermatitis causing the CAPS-typical urticaria-like rash. In particular when faced with a mild CAPS phenotype, these additional investigations should be considered prior to initiation of targeted therapies.

To our knowledge, the only other initiative aiming to develop and validate diagnostic criteria for inflammatory diseases is the Diagnosis and Classification Criteria for Vasculitis Study that has recruited over 5000 patients—adult vasculitis cases and vasculitis mimic controls—from 129 sites worldwide.[24] In both

disease entities, vasculitis and CAPS, a rapid diagnosis and initiation of targeted therapy is essential to prevent organ damage from inflammation.

The study has several limitations. The number of CAPS cases and controls was limited and not all possible differential diagnoses of CAPS may have been included, potentially leading to an overestimation of the specificity of the proposed model. However, the group dedicated long, thorough discussions to the identification of clinically relevant control populations. Importantly, the team collected the largest number of CAPS cases and controls studied to date. Not all subspecialists involved in the care of children and adults with CAPS were part of the team. The group did not identify any ear–nose–throat or ophthalmology CAPS experts, which may have caused an underrepresentation of clinical CAPS items generated from these subspecialists. However, missing expertise should have been partially compensated by items generated from the systematic literature review. Also, all team members provide care in an interdisciplinary team and felt that all specific organ-related items were well integrated.

CONCLUSION

The CAPS diagnosis model is the result of a unique collaborative team approach. It captures all diseases in the spectrum of CAPS and therefore enables a rapid diagnosis and initiation of treatment for children and adults with CAPS, a rare, heterogeneous inflammatory disease. The novel approach integrated traditional methods of evidence synthesis with expert consensus, web-based decision tools and innovative statistical methods and may serve as a model for developing diagnostic criteria for other rare diseases.

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Contributors JBK-D, SO and SMB conceived of the study, organised the project, wrote and revised the manuscript; PNT conducted the statistical analyses; IK-P, RG-M, HL, NB, HMH, EW-R, BH, TK, MG, AG, FD and LC participated in the expert surveys and consensus conferences; NTH and MO conducted the standardised literature search and evaluated the papers for scientific content applying to this study.

Competing interests JBK-D performed clinical studies with Novartis and received speaking honoraria from Novartis and SOBI.

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2016 update of the EULAR recommendations for the management of early arthritis

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ABSTRACT

Objectives Since the 2007 recommendations for the management of early arthritis have been presented, considerable research has been published in the field of early arthritis, mandating an update of the 2007 European League Against Rheumatism (EULAR) recommendations for management of early arthritis.

Methods In accordance with the 2014 EULAR Standardised Operating Procedures, the expert committee pursued an approach that was based on evidence in the literature and on expert opinion. The committee involved 20 rheumatologists, 2 patients and 1 healthcare professional representing 12 European countries. The group defined the focus of the expert committee and target population, formulated a definition of 'management' and selected the research questions. A systematic literature research (SLR) was performed by two fellows with the help of a skilled librarian. A set of draft recommendations was proposed on the basis of the research questions and the results of the SLR. For each recommendation, the categories of evidence were identified, the strength of recommendations was derived and the level of agreement was determined through a voting process.

Results The updated recommendations comprise 3 overarching principles and 12 recommendations for managing early arthritis. The selected statements involve the recognition of arthritis, referral, diagnosis, prognostication, treatment (information, education, pharmacological and non-pharmacological interventions), monitoring and strategy. Eighteen items were identified as relevant for future research.

Conclusions These recommendations provide rheumatologists, general practitioners, healthcare professionals, patients and other stakeholders with an updated EULAR consensus on the entire management of early arthritis.

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Peripheral inflammatory arthritis is among the most common features with which patients present in clinical rheumatology. Identifying the underlying disease can be difficult, particularly at an early stage. In clinical practice, early inflammatory arthritis is frequently undifferentiated.[1] Early arthritis can develop into established rheumatoid arthritis (RA) or another definite arthropathy, can resolve spontaneously, or may remain undifferentiated for indefinite periods. To better evaluate diagnosis and outcome in arthritis, it has been proposed to first recognise inflammatory arthritis; then search for a definite diagnosis (eg, peripheral or axial spondyloarthritis; psoriatic arthritis (PsA); systemic lupus erythematosus, etc), and finally estimate the risk of developing persistent and/or erosive arthritis and propose an optimal therapeutic strategy.[2 3] Although the prognosis of early arthritis is still difficult to define, a combination of clinical, laboratory and radiographic parameters may help to predict patients' outcomes with acceptable accuracy.

The management of early arthritis has changed considerably in the past few years under the influence of new concepts for diagnosis and new effective therapies. Conventional synthetic disease-modifying antirheumatic drugs (csDMARDs) have been shown to slow disease progression in chronic inflammatory arthritides such as RA and PsA.[4–6] Furthermore, biological (b) DMARDs have demonstrated rapid and sustained disease control associated with an arrest of joint destruction.[7 8] A large body of evidence points to the usefulness of very early DMARD-start for early chronic inflammatory arthritis, preferably before the onset of erosions, in order to reduce or even prevent the risk of (further) joint damage and disability.[5 9 10] Also, the assessment and tight monitoring of patients with early arthritis serves to better adapt therapeutic strategies.[9 11] Beyond doubt, the treatment goal of early arthritis should now be clinical remission and prevention of joint destruction.

Patients with early arthritis should be identified and referred to rheumatologists to confirm the presence of arthritis, the (potential) diagnosis and its prognosis and initiate appropriate treatment strategies based on these findings. Furthermore, management of early arthritis should include more than drug treatment alone, with education, shared decision making and the role of allied healthcare professionals as important themes.

A set of recommendations for the management of arthritis should address all these different aspects.

The European League Against Rheumatism (EULAR) recommendations for the management of early arthritis have been published in 2007.[9] In 2010, EULAR presented recommendations for the management of RA with synthetic and biological DMARDs, which have been updated in 2013 and

2016;[12 13] in addition, recommendations for the management of PsA were recently published.[6] While the latter recommendations focused on the pharmacological treatments of PsA and RA, both in advanced and in early disease, the 2007 recommendations for the management of early arthritis covered the entire spectrum of management of early arthritis, including the recognition of arthritis, referral, diagnosis, prognosis, classification, information, education, non-pharmacological interventions and monitoring of the disease process as well as pharmacological treatment. The systematic literature review (SLR) that has guided the 2007 EULAR recommendations included publications up to January 2005.[9] Between 2005 and 2015, research in early arthritis has been a major focus, and many studies have appeared in the peer-reviewed literature. This literature includes —but is not limited to—topics such as diagnosis and classification criteria, window of opportunity, imaging, prognostication, treatments and therapeutic strategies.

These developments mandated an update of the existing EULAR recommendations on early arthritis, which is reported here.

METHODS

The update of the EULAR recommendations for the management of early arthritis has followed the 2014 EULAR Standardised Operating Procedures.[14] The definitions (eg, management and early arthritis) of and the target populations (rheumatologists, general practitioners, medical students, healthcare professionals, patients) addressed by the 2007 expert committee[9] were considered. Briefly, the term 'management' was defined as 'all organisational, diagnostic, medical and educational procedures related to patients seeking help for arthritis of a peripheral joint' and 'early arthritis' was restricted to 'early inflammatory joint disease'.

The expert committee

The expert committee comprised 20 rheumatologists, including 2 research fellows (CID and CH), 1 healthcare professional and 2 patients, from 12 European countries.

Fifteen research questions derived from the 2007 process were proposed by the convenor (BC) and the methodologist (RL), and subsequently amended and approved by the whole committee. The selected topics included recognition of arthritis, referral, diagnosis, prognostics, classification, information, education, non-pharmacological interventions, pharmacological treatments, monitoring of the disease process, strategy and prevention.

Evidence-based approach

The research questions were adjusted for further literature research if appropriate, and structured according to the Patients-Intervention-Comparator-Outcome systematic by four of the authors (CID, CH, BC, RL). Eligible study types were also defined.

A systematic search of PubMed, Medline, Embase, CINAHL and the Cochrane library was performed, with the help of a skilled librarian (Louise Falzon, Columbia University Medical Centre, USA). All articles published in English up to December 2015 were included. Abstracts from the 2014 and 2015 EULAR and American College of Rheumatology (ACR) conferences were also considered. The search was completed by a hand search and by questioning experts for additional references. The SLR process is reported in detail in two separate articles.[15 16]

Expert opinion approach

Each member of the expert committee obtained insight into the results of the literature search and the accompanying levels of evidence before a meeting in January 2016. During the meeting, the results of the SLR were presented to the committee in aggregated format. Three break-out groups, chaired by one expert, were formed to amend the 2007 recommendations (1–4; 5–8 and 9–12) and to propose new recommendations if considered appropriate. Each group then reported its proposals and wording to the entire committee for discussion and consensus, and the final formulation of the recommendations was obtained after a vote with at least 85% agreement for each item's final wording.

After the meeting the recommendations were circulated by email to all expert committee members for further minor amendments if necessary. Categories of evidence and grades of recommendations were then determined (by CID, CH, RL, BC) according to the standards of the Oxford Centre for Evidence-Based Medicine.[17] To determine the level of agreement with recommendations, an anonymised email-based voting on a 0–10 scale was performed, a vote of 0 indicating complete disagreement with a particular recommendation and 10 indicating complete agreement. The means and SDs for scores from the whole group were calculated. The recommendations are presented in box 1 and figure 1.

RESULTS

The discussions of the expert committee resulted in 3 overarching principles and 12 recommendations (box 1) (in 2007, 12 recommendations were formulated).

Overarching principles

The expert committee considered that some of the principles on the care of patients with early arthritis are generic and should be stated first and separated from individual recommendations on diagnosis, prognosis and treatment. The committee decided unanimously on the following three overarching principles (box 1).

Principle A:

> Management of early arthritis should aim at the best care and must be based on a shared decision between the patient and the rheumatologist.

The term 'best care' is obviously a major principle in medicine. The wording 'shared decision between the patient and the rheumatologist' is more than informing the patient; it rather refers to the comprehensive process of communication, knowledge exchange and achieving consensus that should lead to a treatment decision, that is, optimal from the perspectives of both patient and clinical care provider.

Principle B:

> Rheumatologists are the specialists who should primarily care for patients with early arthritis.

This statement, which was part of recommendation 1 in the 2007 recommendations, was also highlighted in the EULAR recommendations for the management of RA[14] and PsA.[6] Its basis is evidence that patients with chronic arthritis under rheumatologists' care receive an earlier diagnosis, start treatment earlier and have better outcomes, in particular with respect to joint damage and physical function.[18–20] Rheumatologists have the expertise to establish an accurate diagnosis of early arthritis, are familiar with monitoring disease activity and with the potential severity of the disease in their patients with inflammatory arthritis and are well aware of the indications, contraindications and adverse effects of specific therapies.

Box 1 2016 update of the EULAR recommendations for management of early arthritis: final recommendations based on evidence and expert opinion

Overarching principles

A. Management of early arthritis should aim at the best care and must be based on a shared decision between the patient and the rheumatologist
B. Rheumatologists are the specialists who should primarily care for patients with early arthritis
C. A definitive diagnosis in a patient with early arthritis should only be made after a careful history taking and clinical examination, which should also guide laboratory testing and additional procedures

Recommendations

1. Patients presenting arthritis (any joint swelling, associated with pain or stiffness) should be referred to, and seen by, a rheumatologist, within 6 weeks after the onset of symptoms
2. Clinical examination is the method of choice for detecting arthritis, which may be confirmed by ultrasonography
3. If a definite diagnosis cannot be reached and the patient has early undifferentiated arthritis, risk factors for persistent and/or erosive disease, including number of swollen joints, acute phase reactants, rheumatoid factor, ACPA and imaging findings, should be considered in management decisions
4. Patients at risk of persistent arthritis should be started on DMARDs as early as possible (ideally within 3 months), even if they do not fulfil classification criteria for an inflammatory rheumatologic disease
5. Among the DMARDs, methotrexate is considered to be the anchor drug and, unless contraindicated, should be part of the first treatment strategy in patients at risk of persistent disease
6. NSAIDs are effective symptomatic therapies but should be used at the minimum effective dose for the shortest time possible, after evaluation of gastrointestinal, renal and cardiovascular risks
7. Systemic glucocorticoids reduce pain, swelling and structural progression, but in view of their cumulative side effects, they should be used at the lowest dose necessary as temporary (<6 months) adjunctive treatment. Intra-articular glucocorticoid injections should be considered for the relief of local symptoms of inflammation
8. The main goal of DMARD treatment is to achieve clinical remission, and regular monitoring of disease activity, adverse events and comorbidities should guide decisions on choice and changes in treatment strategies to reach this target
9. Monitoring of disease activity should include tender and swollen joint counts, patient and physician global assessments, ESR and CRP, usually by applying a composite measure. Arthritis activity should be assessed at 1-month to 3-month intervals until the treatment target has been reached. Radiographic and patient-reported outcome measures, such as functional assessments, can be used to complement disease activity monitoring
10. Non-pharmacological interventions, such as dynamic exercises and occupational therapy, should be considered as adjuncts to drug treatment in patients with early arthritis
11. In patients with early arthritis smoking cessation, dental care, weight control, assessment of vaccination status and management of comorbidities should be part of overall patient care
12. Patient information concerning the disease, its outcome (including comorbidities) and its treatment is important. Education programmes aimed at coping with pain, disability, maintenance of ability to work and social participation may be used as adjunct interventions

ACPA, anticitrullinated peptide antibodies; CRP, C reactive protein; DMARD, disease-modifying antirheumatic drug; ESR, erythrocyte sedimentation rate; EULAR, European League Against Rheumatism; NSAIDs, non-steroidal anti-inflammatory drugs.

However, the expert committee intentionally added the term 'primarily' to this statement for three reasons: (1) the management of patients with early arthritis includes the care by primary care physicians and other healthcare professionals in a multidisciplinary approach; (2) in some places care by rheumatologists is not always available and accessible. Some countries have a shortage of rheumatologists, and in such situations patients should receive treatment from other healthcare providers with experience in the care of patients with inflammatory arthritis; (3) in some countries, task shifting from rheumatologists to other healthcare professionals is actively supported in order to facilitate early access and optimal quality of care, and to make care cheaper. Such care is still primarily under the responsibility and supervision of rheumatologists, but may be provided by other care providers.

Principle C:

A definite diagnosis in a patient with early arthritis should only be made after a careful history taking and clinical examination, which should also guide laboratory testing and additional procedures.

In the 2007 recommendations, this important statement was included as bullet point 3. It was considered that 'good clinical practice' and a 'high level of training' suffices an opinion that was entirely expert-based. The expert group was of the unanimous opinion that the statement is so generic that it represents an overarching principle rather than a recommendation. To establish a definite diagnosis in a patient with early arthritis, the group proposed that the minimum diagnostic procedures should include careful history taking and clinical examination, keeping the different possible causes of inflammatory arthritis in mind. After excluding other causes of joint swelling and pain (eg, septic arthritis, trauma, osteoarthritis, gout), particular attention should be paid to age, geographical area and travel history, number and pattern of involved joints, axial/entheseal involvement and extra-articular features (eg, eye, skin, genitourinal and gastrointestinal symptoms), including recent infections.[1] A minimal laboratory testing panel was proposed in the 2007 recommendations and should include testing for C reactive protein (CRP)/erythrocyte sedimentation rate (ESR), full blood cell count, transaminase levels, renal function and urine analysis,

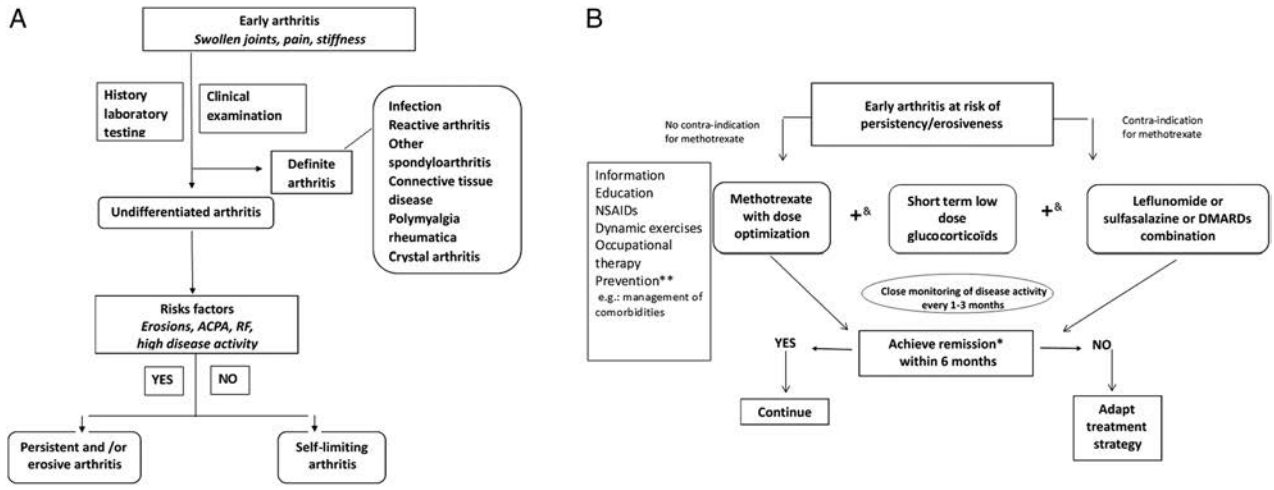

Figure 1 Algorithms based on the 2016 update of the European League Against Rheumatism recommendations for management of early arthritis. (A) Diagnosis and prognosis. (B) Treatment and strategy. &Combination with glucocorticoids preferred. *Low disease activity could be an alternative target in rare occasions. **Should also include weight loss, smoking cessation, dental care and vaccination. ACPA, anticitrullinated peptide antibodies; DMARD, disease-modifying antirheumatic drug; NSAID, non-steroidal anti-inflammatory drug, RF, rheumatoid factor.

rheumatoid factor (RF), anticitrullinated peptide antibodies (ACPA) and antinuclear antibodies. In addition, the diagnostic procedure may be expanded with microbiology and/or serological tests (reactive arthritis, synovial fluid microbial culture, Lyme disease, parvovirus infection, hepatitis B or C), uric acid testing, synovial fluid analysis (cell count and polarised light microscopy if needed), chest and joint radiographs, but dependent on the context and the country.

Recommendations

The discussions of the expert committee culminated into 12 recommendations (box 1). In comparison with 2007, the previous recommendation 3 was transformed into overarching principle C, while a recommendation for prevention (no. 11) was added. In addition, the order of the bullet points was slightly amended in order to better assure a logical sequence (and not for reasons of prioritisation). Table 1 displays the levels of evidence and grades for the following recommendations based on the Oxford Levels of Evidence assessment as well as level of agreement after anonymised voting by the expert committee.

Recommendation 1:

> Patients presenting with arthritis (any joint swelling, associated with pain or stiffness) should be referred to, and seen by, a rheumatologist, within 6 weeks after the onset of symptoms.

This recommendation is almost identical to its 2007 counterpart, but with subtle changes in the wording. After 2005, two studies have confirmed that patients with inflammatory arthritis in general, and those with suspected RA in particular, should be referred to rheumatologists as early as possible.[19 20] A delay in referral is one of the most important causes of late diagnosis and late start of effective treatment. Patients with early arthritis referred to a specialist within 3 months show better outcomes in terms of drug-free remission, radiographic damage and (less) need for orthopaedic surgery than those with late referral.[15] This is also fully in line with standards of care developed for patients with RA and quality indicators as established by European Expert committees.[21] On the basis of these data as well as the clinical experience of the committee members, it was recommended that diagnosis and start of treatment, both by a rheumatologist, should be established within a relatively short period after the onset of complaints which justifies the wording 'within 6 weeks' in this recommendation.

Joint swelling not due to trauma or bony swelling suggests early inflammatory arthritis, especially if associated with pain and morning stiffness >30 min.[22] Several referral questionnaires evaluating swelling, pain and stiffness have been developed to aid in the detection of early arthritis.[15] These questionnaires have a good sensitivity (86%–90%) and specificity (90%), but have been tested only in small patient samples and lack confirmation in independent validation cohorts. The committee was of the opinion that an appropriately validated tool to help general practitioners in adequately diagnosing and referring patients

Table 1 Updated EULAR recommendations for management of early arthritis, with LoE, GoR and LoA

		LoE*	GoR*	LoA*
A.	Shared decision	na	na	9.87±0.46
B.	Rheumatologists	na	na	9.78±0.67
C.	Diagnosis	na	na	9.78±0.67
1.	Early referral	Ib	B	9.43±1.16
2.	Clinical examination	IIb	C	9.48±0.99
3.	Prognosis	IIb	C	9.83±0.49
4.	Early treatment start	Ia	A	9.35±1.07
5.	MTX, the anchor drug	Ia	A	9.52±0.99
6.	NSAIDs	IV	D	9.00±1.13
7.	Glucocorticoids	Ia	A	9.00±1.28
8.	Remission and treatment strategies	Ib, IV†	A, D	9.52±0.9
9.	Regular monitoring	Ia, IV	A, D‡	9.13±1.06
10.	Non-pharmaceutical interventions	Ia	B	8.96±1.26
11.	Prevention	IIb, IV	C, D‡	8.96±1.19
12.	Patient information	Ia, Ib	B	9.35±0.98

*LoE and GoR are based on the recommendations of the Oxford Centre for Evidence-Based Medicine. LoA was based on an anonymised email voting system with a 0–10 scale by all members of the expert committee (data are mean±SD; 100% of voters).
†The general statement is evidence-based.
‡The place in the treatment algorithm is based on expert consensus.
EULAR, European League Against Rheumatism; GoR, grade of recommendation; LoA, level of agreement; LoE, level of evidence; MTX, methotrexate; na, not applicable; NSAIDs, non-steroidal anti-inflammatory drugs.

with early arthritis is currently lacking. The strength of this recommendation was considered 'good' (category B) (table 1).

Recommendation 2:

Clinical examination is the method of choice for detecting arthritis, which may be confirmed by ultrasonography (US).

The expert committee unanimously appreciated the pivotal role of clinical examination. Clinical examination is still the cornerstone of detecting synovitis. This appreciation does not preclude that imaging modalities may be more sensitive in the detection of synovitis. US, including power Doppler techniques, may suggest synovitis by showing thickening of the synovial membrane, bursae and/or tendon sheaths with enhanced vascularity.[15] Several controlled studies have suggested a greater sensitivity of US than clinical examination in detecting synovitis in the knee and in small joints. US has been evaluated in detail in the 'EULAR recommendations for the use of imaging of the joints in the clinical management of rheumatoid arthritis'.[23] The expert committee did not recommend a more prominent role for US in the detection of synovitis, since it was broadly felt that potentially decreased specificity and lack of knowledge regarding the long-term consequences of positive US in individual patients did not currently justify a more prominent position for US. Furthermore, wording specifically referring to power Doppler was deleted, because the group considered that power Doppler should be part of every US joint examination anyway.

MRI has also been suggested to be more sensitive than clinical examination in the early detection of synovitis,[23–25] but may face a lack of specificity as suggested by the prevalence of MRI abnormalities in the normal population.[26] In contrast with US, which is now a common tool in many rheumatologist practices, the long scanning time, limited access and the relatively high costs limit the widespread use of MRI. Therefore, the expert committee considered that MRI should be proposed only in very difficult cases or in patients with specific forms of arthritis, and that further research is needed to better determine the place of this imaging modality in the diagnosis of patients with early arthritis. MRI was part of the 2007 recommendations but was deleted from the current set.

Recommendation 3:

If a definite diagnosis cannot be reached and the patient has early undifferentiated arthritis, risk factors for persistent and/or erosive disease, including number of swollen joints, acute-phase reactants, RF, ACPA and imaging findings, should be considered in management decisions.

This recommendation was slightly rephrased because the group wanted to highlight that early undifferentiated arthritis should be clearly differentiated from early RA. In addition, 'imaging' was used instead of 'radiographic' to show that imaging modalities other than plain radiographs may provide prognostic information. For patients with early arthritis, after the exclusion of specific forms of arthritis, the working diagnosis is often undifferentiated arthritis. The next step in the diagnostic procedure is to evaluate the risk of persistent and/or erosive arthritis, usually corresponding to the definition of RA, in an individual patient.[27] This prognostic typing is now considered crucial to guide the optimal therapeutic strategy.

Since the 2007 exercise, many observational studies have evaluated the prognostic value of laboratory and imaging procedures for early arthritis. Most prognostic factors were analysed in a multivariate manner in these studies, to test their independent contribution. Commonly tested dependent variables were persistence, erosiveness or radiographic progression.

In most of the studies, ACPA and RF positivity and ACPA and RF levels have shown some predictive value for the development of persistent and erosive arthritis. This observation was clearly highlighted by EULAR and ACR since ACPAs, in addition to RF, have obtained an important weight in the 2010 ACR/EULAR classification criteria for RA.[27 28] In addition, several recent studies have confirmed the independent association of ACPAs with a diagnosis of RA as well as with radiographic progression in patients with early arthritis.[29–33] RF has been assigned a similar weight as ACPAs in the 2010 ACR/EULAR classification criteria for RA, although recent publications stemming from early arthritis cohorts and observational studies have suggested a lower predictive and diagnostic value of RF compared with ACPAs but RF has a stronger association with disease activity independent of the presence of ACPA.[15] The combination of RF and ACPAs does not provide additional value to RF or ACPAs alone.[28] In addition to ACPA, the number of swollen joints and the level of CRP and ESR are independent contributory factors.

Early erosion typical of RA is still a major prognostic factor in early arthritis and automatically leads to a classification of RA.[27 34] Synovitis and erosion detected by MRI or US may predict further joint damage in early arthritis, but false positivity has been reported.[26 35] MRI-detected bone marrow oedema and osteitis are independent predictors of radiographic progression in early RA,[23 24] but data are limited in early arthritis. Finally, two recent studies have shown that hand flexor or extensor tenosynovitis on US[36] or MRI[25] may be a specific—although not very sensitive—marker for RA classification.

Several combinations of diagnostic markers have been evaluated, but no one has been formally validated.[15] In addition, multibiomarker tests have been proposed to evaluate disease activity, prognosis and response to therapy, but current data are not convincing and further research is warranted.[15] Finally, it has been reported that substituting MRI for clinical examination in the 2010 ACR/EULAR criteria increases the sensitivity but decreases the specificity for a diagnosis of RA.[15] MRI is therefore of limited value in making a diagnosis of RA and is not recommended as a standard procedure.

Recommendation 4:

Patients at risk of persistent arthritis should be started on DMARDs as early as possible (ideally within 3 months), even if they do not fulfil classification criteria for an inflammatory rheumatologic disease.

This recommendation was slightly reworded and reiterates the unanimous opinion of the committee that an early treatment start is pivotal in the management of patients with early chronic arthritis such as early RA, early PsA or those at risk to develop persistent and erosive disease. The wording 'RA' is not used in this statement, but the implicit meaning is that persistent and/or erosive disease is factually synonymous to RA (see previous item) and justifies an early start with DMARDs. A new element is the maximum delay of 3 months after the onset of symptoms before starting the first DMARD. The expert committee was of the opinion that this time frame constitutes a 'window of opportunity' that should be considered to provide an optimal outcome in the patients at risk. Eight recent studies have endorsed an early treatment start. Four studies showed that introducing DMARDs within 3 months after the onset of symptoms leads to better outcome (remission, response to treatment, Health Assessment Questionnaire disability score or radiographic progression).[37–40] Very recently, van Nies *et al*[41] have suggested, based on data in the Etude et Suivi des Polyarthrites Indifferenciées Recentes (ESPOIR) and Leiden early arthritis cohorts, that 12–14 weeks represent an appropriate window within which therapy should be started in order to prevent

arthritis persistence. In addition, disease duration at the time of DMARD initiation was the most important determinant of response to DMARD therapy in another study.[15] This statement may raise questions about the best definition for 'early RA'. A duration of 3 months after the onset of symptoms may be the longest allowable delay in prescribing the first DMARD. However, this maximum delay is still difficult to meet in daily practice, while most of the recent 'early RA cohorts' allowed a delay of 6 months from the onset of symptoms (joint swelling usually) for inclusion.[28 29 41] A delay of not more than 6 months was also proposed in recent RA guidelines.[42] A delay of more than 1 year from symptom onset must not be considered 'early' anymore.

Recommendation 5:

> Among the DMARDs, methotrexate (MTX) is considered the anchor drug and unless contraindicated, should be part of the first treatment strategy in patients at risk of persistent disease.

This recommendation (previously no. 9) remains almost unchanged. Previous SLRs have confirmed the clinical and structural efficacy as well as the good safety profile of MTX.[4 43 44] An important argument to consider MTX an anchor drug as part of the first treatment strategy in patients at risk of persistent arthritis (eg, at risk of RA) is its good efficacy in early RA, and its 'practicability', both as monotherapy and in combination with glucocorticoids (GC), other csDMARDs and bDMARDs.[4 13 45] Recent trials in early DMARD-naïve patients with RA have evaluated MTX monotherapy versus csDMARDs combined with different dosages and routes of administration of GC. Verschueren *et al*[46] have recently reported similar 16-week remission rates in high-risk patients with early RA receiving MTX monotherapy, MTX plus sulfasalazine (SSZ) or MTX plus leflunomide (LEF), all in combination with high-dose prednisone bridging strategies. In another trial, MTX plus temporary high-dose prednisone was not less effective than MTX plus SSZ plus temporary high-dose prednisone after 26 weeks.[47] The Treatment in the Rotterdam Early Arthritis CoHort (tREACH) trial suggested short-lived superiority of MTX combined with SSZ, hydroxychloroquine and GC versus MTX and GC, but this superiority was not seen in all aspects, was not clinically meaningful and did ultimately not sustain after 1 year.[48] The Treatment of Early Aggressive Rheumatoid Arthritis (TEAR) trial did not support a benefit of an intensive csDMARDs combination regimen over MTX monotherapy either.[49] In the absence of clear signals for superiority of a csDMARDs combination regimen, and guided by a trend towards lower tolerability for csDMARD combination,[16] the committee was of the opinion that the first treatment strategy should be MTX monotherapy with or without short-term high-dose GC as bridging therapy for most patients. In that regard, dose optimisation is an important aspect of first-line DMARD strategy, as previously reported[4 45] (MTX should be titrated rapidly to 20–30 mg/week, depending on clinical response and tolerability; parenteral administration should be considered in case of inadequate clinical response or intolerance).

The superiority of bDMARDs plus MTX over MTX monotherapy has been proven in many randomised controlled trials (RCTs) and was confirmed by eight recent studies in the current SLR.[16] In addition, two targeted synthetic DMARDs have recently demonstrated superiority to MTX, both used as monotherapy, in patients with early RA.[50 51] Nevertheless, because the benefit-to-risk ratio of these biological and targeted synthetic DMARDs was not convincingly favourable in patients with early disease, because tight monitoring is anyway part of the current treatment strategy to identify those in need of adding biologics and also because of their high cost, the expert committee considered their use as a first treatment strategy inappropriate, except in rare situations.

Recent RCTs comparing other csDMARDs with MTX were lacking. The clinical efficacy of LEF, and to a lesser extent SSZ, is similar to MTX in established and recent RA.[9] LEF is as effective as MTX in slowing radiographic damage, and its therapeutic maintenance is similar to that of MTX.[9] In contrast, SSZ may be inferior to LEF and MTX in the long term. Although formal evidence prioritising MTX over other csDMARDs as the first DMARD used in early arthritis and/or early RA is lacking, the expert committee does recommend MTX as first-choice treatment (unless contraindicated) in patients at risk of persistent disease. LEF and (to a lesser extent) SSZ are considered the best alternatives. Of note, SSZ is considered safe during pregnancy in contrast to MTX and LEF. Finally, the committee is of the opinion that antimalarial drugs, which have shown less clinical efficacy and may not retard radiographic progression in patients with RA but may have positive metabolic effects, can be considered as partner in combination therapy or as DMARD monotherapy in patients with mild disease and comorbidities or with persistent arthritis other than RA.[52]

Recommendation 6:

> Non-steroidal anti-inflammatory drugs (NSAIDs) are effective symptomatic therapies, but should be used at the minimum effective dose for the shortest time possible, after evaluation of gastrointestinal, renal and cardiovascular risks.

The SLR did not yield new data on NSAIDs in patients with early arthritis. The expert committee felt that symptomatic therapy with NSAIDs is still of value in patients presenting with early arthritis, but only after a careful consideration of gastrointestinal, renal and cardiovascular contraindications. In addition to the previous item no. 7 about NSAIDs, the group now reinforces the need to follow the US Food and Drug Administration and European Medicines Agency guidelines about NSAIDs, which includes wording about the shortest possible treatment duration, the minimum effective dose and the contraindications for patients at risk (http://www.fda.gov; http://www.ema.europa.eu).

Recommendation 7:

> Systemic GC reduce pain, swelling and structural progression, but in view of their cumulative side effects, they should be used at the lowest dose necessary as temporary (<6 months) adjunctive treatment. Intra-articular glucocorticoid injections should be considered for the relief of local symptoms of inflammation.

The expert committee has intensively debated the role of GC in the management of early arthritis. This discussion was based on expert opinion and on new information obtained by the SLR.[16] Recently, one meta-analysis of 14 RCTs in patients with RA and 2 RCTs in patients with 'early RA' has confirmed that systemic GC improve clinical and radiographic outcomes.[16 53 54] Preferably, therapy with systemic GC is temporary because of the risk of side effects, including weight gain, hypertension, diabetes, cataracts and osteoporosis, which justify careful monitoring and appropriate prevention. New data stemming from registries, observational studies and extensions of RCTs have also suggested an increased risk of severe infections, cardiovascular events and mortality.[16 55–60] In addition, there is evidence that intra-articular steroids may be an effective adjunct to DMARDs in relieving joint symptoms in patients presenting with early arthritis and may improve disease activity up to 24 months.[16]

The committee has reworded this item (no. 8 in the previous recommendations) in order to highlight the effectiveness of

systemic GC for relieving symptoms and disease progression but also in order to point to the risks of cumulative side effects in the medium to long term. The committee is of the opinion that GC can only be justified if used at the lowest possible cumulative dose, for the shortest possible duration and exclusively as adjunct (or bridge) therapy to csDMARDs. GC monotherapy may mask disease activity before a diagnosis has been established and should be avoided in patients with early arthritis, in order to expedite a proper diagnosis, and secure an adequate prognosis and a prompt DMARD treatment start. Despite a fierce debate, this recommendation was finally approved by 95% of the members and obtained a high level of agreement (mean of 9.00 ±1.28) with anonymous voting. The wording 'low dose' and the optimal regimen (low daily dose or high dose then step-down or parenteral boosts) in early arthritis are still under debate and will be mentioned in the research agenda (box 2).

Recommendation 8:

The main goal of DMARD treatment is to achieve clinical remission, and regular monitoring of disease activity, adverse events and comorbidities should guide decisions on choice and changes in treatment strategies to reach this target.

The 2007 recommendations for patients with early arthritis were among the first guidelines to highlight clinical remission as the main objective in the care of these patients. In the past 10 years, accumulating data have supported this as a major goal for the treatment of RA and other inflammatory arthritides.[6 9 11 13 61]

The expert committee has decided to keep the wording of the previous recommendation no. 10 unchanged. A few new studies have confirmed that achieving clinical remission as early as possible results in better clinical outcomes and quality of life, and helps to prevent further structural damage, functional disability and job loss in patients with early arthritis and early RA.[62] Which particular remission criteria should be used in practice remains unclear. Composite scores (disease activity score (DAS), DAS28, Clinical Disease Activity Index, Simplified Disease Activity Index (SDAI)) should be used, and the ACR-EULAR remission criteria (Boolean or SDAI) is likely the most stringent.[63] An interesting definition for daily practice is 'the absence of signs and symptoms of significant inflammatory disease activity'.[11] Recent evidence has suggested that remission leads to a better outcome than low disease activity (LDA),[62 64 65] and the committee was of the opinion that clinical remission according to the ACR-EULAR Boolean or index-based definition is the target for every patient presenting with early arthritis. A LDA state could be an appropriate alternative goal only in cases in which remission is considered unfeasible. In this respect, factors such as comorbidities, age or adverse events must be considered, and may determine the desired treatment target, which will form the basis for the process of shared decision making with the patient.

The expert committee also discussed whether imaging remission should be included in the target, as suggested by some recent recommendations.[23] Studies have suggested that ongoing inflammation seen by US, and to a lesser extent by MRI, in patients with clinical remission may predict structural progression. However, the significance thereof and its clinical utility are questionable and is associated with significant overtreatment and thus potential waste of societal resources;[66] the SLR did not yield new information.[15 16] Therefore, the expert committee suggested that the value of imaging remission should be part of the research agenda.

Finally, the committee felt that disease activity should be closely monitored in order to allow a timely change in DMARD therapy when necessary. The benefits of the treat-to-target approach have now amply been shown in patients with RA and PsA[11 67] and there is no reason to assume that the situation is different for early arthritis.

Recommendation 9:

Monitoring of disease activity should include tender and swollen joint counts, patient's and physician's global assessments, ESR and CRP, usually by applying a composite measure. Arthritis activity should be assessed at 1-month to 3-month intervals until the treatment target has been reached.

Box 2 Research agenda for management of early arthritis

Diagnosis and prognosis

1. Which tools could help general practitioners to diagnose early arthritis and prioritise referral?
2. Can we better define the diagnostic and prognostic value of ultrasonography in early arthritis?
3. Can we better define the diagnostic and prognostic value of MRI in early arthritis?
4. What is the diagnostic value of the systematic screening of antinuclear antibodies in early arthritis?
5. Which new biomarkers/multibiomarkers may help to better evaluate disease activity, the prognosis and treatment response in early arthritis?

Treatment and outcome

1. Can we develop prediction models to better define the therapeutic strategy in early arthritis?
2. Can we define at what level of risk (for developing persistent arthritis) different pharmacological interventions have a favourable benefit-to-risk ratios?
3. Do combinations of csDMARDs provide a better benefit-to-risk ratio than csDMARD monotherapy in early arthritis?
4. Can we better define 'low dose' and 'short term' use of glucocorticoids for an optimal medium-term to long-term benefit-to-risk ratio?
5. What is the optimal regimen (low daily dosage or high dose then step-down, or parenteral boosts) of glucocorticoids for better outcome in early arthritis?
6. Does imaging remission have an added benefit to clinical remission in treatment decisions?
7. What is the optimal interval at which to monitor radiographic progression in early chronic inflammatory arthritis?
8. What is the effectiveness of different non-pharmacological interventions in early arthritis?
9. Can physical activity/exercise reduce cardiovascular risk in early chronic arthritis?
10. Which study designs can best be used to investigate the comparative effectiveness and cost-effectiveness of different therapeutic strategies?
11. Is smoking cessation, oral hygiene, diets or psychological interventions beneficial for the outcome of patients with early arthritis?
12. What are the most efficient and effective information and education interventions and exercise programmes for early arthritis?

csDMARDs, conventional synthetic disease-modifying anti-rheumatic drugs; DMARD, disease-modifying antirheumatic drug.

Radiographic and patient-reported outcome measures, such as functional assessments can be used to complement disease activity monitoring.

In every patient with active arthritis, closely monitoring disease activity is now considered of particular importance in the therapeutic strategy to provide a good outcome and this is highlighted by all of the most recent recommendations.[6 9 11 13 42 61] Monitoring disease activity should be as frequent as the level of disease activity mandates, usually every 1–3 months, then potentially less frequently (such as every 6–12 months) once the treatment target has been achieved.

Nevertheless, three changes were proposed to this item (previously no. 12). First, a composite measure was recommended as the method of choice to monitor disease activity; second, a specific time frame for monitoring structural damage was deliberately left out and third, patient-reported outcomes were expanded beyond functional assessments.

Swollen joint count and progression of joint damage have been consistently found to be associated.[68 69] In addition, many trials have supported the use of a tight control of disease activity assessed via composite measures that include joint count evaluation.[11 16 67 70] Although it is difficult to formally investigate, the expert committee was of the opinion that monitoring the occurrence of radiographic progression is useful in view of one of the key objectives of managing early arthritis: the prevention of joint destruction. The determination of an optimal window for monitoring progression was added as an item for the research agenda (box 2).

Finally, patient-reported outcomes such as quality of life, fatigue and physical function are key to evaluate outcome[71 72] and the committee has mandated them as part of disease monitoring.

Recommendation 10:

Non-pharmacological interventions, such as dynamic exercises and occupational therapy, should be considered as adjuncts to drug treatment in patients with early arthritis.

This recommendation has remained almost unchanged. The efficacy of non-pharmacological therapy has not been investigated in early arthritis and can only be extrapolated from the results of several RCTs in established RA. Hydrotherapy in patients with RA has been evaluated in some studies,[73 74] but with insufficient evidence to support a strong recommendation; consequently, hydrotherapy was not included in the current statement but may be considered at the individual patient level. Previous RCTs have shown that joint-specific dynamic exercises may improve strength and physical function in RA, but the current SLR identified some controversial effects on disease activity.[16 74] Occupational therapy may improve functional ability and self-management but does not have a positive effect on disease activity; recent studies were not found.[75]

Finally, psychological counselling can be considered in selected patients, but trials investigating the efficacy of psychological interventions are lacking, and the committee did not include counselling in the statement. Furthermore, the SLR did not identify appropriate trials that evaluated the effectiveness of diets.

Since dynamic exercises, occupational therapy and to a lesser extent hydrotherapy have been associated with symptom relief in patients with established RA, the expert committee has decided to include them as adjunct therapies to pharmaceutical therapies in patients with early arthritis.

Recommendation 11:

In patients with early arthritis, smoking cessation, dental care, weight control, assessment of vaccination status and management of comorbidities should be part of overall patient care.

This recommendation is new and largely based on expert opinion. The expert committee felt that during the last decade evidence has accumulated that highlights the importance of the management of comorbidities (eg, cardiovascular diseases, metabolic conditions (eg, hyperlipidaemia, diabetes), lung diseases, infections, malignancies, osteoporosis and depression) in the context of the management of early arthritis.[76–82] Comorbidities may affect life expectancy and outcomes (physical function, quality of life) independently of disease activity in patients with inflammatory arthritis. In addition, coexisting diseases may affect the efficacy and safety of antirheumatic therapies.[82] Obesity and smoking may affect the response to treatment in inflammatory arthritis.[80] Prevention is now considered key in the management of chronic inflammatory rheumatic diseases, but comorbidities are still not optimally managed.[76] Smoking is the best-established modifiable risk factor in the development of RA and spondyloarthritis.[83 84] Furthermore, tobacco use has been associated with the presence of extra-articular manifestations such as rheumatoid nodules and also serum RF and ACPAs. While smoking does not seem to be associated with the perpetuation of disease activity or progression of RA,[85] it may affect the outcome of spondyloarthritis.[84]

RA is associated with periodontal disease, although the direction of the relationship still remains unclear.[86] The microbiome may play a role in chronic arthritis risk and progression, and *Porphyromonas gingivalis* infection could promote aberrant citrullination and a local breach of tolerance to citrullinated peptides. The potentially beneficial contribution of oral hygiene has been put on the research agenda.

Although current data do not prove that risk-factor modification is beneficial to patients, the modifiable risk factors identified in the SLR are so generic in nature that the committee was unanimously of the opinion that a recommendation aiming at abolishing their potential influence on arthritis (and general health) would not harm patients and may convey some benefits.

In addition, the expert committee noted that fewer patients with chronic arthritis than recommended are currently vaccinated,[87] and that this should be specifically mentioned.

Recommendation 12:

Patient information concerning the disease, its outcome (including comorbidities) and its treatment is important. Education programmes aimed at coping with pain, disability, maintenance of ability to work and social participation may be used as adjunct interventions.

This recommendation was very similar to the previous item no. 6. Obviously, full transparency about the disease and its treatment options should be an integral part of the management of any chronic disease, and constitutes the core of overarching principle A. Other healthcare providers share the responsibility in the provision of information. Studies have suggested that adherence to treatment is dependent on the quality of information exchange and the quality of the interaction between the patient and healthcare professionals, including rheumatologists.[16]

EULAR has recently recommended that 'people with inflammatory arthritis should have access to and be offered patient education throughout the course of their disease, including as a minimum, at diagnosis, at pharmacological treatment change and when required by the patient's physical or psychological condition'.[88] The content and delivery of patient education should be individually tailored, with individual and group sessions representing different approaches to delivery. It is impossible to prioritise a single educational intervention since all tested interventions have only short-term benefits and feature

cross-national and cultural variations.[16] Improved quality of life is a major aim for patients and the committee proposed to add 'social participation' as one of the objectives of these education programmes. The expert committee also felt that patients should be aware that comorbidities may affect the outcome and treatment of inflammatory arthritis, and that their screening and management should be part of the global management of early arthritis.

DISCUSSION

The update of the EULAR recommendations for the management of early arthritis followed the 2014 EULAR Standardised Operating Procedures.[14] The committee has proposed an important revision of the items, but obviously most major recommendations have remained intact. These updated recommendations for management of early arthritis contain 3 overarching principles, 12 recommendations and 2 algorithms that integrate all the recent developments in the management of early arthritis. The definition of the term 'management' was unchanged and includes all spectra of management of early arthritis, including referral, diagnosis, prognosis, classification, information, education, non-pharmacological interventions and pharmacological treatments and monitoring of the disease. The term 'early arthritis' was restricted to 'early inflammatory arthritis' and mainly, but not only, focused on the risk of chronic arthritis.

The expert committee had to face a limitation in that most of the published data on treatment and strategy on which they could build their recommendations involved studies in patients with early RA or established RA, rather than specific studies of early arthritis. Despite this limitation, the committee considered much of the data for early RA sufficiently robust and relevant for extrapolating to 'early arthritis with a certain propensity to become persistent.' The scope was different compared with the EULAR recommendations for the management of RA,[13] which focussed on the use of DMARDs in both early and established disease. However, there are overlaps with regard to the first-line therapy for early arthritis at risk of persistence (figure 1) and for early RA (DMARD-naïve and usually <6 months disease duration). Not surprisingly, the two sets of recommendations are very congruent on these specific points.

These recommendations have important strengths including the composition of the expert committee comprising 20 rheumatologists, including 2 research fellows, from 12 European countries and new addition of 1 healthcare professional and 2 patient representatives. The committee chose to grade the level of evidence provided by every study, which was based on the methodology of the study, and took this grading into consideration when discussing the content and the strength of the recommendations. An important consideration in the discussions was always whether the type of study fitted the content of the research question that was at the basis of the literature search. The recommendations were based on the most recent evidence and on expert opinion. For example, the expert committee felt that evidence supported comorbidities as possibly affecting the outcome of arthritis and also treatment efficacy and safety and should be considered in the management of all early arthritis cases. Despite the sparse evidence, the expert committee also wanted to indicate that smoking cessation and dental care could be proposed to patients with early arthritis, and that both patients and healthcare professionals should be aware of the importance to improve vaccination coverage. In this respect, a new recommendation on prevention was added (item no. 11). Of note, the level of agreement among the experts was high for each item (means of 9.0–9.9), which support the appropriateness and validity of the recommendations.

In light of the current literature and despite important recent advances, the committee felt that further development of new tools is needed for early and accurate diagnosis and prognosis, including new biomarkers, better understanding of the added value of US and MRI and creation of prediction algorithms for long-term outcome (box 2). Finally, the expert committee felt that the comparative effectiveness and cost-effectiveness of the different strategic modalities in early arthritis, including the effectiveness of non-pharmacological interventions, need additional research.

While these 'recommendations' are deliberately not called 'guidelines', they do reflect a strong view of many European experts including patient representatives. They should provide rheumatologists, general practitioners, medical students, healthcare professionals, health authorities and patients a practical approach to the management of early arthritis, even though each healthcare professional should choose the most appropriate management strategy for each individual patient. To that end, it is hoped that the recommendations will be widely disseminated and discussed within the community of rheumatologists and other healthcare professionals caring for patients with early arthritis and that they will help improve the standard of care for patients with arthritis across different healthcare systems. Obviously, these recommendations will probably need amendment after about 5 years to incorporate new scientific evidence.

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EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2016 update

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ABSTRACT

Recent insights in rheumatoid arthritis (RA) necessitated updating the European League Against Rheumatism (EULAR) RA management recommendations. A large international Task Force based decisions on evidence from 3 systematic literature reviews, developing 4 overarching principles and 12 recommendations (vs 3 and 14, respectively, in 2013). The recommendations address conventional synthetic (cs) disease-modifying antirheumatic drugs (DMARDs) (methotrexate (MTX), leflunomide, sulfasalazine); glucocorticoids (GC); biological (b) DMARDs (tumour necrosis factor (TNF)-inhibitors (adalimumab, certolizumab pegol, etanercept, golimumab, infliximab), abatacept, rituximab, tocilizumab, clazakizumab, sarilumab and sirukumab and biosimilar (bs) DMARDs) and targeted synthetic (ts) DMARDs (Janus kinase (Jak) inhibitors tofacitinib, baricitinib). Monotherapy, combination therapy, treatment strategies (treat-to-target) and the targets of sustained clinical remission (as defined by the American College of Rheumatology-(ACR)-EULAR Boolean or index criteria) or low disease activity are discussed. Cost aspects were taken into consideration. As first strategy, the Task Force recommends MTX (rapid escalation to 25 mg/week) plus short-term GC, aiming at >50% improvement within 3 and target attainment within 6 months. If this fails stratification is recommended. Without unfavourable prognostic markers, switching to—or adding—another csDMARDs (plus short-term GC) is suggested. In the presence of unfavourable prognostic markers (autoantibodies, high disease activity, early erosions, failure of 2 csDMARDs), any bDMARD (current practice) or Jak-inhibitor should be added to the csDMARD. If this fails, any other bDMARD or tsDMARD is recommended. If a patient is in sustained remission, bDMARDs can be tapered. For each recommendation, levels of evidence and Task Force agreement are provided, both mostly very high. These recommendations intend informing rheumatologists, patients, national rheumatology societies, hospital officials, social security agencies and regulators about EULAR's most recent consensus on the management of RA, aimed at attaining best outcomes with current therapies.

The management of rheumatoid arthritis (RA) has changed dramatically over the past 30 years. Few therapeutic agents existed then, which were either minimally or not efficacious, because of toxicity and the fact that optimal dosing and onset of action had not yet been elucidated for some agents.[1–4] Available therapies were started late rather than early in the course of the disease.[5 6] Early arthritis clinics were emerging,[7–9] and their successes fuelled reappraisal of the classification criteria then available that focused primarily on long-standing disease.[10] A therapeutic target had not yet been defined, because relief of symptoms appeared to be the most important goal and the concept of aiming at disease states like remission or low disease activity was at best aspirational.[11]

To date, we have available numerous efficacious agents. Among the conventional synthetic (cs) disease-modifying antirheumatic drugs (DMARDs),[12] we adopted methotrexate (MTX), on its optimal use, as the anchor drug[4]; in addition, a number of biological (b) DMARDs have been approved, more recently followed (in many countries) by approval of the first targeted synthetic (ts) DMARD, with more in development.[13] Today, new classification criteria for RA promote the study of patients earlier in their disease course than before[14] and recommendations have been developed to treat patients with RA via strategic algorithms targeting an optimal outcome, irrespective of the types of available therapies.[15–17]

A limited number of measures to assess response in clinical trials and follow disease activity in

clinical practice are widely used[18–21] and the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) have jointly developed new definitions for remission which provide an optimal clinical outcome and can be achieved in a significant proportion of patients in trials and practice.[22] Attaining remission according to these criteria, index-based or Boolean, will prevent joint destruction or at least progression of joint damage irrespective of residual subclinical changes,[23 24] optimise physical function, improve quality of life and work capacity[25 26] and reduce comorbidity risks.[27 28]

With this recent evolution of evidence supporting stringent disease control to improve outcomes, interest in purely symptomatic drugs has significantly decreased today and disease modification has become the pivotal attribute of all modern drugs and treatment strategies. Nevertheless, symptomatic agents as well as physical measures, psychological support and surgery may and do have a place in the overall management of RA. However, disease modification is the mainstay of RA treatment and constitutes an amalgam of characteristics: relief of signs and symptoms; normalisation—or at least important improvement—of impairment in physical function, quality of life and social and work capacity; and—as the foremost distinguishing characteristic of DMARDs compared with symptomatic agents—inhibition of structural damage to cartilage and bone. Therefore, showing inhibition of damage progression by radiography is still a pivotal outcome for the classification of a drug as a DMARD, since radiographs can depict bony and cartilage damage and have proven sensitivity to change even over short-term intervals and at very low levels of overall progression in a population.[29 30] Rapid attainment of the targeted end point is now critical, and to achieve the treatment goal of remission or at least low disease activity within the time frame of 6 months, at least 50% clinical improvement within 3 months is desirable.[31]

With rising standards of care and outcomes, RA management has become increasingly complex over the last decade. Despite the availability of many efficacious agents, treatment strategies that have been developed, and outcomes assessments that allow effective follow-up, the high costs of novel therapies have limited the widespread use of these therapeutic options, creating a significant extent of inequity. Therefore, management recommendations on the approach to treating patients with RA have become increasingly useful in providing physicians, patients, payers, regulators and other healthcare suppliers with evidence-based guidance supported by the views of experts involved in many of these novel developments. Indeed, EULAR has recently updated the standardised operating procedures on the development of recommendations, which include cost aspects in addition to accounting for the assessment of evidence and expert opinion.[32]

EULAR developed a first set of recommendations for the management of RA with DMARDs in 2010 and updated them in 2013. They were originally based on the evidence provided by five (2010) and three (2013)[33–35] systematic literature reviews (SLRs). The EULAR recommendations have been widely used. They have been referred to by national rheumatology societies and regional leagues to inform the development of their own recommendations (such as Canadian, French, German, Mexican, Asia Pacific League of Associations for Rheumatology (APLAR), Pan American League of Associations for Rheumatology (PANLAR)), as well as by regulatory authorities.[36–42]

Consistent with our approach to providing recommendations based on the latest evidence, we have continued to evaluate the literature on clinical trials of new agents, new information on established drugs, new strategic studies, new perceptions on outcomes assessments and new insights related to the research agenda[16] over the last 3 years. An abundance of new information motivated us to now further update the EULAR recommendations for the management of RA with DMARDs.

METHODS

After approval by the EULAR Executive Committee, the Convenor (JSS) and methodologist (RL) invited a Steering Committee and a Task Force to work on this update of the EULAR recommendations for the management of RA. The 2010 recommendations and their 2013 update adhered to the original EULAR standardised operating procedures for the development of recommendations[43]; the 2016 update followed the recently amended version of these standards,[32] which also suggest adherence to the Appraisal of Guidelines for Research & Evaluation (AGREE) recommendations in its updated version (AGREE II).[44]

Steering Committee

The Steering Committee included seven rheumatologists, one patient representative and three fellows. This group initially developed the research questions for the three SLRs. These SLRs focused on (i) efficacy of synthetic (s) DMARDs (as monotherapy or combination therapy, including both csDMARDs and ts DMARDs) and glucocorticoids (GC); (ii) efficacy of bDMARDs (as monotherapy or combined with csDMARDs) and (iii) safety aspects of sDMARDs and biological (b) DMARDs. To this end, the original SLRs obtained in 2013[33–35] served as a starting point and an update on the literature published between 2013 and 2016 was performed. New information on treatment strategies was also evaluated in the present SLRs. Formal economic analyses were not performed, but cost aspects were considered throughout the process in line with the current state of the art of developing recommendations,[45 46] EULAR's own previous SLR on cost aspects in the context of DMARD therapy[47] and the advent of biosimilars.[48] The three rheumatology fellows (KC, JN, SR) performed the SLRs (and checked each other's work) exploiting existing publication databases on randomised controlled trials for efficacy and registry data for safety, and also evaluating recent EULAR and ACR congress abstracts. Summary-of-findings (SoF) tables were generated and levels of evidence (LoE) were determined using the standards of the Oxford Centre for Evidence-Based Medicine.[49] The three SLRs informing the Task Force and a detailed description of their methods are published separately.[50–52]

The SoFs of the SLRs were presented to the Steering Committee that formulated a proposal for an update of the recommendations based on this information. The SLR data and the proposals of the Steering Committee were subsequently presented to the whole Task Force for further discussions and ultimately development of the updated recommendations.

Task Force

The Task Force consisted of 50 individuals, including the Steering Committee members. Among the Task Force members were three patients, two health professionals and two delegates of the EULAR young rheumatologists' network Emerging Eular NETwork (EMEUNET). The rheumatologists were all experienced in the treatment of RA and most had frequently participated in clinical trials; moreover, several of them had experience in patient registries of their countries or in various aspects of outcomes research. The patients and health professionals all had experience in consensus finding activities, as well as most of the rheumatologists. Since we also wished the Task Force's work to be informed by rheumatologists from other

regions of the world, aside from a broad representation from 14 European countries, 2 colleagues from Asia, 1 from Australia, 2 from Latin America and 2 from North America were invited to participate. Several of them had actively participated in developing documents of their regional leagues and/or national societies. All Task Force members declared their potential conflicts of interest before the start of the process.

The Task Force agreed on a few principal considerations upfront. First, all recommendations needed to be discussed in the context of new evidence; where no new evidence was available, the former evidence base was followed. Second, any of the previous recommendations (4 overarching principles and 14 recommendations) could be maintained as they had been presented in the 2013 version, amended, shifted in sequence or deleted. Third, drugs that were not (yet) approved in Europe but used elsewhere in the world, or drugs that had not yet undergone regulatory assessment but for which evidence from clinical trials was available, could be considered in recommendations to allow for some anticipation of a potential uptake in clinical practice, with all respective caveats. Finally, there was agreement that all recommendations of 2013, which were either further supported by new evidence or lacked novel information, should be incorporated as previously worded, unless certain components were now considered inappropriate.

After the presentation of the SLR results and the Steering Committee's proposals for the amendment of the recommendations, the Task Force was split into four breakout groups. One group reviewed bDMARDs, the second group csDMARDs, the third tsDMARDs and the fourth GC; all groups proposed draft language for respective recommendations to the whole Task Force. Safety aspects were addressed in each of these breakout groups.

Consensus finding

Representatives of each breakout group reported the results of the respective deliberations and presented proposals for the wording of individual recommendations to the whole Task Force. Thereafter, the voting process took place.

For an overarching principle or recommendation to be accepted for the final document without further change, a majority of 75% of the votes was required in the first ballot. If this result was not achieved, the respective text was amended and subjected to a second ballot, for which a 67% majority was required. If this ballot was not successful, further textual changes were proposed until a ≥50% majority was attained. The recommendations are presented as finally voted on. The results of the respective last ballot are presented as percentage of voting members. Notes captured the contents of the discussions and the reasoning behind each decision to be presented in the comments accompanying the individual items. For various reasons, not every Task Force member was present in the room throughout the whole meeting and, therefore, there were slight variations in the numbers of votes. However, at every point in time >90% of the members participated in the ballots.

After the face-to-face meeting, the recommendations, as agreed by the Task Force, were subjected to an anonymous vote (by email) on the levels of agreement (LoA). Each recommendation received an adjudication on a scale of 0–10, 0 meaning no agreement whatsoever and 10 absolute agreement. During this process, several weeks after the meeting, one individual withdrew from the Task Force, because the inclusion of csDMARD combination therapy in the recommendations had not found a majority during the preceding voting process. This colleague had been present and voted throughout the face-to-face meeting and the respective votes regarding all recommendations are accounted for accordingly, but ultimately the person declined authorship and no vote was cast on the LoA.

The draft of the manuscript was sent to all Task Force members for their comments. After incorporation of these comments, it was submitted to the EULAR Executive Committee for review and approval; at this time, it was again sent to the Task Force members. Final remarks were obtained from members of the Task Force and the Executive Committee and addressed in the manuscript, which was then submitted with approval by the EULAR Executive Committee.

RESULTS

General aspects

As before, the 2016 update of the EULAR RA management recommendations reflects the balance of clinical, functional and structural efficacy, safety, costs and patients' perceptions as perceived by the Task Force. Aspect of drug toxicity were considered in the overall wording of the recommendations, but data are presented only in the Safety SLR[50] because it is assumed that prescribers are aware of the safety information provided in the manufacturers' package inserts of the various agents. Also, EULAR has developed a series of documents dealing with safety aspects of RA drugs,[53–58] and various other publications have addressed these aspects.[59–62] In particular, as also suggested by the safety SLR,[50] the major risk of bDMARDs (and also tsDMARDs) is related to infections, and recommendations for vaccination[56] as well as a score allowing to calculate the risk of infection in patients exposed to bDMARDs have been recently developed.[63 64] For all medications discussed in this paper, the summary of product characteristics document provides valuable information on risks, side effects and need for monitoring. The recommendations given here should in no way be construed so as to detract from that information. In any case, when toxicity constitutes a major issue, a specific warning is provided within the respective recommendation or the accompanying comments. Of note, the three SLRs as well as the text accompanying each item should be regarded as part and parcel of the recommendation. The individual bullet points represent abbreviated conclusions from the discussions and, as such, do not capture all aspects related to a particular theme; rather, such aspects are elucidated in more detail in the respective explanatory part of the Results section.

When classifying DMARDs, the Task Force adhered to the previously used nomenclature[12 16] as shown in table 1. Table 1 also provides a glossary for terms employed in the recommendations. The Task Force did not distinguish between early and established RA regarding the recommendation of the types of drugs, but rather discerned phases of the treatment process by differentiating between patients who are naïve to any DMARD therapy, patients who had an insufficient response (IR) to initial course(s) of csDMARDs and those who had an IR to bDMARDs. There is currently no evidence for differential responses solely based on disease duration, when leaving differences in baseline damage due to delayed treatment initiation aside. Indeed, trials on MTX-naïve patients with RA used different disease durations for inclusion, which ranged from a few months to several years, without appreciable differences in outcomes on indirect comparison.[65–68] However, the Task Force distinguished between early and established RA in terms of the targeted outcome (see recommendation 2). The Task Force also took prognostic factors (table 1) into account, which have similar predictive power irrespective of disease duration.[69] Of note, recommendations for the management of early arthritis, including undifferentiated arthritis, have been recently

Table 1 Glossary and definitions

Term	Definition	
Poor prognostic factors	▶ Moderate (after csDMARD therapy) to high disease activity according to composite measures[71] ▶ High acute phase reactant levels[72 73] ▶ High swollen joint counts[72–74] ▶ Presence of RF and/or ACPA, especially at high levels[72 75] ▶ Combinations of the above[69 76] ▶ Presence of early erosions[72] ▶ Failure of two or more csDMARDs[77]	
Low-dose glucocorticoid	▶ ≤7.5 mg/day (prednisone equivalent)[57 78]	
Meanings of treatment reduction		
Tapering	▶ Usually reduction of drug dose or increase of application interval ('spacing') ▶ May include discontinuation (tapering to 0), but then only after slow reduction	
Cessation, discontinuation	Stopping of a particular drug	
Disease activity states		
Remission	ACR-EULAR Boolean or index-based remission definition[22]	
Low disease activity	Low disease activity state according to any of the validated composite disease activity measures that include joint counts[79–81]	
Moderate, high disease activity	Respective disease activity state according to any of the validated composite disease activity measures that include joint counts[79–81]	
DMARD nomenclature[12]		
Synthetic DMARDs	▶ Conventional synthetic DMARDs (csDMARDs)	For example, methotrexate, leflunomide, sulfasalazine, hydroxychloroquine
	▶ Targeted synthetic DMARDs (tsDMARDs)	For example, tofacitinib, baricitinib
Biological DMARDs	▶ Biological originator DMARDs (boDMARDs)	
	▶ Biosimilar DMARDs (bsDMARDs)	

ACPA, anticitrullinated protein antibody; ACR, American College of Rheumatology; DMARDs, disease-modifying antirheumatic drugs; EULAR, European League Against Rheumatism; RF, rheumatoid factor.

updated.[70] The present recommendations address the management of patients with RA from the time of its diagnosis and not pre-RA or undifferentiated arthritis.

Overarching principles

As in previous versions, the Task Force endorsed the presentation of general principles for the treatment of patients with RA as overarching (table 2). Their nature is so generic that there was no requirement to base them on specific searches or LoE, but at the same time the group believed it is crucial to communicate them as a foundation on which the actual recommendations were based. However, while all three former overarching principles were maintained as formulated in 2010, the Task Force added a fourth one as overarching principle B.

A. *Treatment of patients with RA should aim at the best care and must be based on a shared decision between the patient and the rheumatologist*. This principle remained unchanged both in its textual details and in its place as item A, a prominent position within the recommendations. Shared decision-making between patient and rheumatologist involves all aspects of the disease: information on the disease and its risks, the modalities of disease assessment, decisions on the therapeutic target and the potential means to reach the target, the development of a management plan and discussions on the benefits and risks of individual therapies. These aspects have also been detailed in recommendations on standards of care.[82] Naturally, 'best care' refers to the recommendations presented here and inherently 'shared decision' relates to all individual recommendations. To this end also quality indicators have been developed more recently.[83]

B. *Treatment decisions are based on disease activity and other patient factors, such as progression of structural damage, comorbidities and safety issues*. This is a new principle. It derives from previous recommendation 14, the last item of the 2013 version, which was deemed by the current Task Force to represent such a central and self-evident rule to any therapeutic approach that it should constitute an overarching principle rather than a recommendation. Indeed, in line with these considerations, the level of evidence of this recommendation had been rather low in 2013. Withdrawing this item from the recommendations elicited some discussions. Especially the patients brought forward that ending the list of recommendations with an item on patient-related factors would convey prominence to patient preferences and patient aspects in the management of RA. However, the reasoning that this item would even benefit more from being a general principle than a recommendation, which was unlikely to ever be studied in all its subtleties, prevailed to an extent that principle B was unanimously accepted (table 2).

C. *Rheumatologists are the specialists who should primarily care for patients with RA*. Originally presented as item B, the wording of this principle was not changed. Of interest, in 2010 this was even presented as overarching principle A. However, over the last years, it was recognised that shared decision-making and considerations of patient factors should receive the most prominent recognition. Whether positioned as A, B or C, this item addresses the importance of specialty care for a complex disease like RA. There is compelling evidence that being cared for by a rheumatologist is advantageous for the patients in terms of early initiation of therapy, prevention of damage and reduction in surgical procedures.[84–88] Moreover, rheumatologists have the most profound experience regarding the use of csDMARDs and bDMARDs. This includes the adverse event profiles of these drugs, as well as awareness of and experience with comorbidities in RA. Therefore, rheumatologists

Table 2 The 2016 EULAR updated recommendations

	Overarching principles
A	Treatment of patients with RA should aim at the best care and must be based on a shared decision between the patient and the rheumatologist
B	Treatment decisions are based on disease activity and other patient factors, such as progression of structural damage, comorbidities and safety issues
C	Rheumatologists are the specialists who should primarily care for patients with RA
D	RA incurs high individual, medical and societal costs, all of which should be considered in its management by the treating rheumatologist
	Recommendations
1.	Therapy with DMARDs should be started as soon as the diagnosis of RA is made
2.	Treatment should be aimed at reaching a target of sustained remission or low disease activity in every patient
3.	Monitoring should be frequent in active disease (every 1–3 months); if there is no improvement by at most 3 months after the start of treatment or the target has not been reached by 6 months, therapy should be adjusted
4.	MTX should be part of the first treatment strategy
5.	In patients with a contraindication to MTX (or early intolerance), leflunomide or sulfasalazine should be considered as part of the (first) treatment strategy
6.	Short-term glucocorticoids should be considered when initiating or changing csDMARDs, in different dose regimens and routes of administration, but should be tapered as rapidly as clinically feasible
7.	If the treatment target is not achieved with the first csDMARD strategy, in the absence of poor prognostic factors, other csDMARDs should be considered
8.	If the treatment target is not achieved with the first csDMARD strategy, when poor prognostic factors are present, addition of a bDMARD*[1,2] or a tsDMARD*[3] should be considered; current practice would be to start a bDMARD[§]
9.	bDMARDs*[1,2] and tsDMARDs[#3] should be combined with a csDMARD; in patients who cannot use csDMARDs as comedication, IL-6 pathway inhibitors and tsDMARDs may have some advantages compared with other bDMARDs
10.	If a bDMARD* or tsDMARD[§] has failed, treatment with another bDMARD or a tsDMARD should be considered; if one TNF-inhibitor therapy has failed, patients may receive another TNF-inhibitor or an agent with another mode of action
11.	If a patient is in persistent remission after having tapered glucocorticoids, one can consider tapering bDMARDs, especially if this treatment is combined with a csDMARD
12.	If a patient is in persistent remission, tapering the csDMARD could be considered

The symbols (*, §, #) indicate different levels of evidence which are correspondingly provided together with voting results and levels of agreement in table 3.
[1]TNF-inhibitors: adalimumab, certolizumab pegol, etanercept, golimumb, infliximab boDMARDs or the respective EMA-approved/FDA-approved biosimilars.
[2]Abatacept, rituximab (as first bDMARD under special circumstances—see text), or tocilizumab or respective EMA-approved/FDA-approved biosimilars, as well as other IL-6 pathway inhibitors, sarilumab and/or sirukumab, once approved.
[3]Jak-inhibitors (where approved).
boDMARDs, biological originator DMARDs; bsDMARD, biosimilar DMARDs; csDMARDs, conventional synthetic DMARDs; DMARDs, disease-modifying antirheumatic drugs; EULAR, European League Against Rheumatism; Jak, Janus kinase; MTX, methotrexate; RA, rheumatoid arthritis; TNF, tumour necrosis factor; tsDMARDs, targeted synthetic DMARDs.

can provide the 'best care' in accordance with item A, in the sense of a holistic approach. The reasoning behind the term 'primarily' has been discussed amply in previous versions of the recommendations and relates to considerations of multidisciplinary care, including specialty nurses, and to the fact that in certain areas of the world rheumatology training is not sufficiently provided and other experts may have experience in the management of RA. Moreover, some comorbidities, such as chronic hepatitis or interstitial lung disease, may require consultation of, and treatment by, other specialists.

Table 3 Evidence levels, voting results and agreement

	LoE	SoR	Final vote (%)	Level of agreement (0–10)
A	n.a.	n.a.	100	9.9
B	n.a.	n.a.	100	9.9
C	n.a.	n.a.	100	9.8
D	n.a.	n.a.	98	9.7
1.	1a	A	96	9.9
2.	1a	A	91	9.6
3.	2b		100	9.5
4.	1a	A	71	9.8
5.	1a	A	85	9.0
6.	1a	A	98	8.7
7.	5	D	94	8.5
8.	*1b §5	*A §D	96	9.0
9.	*1a #1b	*A #A	96	9.2
10.	*1a §5	A* §D	71	9.1
11.	2b	B	86	9.0
12.	4	C	86	8.5

The symbols (*, §, #) relate to the corresponding symbols in the recommendations (table 2), showing the respective LoE.
LoE, levels of evidence; n.a., not available; SoR, strength of recommendation.

D. *RA incurs high individual, medical and societal costs, all of which should be considered in its management by the treating rheumatologist*. Again, this principle is worded exactly as last time, except that it was item C, but also last.[16] It is meant to remind all stakeholders that effective RA therapy—in spite of its direct costs—will reduce the economic burden on the individual patients, their families and society, which includes direct medical costs and indirect costs such as work disability and premature retirement. In this context, it must be borne in mind that direct medical costs accrue beyond those attributed to directly treating the overt manifestations of RA and include costs ensuing from comorbidities related to the inflammatory process. This point, however, is also meant to echo that cost-effective treatment approaches must be preferred as long as safety and outcomes are similar compared with more costly ones and in line with the therapeutic paradigms.[46] In some countries, the high cost of treatment is an important factor limiting the availability of modern therapies (inequity), and this factor has to be considered when choosing a treatment strategy.[89] In this respect, the advent of biosimilars provides potential for reduction of pressure on healthcare budgets.[48] At this point, it also must be considered that many patients still do not attain the therapeutic targets, despite all of our modern therapies and therapeutic strategies. Furthermore, any of the bDMARDs, if applied after at least one csDMARD and a bDMARD has failed, leads to only about 10% good treatment responses in terms of ACR70 rates.[90] These aspects impose the need to continue the search for new therapies or strategies.

Recommendations

General aspects

The Task Force's deliberative process resulted in 12 recommendations. The reduction by two recommendations compared with the past EULAR document may be somewhat surprising given

the allegedly increasing intricacy of therapeutic modalities and strategies. However, the content of recommendation 14 was shifted into the overarching principles as discussed above. Moreover, item 11 of the 2013 version, which addressed the use of tofacitinib, was deleted as a separate item, because Janus kinase (Jak) inhibitors as tsDMARDs have now entered into and expanded other recommendations; this will be discussed in more detail in the context of items 8, 9 and 10. Also former recommendation 6, which addressed the use of csDMARD combinations, was deleted by the Task Force; combination therapy with csDMARDs and the reasons to remove it from its previous prominence within the list of recommendations and the algorithm will be addressed in the discussion on recommendations 4 and 5. While three of the 2013 recommendations were deleted via either complete omission or incorporation into other items, former recommendation 8 which addressed the absence or presence of prognostic risk factors was split into new recommendations 7 and 8; a detailed rationale for this decision is discussed below.

The 12 recommendations form a logical sequence. They start with the need to initiate effective therapy immediately after diagnosis and the requirement to set a treatment target and to assess the disease on the way towards that target, employing a treat-to-target strategy. Such strategy has been strongly embedded into the recommendations since their first version in 2010. With these prerequisites in mind, different drugs or combinations of agents are recommended in the course of the therapeutic procedures, with suggested sequential increments, taking prognostic factors and all approved agents into account. They also mention some agents of potential future interest, even though not yet approved by international regulatory authorities. Thus, the recommendations also include a prospective view on drugs that have undergone phase III trials and were available for evidence assessment; obviously their actual prescription will depend on the regulatory approval status in individual countries. The set of recommendations concludes with suggestions towards reduction of therapy and even withdrawal of some drugs when the desired target has been attained and is sustained.

Individual recommendations

1. *Therapy with DMARDs should be started as soon as the diagnosis of RA is made*. This recommendation remained unchanged compared with 2013 and is one of the mainstays of any treatment approach to RA. It implies (i) the necessity to establish a diagnosis as early as possible, as has been reflected also in the 2010 ACR-EULAR classification criteria[14 91 92] and (ii) the advantage of early initiation of DMARD treatment ('as soon as possible'), which enables prevention of damage in a large proportion of patients.[87 93–95] Because of the generic nature of this bullet point, the Task Force did not specify the type of DMARD here. Indeed, all DMARDs enable a better long-term outcome on early, compared with delayed institution, and the sequence of the types of DMARD therapies is addressed in subsequent recommendations. The Task Force did not deal with pre-RA or undifferentiated arthritis and thus assumed that a diagnosis of RA had already been made. However, it should be borne in mind that any chronic arthritis, even if undifferentiated, requires appropriate treatment, including consideration of DMARD therapy, because it usually does not subside spontaneously,[96 97] and an update of the recommendations for management of early arthritis has just been presented by EULAR.[70] With a LoA of 9.9, this recommendation achieved the highest agreement of all items (table 2). LoE 1a; LoA 9.9.

2. *Treatment should be aimed at reaching a target of sustained remission or low disease activity in every patient*. This recommendation addresses two treatment targets: remission, especially in DMARD-naïve patients, and low disease activity, primarily in patients who failed previous therapies. Since clinical remission or low disease activity are mentioned as the sole therapeutic targets, any higher disease activity state has to be regarded as inadequate disease control, thus mandating a therapeutic change, obviously unless patient factors preclude this.[15] Communication with the patient to clarify and agree on the treatment goal and the means to attain this goal is of utmost importance. It allows alignment of the patient's and provider's considerations and aims and enhances adherence. In 2010, the notion 'as soon as possible' was also part of this item[98] and in the current discussion it was specifically decided to mention that the treatment target should be rapidly attained rather than aiming to achieve it in a more distant future. Indeed, there is sufficient evidence that most patients who do not attain significant improvement within 3 months, or do not achieve the treatment target within 6 months, will not reach the desired state subsequently[31 99–101]; exceptions pertain to those patients whose disease activity has been reduced to a level close to the treatment target.

Regarding remission, EULAR and ACR have agreed on Boolean and index-based definitions, the latter based on the Simplified or Clinical Disease Activity Index (SDAI, CDAI).[22] Both correlate highly with the absence of subclinical synovitis by MRI and sonography[102 103] and absence of progression of joint damage.[23] They can even be reliably used when drugs that interfere directly with the acute phase response are employed.[104–107] Moreover, recent strategic clinical trials that compared targeting sonographic remission with targeting clinical remission or low disease activity resulted in the conclusions that aiming at imaging remission had no advantages over the clinical target, but had economic disadvantages.[108 109] Low disease activity also needs to be properly defined and measured. Measures that highly weigh C reactive protein or erythrocyte sedimentation rate (eg, the disease activity score (DAS)28) may not convey sufficiently reliable results when used with agents that interfere with the acute phase response, such as anticytokine agents (especially interleukin (IL)-6 inhibitors) or Jak-inhibitors.[104 107 110]

It is important that the target-state should be sustained. The term 'sustained' is still not defined precisely, and different studies have used different definitions, but some voices in the Task Force suggested at least 6 months as a minimal time frame. This requires follow-up and a strategy to adapt therapy intensity upward or downward, aspects that are dealt with in subsequent recommendations. However, treatment intensification must take patient factors into consideration, especially risks and comorbidities (overarching principle B). LoE 1a; LoA 9.6.

3. *Monitoring should be frequent in active disease (every 1–3 months); if there is no improvement by at most 3 months after the start of treatment or the target has not been reached by 6 months, therapy should be adjusted*. This recommendation on treat-to-target is unchanged in position and formulation from the 2013 version. The frequencies of follow-up examinations should be adjusted in accordance with the level of disease activity, namely more frequently, such as monthly, when patients have high disease activity, and less frequently, such as every 6–12 months when the treatment target has been attained and sustained. EULAR generally recommends the use of a composite measure of disease activity that includes joint counts and the ACR-EULAR definitions for remission.[22 111] Improvement by

3 months refers to the fact that if a minimal change is not achieved, there is only a low likelihood of reaching the treatment target. Thus, a change to a better disease activity state should be seen at 3 months or a relative improvement, pertaining to at least 50% improvement in activity by a composite score, at that point in time, in order to have a considerable chance of reaching the target.[31 100 112 113] Of note, adjustment of therapy includes the optimisation of MTX (or other csDMARD) dose or route of administration,[4] or intra-articular injections of GC in the presence of one or few residual active joints, and refers to a change of drugs only if these measures have not been successful or are not appropriate. Furthermore, in an individual patient the treatment target may not have been fully achieved yet at 6 months. But if disease activity is close to the target, one may think about continuing the effective therapy for a few more weeks to make a final judgement, especially since a considerable proportion of patients may attain the target at a slightly later time point than at 6 months.[114 115] Consequently, the change in disease activity from baseline, and its slope should be considered when making treatment decisions. LoE 2b; LoA 9.5.

4. *MTX should be part of the first treatment strategy.* Compared with 2013, when this item read 'MTX should be part of the first treatment strategy in patients with active RA', the recommendation was slightly shortened. The Task Force felt that pointing to active disease was not necessary, since the EULAR recommendations primarily address patients with active disease. Based on its efficacy, safety (especially in the presence of folic acid), the possibility to individualise dose and method of administration as well as relatively low costs, MTX continues to be the anchor ('first') drug for patients with RA both as monotherapy as well as in combination with other drugs ('treatment strategy'; see below). Moreover, MTX appears to reduce comorbidities and mortality in RA.[116 117] In clinical trials of bDMARDs in early arthritis patients, MTX monotherapy has been associated with 25% ACR70 response rates (which brings patients into the range of low disease activity) within 6 months, even though it had not been combined with de novo GC in these trials.[90] MTX should be rapidly escalated, usually to 25–30 mg/week, orally or subcutaneously administered, with folic acid supplementation,[4] and the maximal MTX dose, if tolerated, should be sustained for about 8–12 weeks to judge the MTX treatment response. Indeed, when MTX is rapidly escalated to 25 mg/week, the response rate may even be higher (~40% low disease activity).[118] Of course, contraindications and the potential of early toxicity have to be taken into account; this is addressed in item 5. The doses mentioned here do not pertain to Asian patients. In China, it is not recommended to exceed 20 mg/week[115] and in Japan the maximum recommended dose for MTX is 16 mg/week.[119]

Of note, at this point in time the Task Force decided to delete previous recommendation 6 ('in DMARD-naïve patients, irrespective of the addition of GC, csDMARD monotherapy or combination therapy of csDMARDs should be used'). The inclusion or exclusion of combinations of csDMARDs within the bullet points elicited long debates within the respective breakout group and the whole Task Force (and the withdrawal of one Task Force member).

The first ballot of the Task Force involved a choice of the following two wordings: (a) 'MTX should be part of the first treatment strategy' and (b) 'in DMARD-naïve patients, irrespective of the addition of GC, csDMARD monotherapy or combination therapy of csDMARDs should be used' (identical with the respective 2013 recommendation), with 23 votes favouring (a), 22 votes favouring (b) and one abstention. Therefore, further discussions took place. Advocates in favour of including combination therapy referred to publications suggesting its superior efficacy compared with csDMARD monotherapy and similar efficacy compared with biological agents[120–124]; moreover, in some countries, csDMARD combination therapy is recommended by the national societies as preferred initial therapy.

Other Task Force members pointed to trials that did not show a real benefit of combination therapy (especially when csDMARD monotherapy was combined with GC in the comparator arms)[125–127]; differences in GC cointervention between combination and monotherapy arms in previous trials[128]; issues concerning the design of some investigator initiated trials suggesting superiority of csDMARD combinations[129]; the significantly higher rate of profound responses on combination with bDMARDs compared with the combination with csDMARD therapy after IR to MTX[123] and the higher level of toxicity of csDMARD combinations versus monotherapy.[126 130]

It was also argued that a higher prevalence of adverse events when using combination therapy, even though often mild, may preclude escalation of therapy and result in not reaching a full dose of some of the drugs. Also, the SLR on csDMARDs did not show evidence for superiority of csDMARD combinations compared with csDMARD monotherapy.[52] Moreover, the ACR Committee on the 2015 update of the ACR management guideline, in contrast to previous versions,[131] did not longer recommend csDMARD combination as initial therapy, but prioritised MTX monotherapy.[17] In line, the updated EULAR recommendations for the management of early arthritis do not advocate the use of csDMARD combination therapy.[70] It was also pointed out that choice (a) included the term 'treatment strategy' and thus comprised the option to use csDMARD combinations. These discussions resulted in a new ballot between two versions for recommendation 4: (a) 'MTX should be part of the first treatment strategy' (as above) and (b) 'MTX should be the first csDMARD, either as monotherapy or in combination with other csDMARDs'. In this second ballot a 71% majority voted for version (a). Thus, csDMARD combination therapy is no longer presented explicitly as initial treatment suggestion within the abbreviated list of recommendations. However, it should be mentioned that the simple fact that csDMARD combination therapy is not included in the bullet point anymore does not preclude using it. This is obviously at the discretion of the physician and the patient in light of all pros and cons that had been discussed ('shared decision').

This recommendation ultimately attained a very high LoA (9.8). The Task Force was well aware that in some countries, such as in the UK or Canada, rheumatologists are required to use at least two csDMARDs before the application of bDMARDs is approved by the payers and that combinations of two or three csDMARDs are accepted in lieu of two csDMARD courses. However, for the reasons just mentioned, the Task Force was not in favour of the practice to define an IR to a combination of csDMARDs as a failure of two or more csDMARDs (when in reality it constitutes only one therapeutic strategy) nor to preclude the approval of bDMARD use when a first csDMARD has failed and the patient has bad prognostic markers (see below item 8 and table 1). LoE 1a; LoA 9.8.

5. *In patients with a contraindication to MTX (or early intolerance), leflunomide or sulfasalazine should be considered as part of the (first) treatment strategy.* The contents of this recommendation were maintained; however, compared with the previous version of item 5, the wording 'in cases of MTX contraindications' was slightly amended, because it is patients

who have contraindications, rather than 'cases'. The Task Force reiterated the relative safety of MTX and it was also discussed that the frequent fears of patients after reading the package insert should be addressed by providing appropriate information (overarching principle A). Nevertheless, there are occasional contraindications (eg, kidney or liver disease) or intolerances. Under these circumstances, leflunomide (dosed at 20 mg/day without loading dose)[132] or sulfasalazine (escalated to 3 g/day) are regarded the best alternatives. Older trials have suggested similar efficacy for both these drugs compared with MTX, although MTX was used at much lower doses than recommended today.[133 134] However, no new trials have been performed to disprove the previous conclusions. Among all the above agents, only sulfasalazine has an acceptable safety profile during pregnancy.[135] In some countries, parenteral gold is still used and, while clinical efficacy is undisputed, there are controversies regarding its safety[136 137]; in other countries, gold salts are not available any more. In contrast, the use of antimalarials, such as hydroxychloroquine and chloroquine, is still substantial, especially in combination therapy[122] or as monotherapy in patients with very mild disease,[138] particularly in China. Interestingly, antimalarials may have significant positive effects on lipid and glucose metabolism[139] and may reduce cardiovascular risk in RA.[140] However, joint damage is not retarded to a similar extent as with other csDMARDs.[141] This recommendation also uses the term 'treatment strategy' implying, as with MTX, that leflunomide and sulfasalazine can be used as monotherapy or in combination with other csDMARDs or biological agents.[142–145] Indeed, step-up combination therapy is frequently employed, even though comparing step-up combination with switching of csDMARD did not reveal significant differences in outcomes.[146] LoE 1a; LoA 9.0.

6. *Short-term GC should be considered when initiating or changing csDMARDs, in different dose regimens and routes of administration, but should be tapered as rapidly as clinically feasible*. The added efficacy of GC when combined with csDMARDs is well established. Indeed, hitherto all trials comparing GC plus csDMARD with bDMARDs plus csDMARD revealed similar efficacy.[146 147] In 2013, GC were dealt with in recommendation 7, but the wording was different: 'low-dose GC should be considered as part of the initial treatment strategy (in combination with one or more csDMARDs) for up to 6 months, but should be tapered as rapidly as clinically feasible'. The current wording constitutes a compromise attempting to accommodate most of the concerns and suggestions raised during the Task Force's debate.

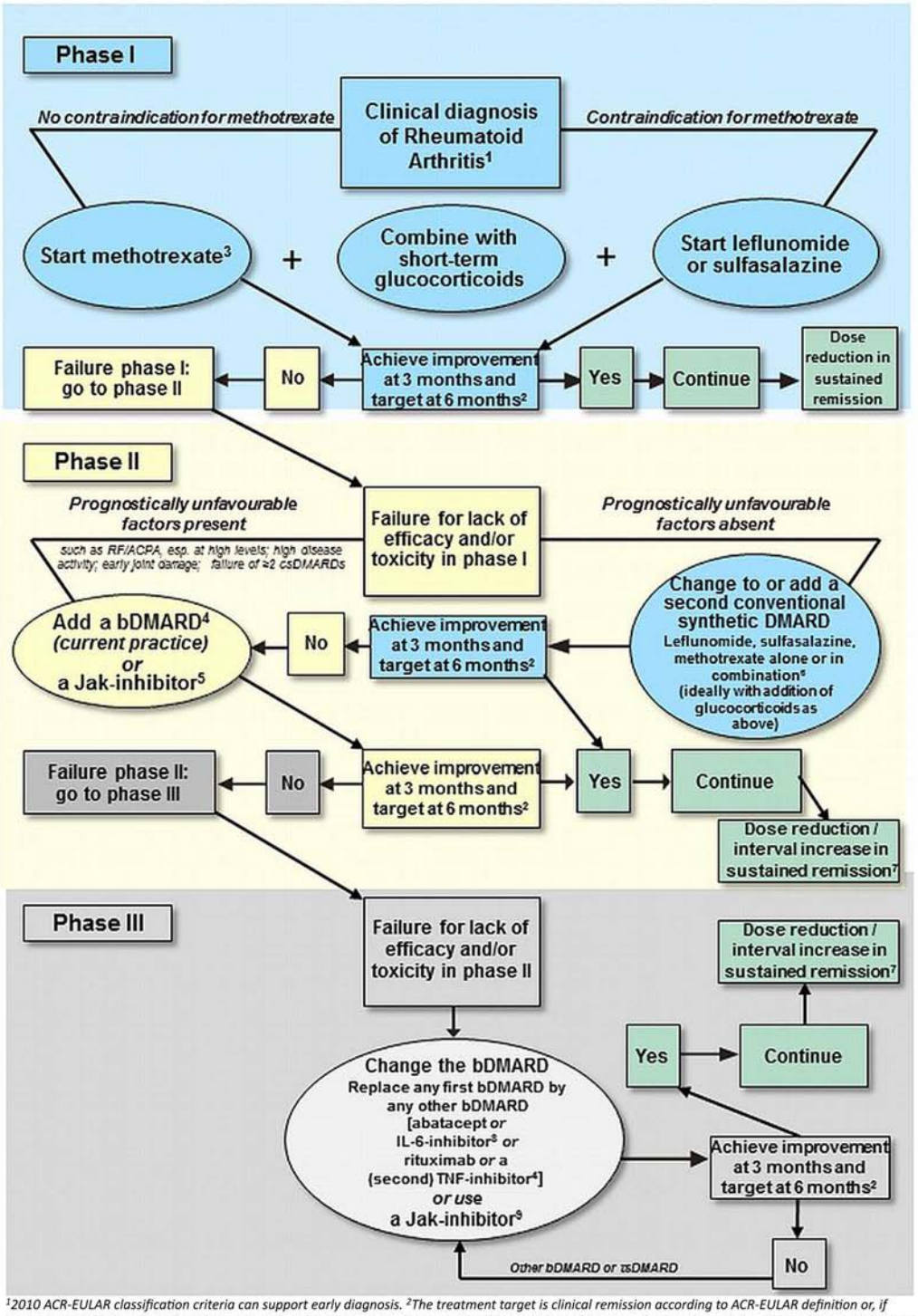
The term 'low-dose' was critically discussed. While all members of the Task Force agreed that high doses of GC should not be used for prolonged periods, it also became clear that the label 'low-dose' (which means a daily dose of 7.5 mg or less prednisone per day),[78 148] while preferred by some Task Force members, does not capture several current ways of GC application. Indeed, recent clinical trials have revealed the efficacy of short-term GC, but at doses >7.5 mg/day, namely orally at 30 mg starting dose,[126] as a single intramuscular injection of 120 mg methylprednisolone[125] or as a single 250 mg intravenous pulse therapy of methylprednisolone.[147] Therefore, the term 'low-dose' was deleted and replaced by 'short-term', leaving the choice about 'dose regimens and routes of administration' (another new piece of wording in this item) to the individual rheumatologist and patient. Indeed, it was argued that a single intramuscular or intravenous application entails a much lower cumulative dose than a few weeks of oral low-dose therapy, but this view was not shared by all Task Force members.

Yet another change involved the replacement of the phrase 'part of the initial treatment strategy' by 'when initiating or changing csDMARDs'. This change clarifies the intention of the Task Force, in that GC should be considered with all csDMARD starts, either as part of a first csDMARD therapy at the time of diagnosis or subsequently if an initial strategy has failed. Finally, the fact that csDMARDs are mentioned specifically implies that GC are typically not needed as a bridging therapy when bDMARDs or tsDMARDs are used, as these usually have a rapid onset of action and the infection risks may be potentiated.[149 150] Thus, it is important to reiterate that the Task Force recommends using GC in combination with csDMARDs primarily as bridging therapy until the csDMARD reaches its maximum effect, and this should be done using one of the dosing and tapering approaches mentioned above, for which respective evidence exists. To reflect the position of the Task Force, the algorithm depicted in figure 1 was modified to show a '+' for the use of GC in the new version rather than a '±' as previously.

By stating '…tapered as rapidly as clinically feasible', the Task Force underlines that GC should be gradually reduced and ultimately stopped, usually within 3 months from treatment start and only exceptionally by 6 months. Long-term use of GC, especially at doses above 5 mg/day, should be avoided because of the many potential risks presented in the SLR.[50 52 57] While some of these risk associations may be due to confounding by indication in patients with high disease activity,[151] the evidence for increased overall and cardiovascular mortality at a dose above a threshold of 7.5 mg/day or a cumulative dose of 40 g is considerable.[152] Of note, applying GC as a sole therapeutic change in patients with IR to csDMARD therapy does not convey good efficacy and is associated with significant adverse events.[153] Moreover, if GC cannot be withdrawn within the time frame mentioned above, the DMARD therapy may have to be considered a failure. Finally, intra-articular GC application may have to be considered in certain instances, such as a residually inflamed or a reactivated joint.

Some Task Force members advocated the chronic use of GC as a possibility for some patients; however, this proposal was not endorsed by the majority. While the bullet point on GC was, as in previous years, most heavily debated, the final wording received a 98% majority vote. However, the LoA was much lower (8.7), in line with previous versions of the recommendations. This relatively low LoA is presumably due to the fact that many Task Force members felt that this point was too liberal and the use of GC should be more restricted, while others were of the opinion that it was too restrictive. LoE 1a; LoA 8.7.

7. *If the treatment target is not achieved with the first csDMARD strategy, in the absence of poor prognostic factors, other csDMARDs should be considered*. This sentence constitutes the first part of previous recommendation 8. It is essentially worded in an identical way, except that the last portion, 'change to another csDMARD strategy should be considered', was reworded as 'other csDMARDs should be considered', in light of the fact that combination with GC has now been recommended clearly also for this step of the treatment algorithm (item 6) and combinations of csDMARDs are not specifically recommended as initial treatment strategy anymore. The poor prognostic factors are presented in table 1. The Task Force also discussed that early intolerance for a csDMARD should not be considered as a treatment failure, which would imply moving immediately to the next phase of the algorithm, but rather require reinstitution of another first csDMARD (replacement). LoE 5; LoA 8.5.



[1]2010 ACR-EULAR classification criteria can support early diagnosis. [2]The treatment target is clinical remission according to ACR-EULAR definition or, if remission is unlikely to be achievable, at least low disease activity; the target should be reached after 6 months, but therapy should be adapted or changed if no sufficient improvement is seen after 3 months. [3]"Methotrexate should be part of the first treatment strategy"; while combination therapy of csDMARDs is not preferred by the Task Force, starting with methotrexate does not exclude its use in combination with other csDMARDs. [4]TNF-inhibitors (adalimumab, certolizumab, etanercept, golimumab, infliximab, including EMA/FDA approved bsDMARDs), abatacept, IL-6-inhibitors, or rituximab; in patients who cannot use csDMARDs as comedication, IL6-inhibitors and tsDMARDs have some advantages. [5]Current practice would be to start with a bDMARD (in combination with MTX or another csDMARD) because of the long-term experience compared with tsDMARDs (Jak-inhibitors). [6]The most frequently used combination comprises methotrexate, sulfasalazine and hydroxychloroquine. [7]Dose reduction or interval increase can be safely done with all bDMARDs with little risk of flares; stopping is associated with high flare rates; most but not all patients can recapture their good state upon re-institution of the same bDMARD. [8]Efficacy and safety of bDMARDs after Jak-inhibitor failure is unknown; also, efficacy and safety of an IL-6 pathway inhibitor after another one has failed is currently unknown. [9]Efficacy and safety of a Jak-inhibitor after insufficient response to a previous Jak-inhibitor is unknown.

Figure 1 Algorithm based on the 2016 European League Against Rheumatism (EULAR) recommendations on rheumatoid arthritis (RA) management. ACPA, anticitrullinated protein antibody; ACR, American College of Rheumatology; bDMARD, biological DMARD; bsDMARD, biosimilar DMARDs; csDMARDs, conventional synthetic DMARDs; DMARDs, disease-modifying antirheumatic drugs; EMA, European Medicines Agency; FDA, Food and Drug Administration; IL, interleukin; MTX, methotrexate; RF, rheumatoid factor; TNF, tumour necrosis factor; tsDMARDs, targeted synthetic DMARDs.

8. *If the treatment target is not achieved with the first csDMARD strategy, when poor prognostic factors are present, addition of a bDMARD* or a tsDMARD* should be considered; current practice would be to start a bDMARD*[§]. The separation of the second part of previous recommendation 8 ('when poor prognostic factors are present, addition of a bDMARD should be considered') and the new item 7 reflect the Task Force's desire to give stratification by prognostic factors more prominence. The bDMARDs currently available include a series of tumour necrosis factor (TNF)-inhibitors (adalimumab, certolizumab, etanercept, golimumab and infliximab); abatacept (a costimulation inhibitor); tocilizumab (an IL-6 receptor blocker, but in the future also possibly another IL-6 receptor inhibitor, sarilumab and IL-6 inhibitors, such as clazakizumab or sirukumab); rituximab (an anti-B-cell agent); both as biological originator (bo) DMARDs and as European Medicines Agency (EMA)-approved or Food and Drug Administration (FDA)-approved biosimilar (bs) DMARDs.

This recommendation was also expanded to include tsDMARDs, namely the Jak-inhibitor tofacitinib and further Jak-inhibitors, such as baricitinib. In the 2013 update, tsDMARDs (then recommendation 11) were recommended for use after a bDMARD had failed. Since then, more data on tofacitinib, especially regarding long-term safety aspects, and new data for baricitinib have been published. The data suggest that baricitinib may be more efficacious than a TNF-inhibitor.[154] Currently, the term tsDMARDs refers only to Jak inhibition. Tofacitinib is approved in many countries, such as in the USA, Latin America and Asia as well as some European countries, but at the time of developing the present recommendations still not in the European Union; baricitinib had completed phase III trials and was under regulatory review at that time and filgotinib and other Jak-inhibitors are undergoing evaluation in clinical trials (in the meantime baricitinib has been approved in the EU). However, similar to the 2010 recommendations, in which TNF-inhibitors had been given a slight preference over other biologics due to availability of long-term registry data for the former but not the latter, preference is given here to bDMARDs over Jak-inhibitors for the same reason. This notion on current practice is an expert opinion and not based on solid evidence. This bullet point still received a very high vote at the meeting and a high LoA.

The recommendation to use these agents in patients who have bad prognostic factors (rather than those who have not) is also not based on solid evidence in the literature. However, in most trials of bDMARDs and tsDMARDs, the existing inclusion criteria, such as high disease activity, presence of autoantibodies and pre-existing joint damage, assured that patients with bad prognostic factors were included. Nevertheless, formal trials comparing the use of any of these agents in patients with and without bad prognostic markers do not exist. On the other hand, several post hoc analyses revealed the value of using TNF-inhibitors in patients with bad prognostic markers (table 1) relative to those without.[69 76]

The footnote to bDMARDs mentions that all approved bDMARDs may be used without hierarchical positioning, and that EMA-approved or FDA-approved bsDMARDs have similar efficacy and safety as the respective boDMARDs, and should be preferred if they are indeed appreciably cheaper than originator or other bDMARDs or tsDMARDs. Since the 2013 update, several bsDMARDs targeting TNF have been approved in Europe and some in the USA.[155–157] Among the bDMARDs, there is no difference in outcomes, irrespective of their target. This conclusion rests on head-to-head trials, meta-analyses, the results of the SLRs[50–52 158] and indirect comparison (the latter being less reliable and therefore least informative).[13 159 160] Of note, the SLR also included available data from clinical trials of sarilumab, a human anti-IL-6 receptor antibody, and sirukumab, a human anti-IL-6 antibody, both of which are not approved at the present time; based on the SLR, the Task Force regarded these two antibodies and tocilizumab as having overall similar efficacy and safety.[51]

While rituximab is approved for use after TNF-inhibitors have failed, there is ample evidence for its efficacy in bDMARD-naïve patients and early RA.[60 159] It is, therefore, frequently used after IR to csDMARDs, especially when there are specific contraindications to other biological agents, such as past lymphoma or demyelinating disorders, given its efficacy in these diseases.[161 162]

The separation of points 7 and 8 was also based on the reason that the previous bullet point comprised two recommendations and that separating them would give the stratification by prognostic factors better visibility. The poor prognostic factors are presented in table 1 and now also include failure of two csDMARDs; if patients have insufficient efficacy to two csDMARD courses, a further csDMARD may have only little additional impact.[77 127]

The Task Force also discussed whether the use of a bDMARD as first-line therapy should be reconsidered, as had been the case in the original 2010 recommendations. Such use has been tested in a large number of randomised trials and has consistently been found to be statistically superior to MTX monotherapy. Importantly, however, none of the respective phase III trials used a combination with de novo GC in the MTX monotherapy arm and the few investigator-initiated studies that compared first-line bDMARDs plus MTX with GC plus MTX (or with a combination of csDMARDs) did not show a clear clinical or structural advantage of early bDMARD therapy.[127 147] Also, embedded within responders to initial treatment with bDMARDs+MTX are 20%–25% good responders to MTX alone, leading to overtreatment of these patients.[13] Finally, it was shown that patients who had an IR to MTX but then rapidly received bDMARD responded to a similar extent as those who had started with the bDMARD plus MTX.[68] Thus, this proposal for the early use of bDMARDs did not find a majority vote.

Nevertheless, it is still conceivable that an induction regimen followed by the subsequent cessation of the bDMARD and continuation of the csDMARD may become a valuable option in the future; there is some support in the literature for such an approach.[68 163–166] However, this would need further confirmation by additional trials before it could be put into place, especially also because the number of initial responders in whom tapering could be considered does not comprise a majority of the patients. The recommendation, as worded above, received 94% of the Task Force members' votes. LoE *1b, [§]5; LoA 9.0.

9. *bDMARDs* and tsDMARDs[#] should be combined with a csDMARD; in patients who cannot use csDMARDs as comedication, IL-6 pathway inhibitors and tsDMARDs may have some advantages compared with other bDMARDs*. This recommendation replaces former no. 9 ('in patients responding insufficiently to MTX and/or other csDMARD strategies, with or without GC, bDMARDs (TNF-inhibitors, abatacept or tocilizumab, and, under certain circumstances, rituximab) should be commenced with MTX'). While the individual bDMARDs and tsDMARDs have been already discussed above, item 9 now refers to the fact that all bDMARDs have superior efficacy when combined with MTX than as monotherapy. Compared with the 2013 update, more evidence has now accrued in favour of combination, even for tocilizumab.[167–169] Also for baricitinib, combination therapy conveys better structural, although not clinical or functional

efficacy than monotherapy.[170] However, regarding signs and symptoms, physical function and joint damage, there are indications for a somewhat better efficacy of tocilizumab monotherapy, and more strongly so for Jak-inhibitors compared with MTX.[170–172] Monotherapy of the other biological agents has not been found clinically superior to MTX monotherapy.[66 67 173] MTX can be used at 7.5–10 mg to provide added efficacy to TNF-inhibitors[174 175] and intolerance at these low doses leading to discontinuation is very rare. Moreover, biologics can also be effectively combined with other csDMARDs.[142 144]

Another aspect, namely the occurrence of antidrug antibodies (immunogenicity), was discussed, especially regarding secondary non-response. In this context, the lack of knowledge about the role of non-adherence and non-persistence was also addressed. The Task Force then discussed routine testing of antidrug antibodies and drug levels and felt that there was little place for these in clinical practice, since a good clinical response would not lead to cessation of therapy even in the presence of antidrug antibodies, or low drug levels, and vice versa. Of note, the use of MTX at the doses mentioned above reduces the incidence of antidrug antibodies.[174 175]

For all these reasons the Task Force felt strongly (96% majority) that bDMARDs (and tsDMARDs) should primarily be added to, that is, combined with csDMARDs, such as MTX or leflunomide, leaving the option of monotherapy, with a preference for certain drugs, as an exception in case of intolerance or contraindication to all csDMARDs. LoE *1a, #1b; LOA 9.2.

10. *If a bDMARD* or tsDMARD§ has failed, treatment with another bDMARD or a tsDMARD should be considered; if one TNF-inhibitor therapy has failed, patients may receive another TNF-inhibitor or an agent with another mode of action*. A similar recommendation was presented in 2013: 'If a first bDMARD has failed, patients should be treated with another bDMARD; if a first TNF-inhibitor therapy has failed, patients may receive another TNF-inhibitor or a biological agent with another mode of action'. Indeed, in a trial published after the elaboration of these recommendations, even primary non-responders to a TNF-inhibitor were shown to have some response to another anti-TNF, making it difficult to draw different conclusions for subsequent therapy for primary compared with secondary failures to TNF-blockers.[176] The addition in the first part ('or tsDMARD') was partly needed because tsDMARDs (Jak inhibition) are now included in the earlier recommendations 8 and 9; 'first' was deleted, because the Task Force did not decide to distinguish between failure of one or more bDMARDs. However, it must be noted that it is currently neither known if a Jak-inhibitor is effective once another one has failed nor established that a second IL-6 receptor inhibitor or inhibitors of the IL-6 ligand are effective if tocilizumab has failed—this is still part of the research agenda. We also lack studies exploring if TNF-inhibitors are efficacious and safe after bDMARDs with other modes of action have failed, and also studies investigating switching between these other modes of action. A few members raised the question if the use of csDMARDs should also be considered when bDMARDs had failed, but this suggestion did not find a majority.

The Task Force was also clear about its recommendations that any bDMARD, including another TNF-inhibitor, could be used if a TNF-inhibitor has previously failed. Thus, drugs with the same or with another mode of action are recommended in this situation. This was based on the data of clinical trials including meta-analyses[158] and on the fact that in contrast to registry data, which may be affected by a variety of confounders, several new prospective studies suggest that there is no difference between these two approaches.[177 178] If a second TNF-inhibitor fails, patients should receive an agent with another mode of action. However, it is self-evident (and supported by the vast majority of the Task Force members) that a bsDMARD of any of the reference boDMARDs should not be used if the respective boDMARD (or another bsDMARD of the same molecule) has failed to induce sufficient efficacy or vice versa. LoE *1a, §5; LoA 9.2.

11. *If a patient is in persistent remission after having tapered GC, one can consider tapering bDMARDs, especially if this treatment is combined with a csDMARD*. This item remained unchanged compared with the 2013 publication. No new data have been published that contest this conclusion. Tapering here means reduction of dose or extension of interval between applications ('spacing'). It does not necessarily imply discontinuation of a bDMARD, which may lead to a recurrence of disease in a majority of patients.[179 180] However, even if treatment is stopped and patients flare, the majority of them (>80%) will recover their previous good outcome on reinstitution of therapy (but some do not),[180 181] and patients should be informed accordingly. There exist certain predictors in whom tapering will be likely successful and these relate primarily to early RA, depth of improvement and duration of remission[182]; prospective trials taking these aspects into consideration are needed in the future. This item also indirectly bolsters recommendation 9 on combination therapy of bDMARDs with MTX or another csDMARD, since it implies that bDMARDs should primarily, if not only, be tapered and possibly discontinued when combined with a csDMARD, while tapering and stopping of bDMARD monotherapy was not yet sufficiently studied. LoE 2b; LoA 9.0.

12. *If a patient is in persistent remission, tapering the csDMARD could be considered*. The 2013 version of the respective point 13 reads: 'In cases of sustained long-term remission, cautious reduction of the csDMARD dose could be considered, as a shared decision between patient and physician'. This item elicited significant discussions, since it would mean leaving patients with RA either without any or with a low dose of a csDMARD. But in general, no new evidence for or against this view has been found over the last years. In the discussion, controversies emerged. It was mentioned that here tapering means primarily reducing the dose and that discontinuing csDMARDs may be possible only in exceptional cases. Many rheumatologists on the Task Force panel expressed a view stating that csDMARDs should never be stopped. Consequently, this item received the lowest LoA (8.5) of all, although still quite high on the scale of 0–10. Of note, the portion worded 'as a shared decision between patient and physician' was now deleted. It was felt by the Task Force that mentioning the shared decision for this item among all 12 would imply that the other recommendations may not need to involve the patient, or single out this specific recommendation in comparison with all other ones and thus offset overarching principle A. Obviously, the removal of this phrase does not mean that shared decision making with the patients is not important, on the contrary: in line with principle A it is of utmost importance for this and for all other recommendations. LoE 4; LoA 8.5.

The updated recommendations are depicted in an abbreviated way in figure 1. Part and parcel of this figure are the respective footnotes as well as the full text as presented here.

DISCUSSION

The 2016 update of the EULAR RA management recommendations was developed by 50 experts, including patients,

rheumatologists and other healthcare professionals. This was the largest Task Force ever convened for the development of EULAR recommendations, both with respect to the overall number of members and the number of European countries involved, and it is also the first EULAR Task Force with a broad international representation, since rheumatologists from several other continents participated in this activity. This allowed us to also include some views from Asia, and Latin America and North America in the development of the recommendations, an input desired given the information provided in the recent publications of the updated ACR and the APLAR recommendations.[17 39]

The 2016 update presents the hitherto 'leanest' EULAR recommendations for RA management. While in 2010 the document comprised 3 overarching principles and 15 recommendations and in 2013 it contained 3 overarching principles and 14 recommendations, the 2016 update arrived with 4 principles and 12 recommendations. Despite this reduction, in light of a continuously increasing spectrum of therapeutic options and new information on existing agents and therapeutic strategies, this update covers more treatment aspects and is built on a better evidence base than ever before. This is due to the availability of at least partial answers to several of the research questions posed in 2013, such as items 4, 6, 9 and 21,[16] and of many new data on established and novel drugs as well as therapeutic strategies.

The Task Force adhered to several principles established in the course of the development of the 2013 update and even in 2010. For example, aside from evidence on efficacy and safety, economic aspects were generally considered in line with respective general specifications.[45 46] Also, agents that have not yet been approved by regulatory authorities but for which data from phase III trials were available, were considered with the caveat that their use would be only possible on such approval. This pertains to bsDMARDs, for which the Task Force relies on the stringency of the regulatory processes of EMA and FDA, for new IL-6 inhibitors and for Jak-inhibitors, the first of which was only licensed in some parts of the world at the time of developing these recommendations, with increasing availability of data on others. However, in the meantime baricitinib has been approved in the European Union. Finally, the Task Force reiterated its previous conclusions on the importance of stratification according to risk factors of adverse RA outcome,[69 76] once an initial therapy has failed.

The individual recommendations are not numbered by importance, but rather by a logical sequence: what is the treatment target and how should the patient be followed? What is the most prudent treatment approach once the diagnosis has been made? How can therapeutic success be maximised? Which therapies should follow a first treatment failure (phase I) and under which circumstances? Which agent or type of drug should be preferred in the course of the development of the treatment strategies?

Consequently, the first three items, which were either left fully unchanged or were only minimally changed, deal with the time point of starting effective therapy (as soon as the diagnosis is made and thus without any loss of time); with the definition of the treatment target (sustained remission or low disease activity); and with monitoring and the need to reach a significant improvement of disease activity within 3 months and attainment of the targeted state within 6 months. The preferred instruments to be used when following patients have been defined in previous EULAR activities[22 111] and comprise composite measures that include joint counts, such as the CDAI, DAS28 and SDAI as well as the ACR/EULAR remission definitions. Of note, instruments weighing acute phase reactants highly may exaggerate response, especially with IL-6 or Jak-inhibitors.

The treatment target (stringent remission or low disease activity) continues to be clinically defined, since focusing at ultrasonographic remission has not shown better outcomes compared with targeting clinical low disease activity or stringent remission, but rather induced overtreatment and thus inefficient use of healthcare resources.[108 109] Moreover, no strategy trial is available comparing the use of the serologic multibiomarker disease activity (MBDA) test with targeting remission using clinical disease activity assessment by a clinical composite measure (with which MBDA correlates anyway); of note, the MBDA test has been reported to improve to a larger extent on using a bDMARD that directly targets a cytokine compared with one that targets T-cell costimulation, despite similar clinical, functional and radiographic outcomes.[183] Moreover, it must be assumed that such test would falsely indicate high disease activity when an infection occurs. For all these reasons, the Task Force recommends to follow patients in clinical practice using a composite measure which comprises joint counts and may include an acute phase reactant. This clinical assessment is pertinent for every therapeutic phase (figure 1).

Subsequent recommendations, however, have undergone some significant changes compared with the 2013 update. While MTX (or in the presence of intolerance another csDMARD) continues to be considered the pivotal drug once the RA diagnosis has been made (item 4), it is recommended more strongly than before to escalate MTX to a dose of 25–30 mg weekly (with folate supplementation), given further recent insights on the high response rate with such strategy.[4 118] Moreover, the combination of csDMARDs, as monotherapy, with GC is more strongly suggested than before in light of increasing evidence that this combination is not surpassed by csDMARD combinations, even if they are applied with GC, or bDMARDs plus MTX in terms of efficacy and safety.[126 147] In the treatment algorithm (figure 1, phase I), this is reflected by the respective change from '±' to '+' for the addition of GC to csDMARDs. The term 'low-dose' GC has now been replaced by 'short-term' GC, given that various modes of application at different doses have shown to be efficacious. Moreover, the most important factors to reduce the risk of adverse event, such as cardiovascular events, infections, diabetes or hypertension,[151 152 184] was deemed to be rapid tapering to discontinuation and a low cumulative dose of GC. This is, indeed, the case with these alternative GC treatment modalities.

In contrast to the 2013 update, csDMARD combination therapy, with or without GC, is no longer an explicit part of the recommendations. This conclusion was based on the accruing evidence that this csDMARD combination therapy may not be superior to MTX monotherapy plus GC, but may be associated with an increase in adverse events.[126 130] A recent indirect-comparison meta-analysis has suggested a superiority of csDMARD combination versus MTX monotherapy.[185] This study was at odds with a previous direct-comparison meta-analysis[35 186] and with our own SLRs,[35 52 133] and indirect comparisons should also be considered with reservation since their rigour and value is insufficiently understood to date. Interestingly, using a somewhat different approach and based on an independent SLR, the ACR guideline has arrived at a similar conclusion as presented here and recommends MTX monotherapy as the first DMARD in early or established RA.[17] However, the use of csDMARD combination therapy is not precluded by the new recommendations, rather it is at the discretion of the

rheumatologist to apply it in the context of the recommendation on the use of MTX as a (first) treatment 'strategy'.

Once phase I has failed to reach the treatment target, either in the presence of bad prognostic markers or in the absence of bad prognostic markers after a second csDMARD strategy has failed, the Task Force recommends to add any bDMARD or, less preferably, a tsDMARD. If phase II as depicted in the algorithm fails to arrive at the treatment target, another bDMARD or a tsDMARD should be used. The Task Force reiterated its position that if a TNF-inhibitor fails, another TNF-inhibitor—but not a biosimilar of the same molecule!—can be as effective as changing the mode of action. Vice versa, an effective biological agent should not be switched to another bDMARD for non-medical reasons. However, important data are missing for some of the drugs; for example, clinical trials did not address the efficacy of a TNF-inhibitor after bDMARDs with other modes of action or a Jak-inhibitor has failed. Similar questions arise for the other agents and also for the use of IL-6R or IL-6 inhibitors, such as sarilumab or sirukumab, after tocilizumab has failed (box 1).

Early bDMARD treatment, including an induction regimen with subsequent withdrawal of bDMARDs as supported by some strategy trials, was discussed but did not find a majority among the Task Force members. This decision was based on the lack of evidence for superiority of such therapy compared with the use of MTX plus GC. Moreover, when placed in the context of a treat-to-target strategy, the initial use of csDMARDs yields equal results in the long-term. Finally, the cost-effectiveness of first-line bDMARD therapy, especially in light of the reasons just mentioned, is very poor.

The 2016 update of the EULAR recommendations is based on the most recent evidence in the area of RA management and on discussions by a large and broadly international Task Force. The recommendations synthesise the current thinking on approaching RA treatment in a set of overarching principles and recommendations. These have been informed by SLRs on the efficacy and safety of the drugs. The Task Force is convinced that adhering to these recommendations, including shared decision making, defining the treatment target, assessing disease activity regularly with appropriate instruments and applying the sequence of drugs as proposed and in a treat-to-target strategy, will maximise the overall outcome in a vast majority of patients with RA. Still, a considerable proportion of patients will not reach the target despite all efforts, and for these patients new drugs will be needed. Also, new information from research activities on treatment strategies, predictive markers and other aspects will become available in the near future and will likely necessitate yet another update of the recommendations in about 3 years; maybe we will then have new data on the research agenda, including precision medicine approaches in RA which allow predicting who will best respond to which drug at which stage of the disease. Until then we hope that the 2016 update will be broadly applied in clinical practice and/or serve as a template for national societies to develop local recommendations.

Box 1 Research agenda

1. How does MTX monotherapy in combination with glucocorticoids compare with monotherapies of sulfasalazine or leflunomide in combination with glucocorticoids, at the doses of csDMARDs as used today?
2. In what proportion of patients is an induction therapy with a bDMARD+MTX with subsequent cessation of the bDMARD effective in inducing sustained remission?
3. Is the application of a TNF-inhibitor after abatacept, tocilizumab, rituximab or a Jak-inhibitor has failed, safe and efficacious?
4. How safe and efficacious are abatacept, tocilizumab and rituximab after any of the other non-TNF-inhibitor-bDMARDs or a tsDMARD has failed?
5. How safe and efficacious is the use of an IL-6 pathway inhibitor if another IL-6 pathway inhibitor/a Jak-inhibitor has failed?
6. How safe and efficacious is the use of a Jak-inhibitor after another IL-6 pathway inhibitor/another Jak-inhibitor has failed?
7. Is the risk stratification as recommended by EULAR after failure of MTX improving outcome in those with risk factors and not harming those without bad prognostic markers? Do patients who lack bad prognostic factors benefit as much from a switch or addition of a csDMARD as from the addition of a bDMARD?
8. Can we find predictors of differential response to the different bDMARDs and tsDMARDs?
9. When starting a DMARD, how can we best predict who will attain the treatment target (remission or low disease activity) and who not?
10. Can we predict who will maintain remission after withdrawal of a bDMARD?
11. Will we be able to develop precision (personalised, stratified) medicine approaches in RA?
12. Is tapering of bDMARD monotherapy, where potentially indicated, comparable with bDMARD tapering in the presence of csDMARDs?
13. Will RCTs on tapering of bDMARDs following the deducted predictors for successful withdrawal of bDMARDs show success?
14. How good is patient adherence to a bDMARD or tsDMARD and can non-adherence explain secondary loss of efficacy?
15. Is measurement of serum drug or antidrug antibody levels useful in clinical practice?
16. Which biomarkers will help to find better predictors of bad outcome or response and which have failed in the numerous clinical trials that evaluated gene-expression and other biomarkers?
17. What is the effect of csDMARD, tsDMARD and bDMARD therapies on cardiovascular outcomes and to which extent is a potential effect dependent on a clinical response?

Is the use of telemedicine or e-medicine approaches as effective as direct contact in the clinic for treat-to-target strategies? bDMARDs, biological DMARDs; csDMARDs, conventional synthetic DMARDs; DMARDs, disease-modifying antirheumatic drugs; EULAR, European League Against Rheumatism; Jak, Janus kinase; MTX, methotrexate; RA, rheumatoid arthritis; RCT, randomised controlled trial; TNF, tumour necrosis factor; tsDMARDs, targeted synthetic DMARDs.

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Correction notice This article has been corrected since it published Online First. At the time of the online publication, baricitinib had received marketing authorisation in the EU; tofacitnib had already received a positive opinion but not yet marketing authorisation in the EU. This has now been obtained between online and print publication.

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2016 update of the ASAS-EULAR management recommendations for axial spondyloarthritis

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ABSTRACT

To update and integrate the recommendations for ankylosing spondylitis and the recommendations for the use of tumour necrosis factor inhibitors (TNFi) in axial spondyloarthritis (axSpA) into one set applicable to the full spectrum of patients with axSpA. Following the latest version of the European League Against Rheumatism (EULAR) Standardised Operating Procedures, two systematic literature reviews first collected the evidence regarding all treatment options (pharmacological and non-pharmacological) that were published since 2009. After a discussion of the results in the steering group and presentation to the task force, overarching principles and recommendations were formulated, and consensus was obtained by informal voting. A total of 5 overarching principles and 13 recommendations were agreed on. The first three recommendations deal with personalised medicine including treatment target and monitoring. Recommendation 4 covers non-pharmacological management. Recommendation 5 describes the central role of non-steroidal anti-inflammatory drugs (NSAIDs) as first-choice drug treatment. Recommendations 6–8 define the rather modest role of analgesics, and disprove glucocorticoids and conventional synthetic disease-modifying antirheumatic drugs (DMARDs) for axSpA patents with predominant axial involvement. Recommendation 9 refers to biological DMARDs (bDMARDs) including TNFi and IL-17 inhibitors (IL-17i) for patients with high disease activity despite the use (or intolerance/contraindication) of at least two NSAIDs. In addition, they should either have an elevated C reactive protein and/or definite inflammation on MRI and/or radiographic evidence of sacroiliitis. Current practice is to start with a TNFi. Switching to another TNFi or an IL-17i is recommended in case TNFi fails (recommendation 10). Tapering, but not stopping a bDMARD, can be considered in patients in sustained remission (recommendation 11). The final two recommendations (12, 13) deal with surgery and spinal fractures. The 2016 Assessment of SpondyloArthritis international Society-EULAR recommendations provide up-to-date guidance on the management of patients with axSpA.

INTRODUCTION

Axial spondyloarthritis (axSpA) is an inflammatory rheumatic disease with a diverse clinical presentation.[1] Chronic back pain is the leading symptom of the disease and often inflammatory in nature with pronounced stiffness and improvement of pain and stiffness with exercise. Other musculoskeletal manifestations of axSpA are arthritis, enthesitis and dactylitis. Extra-articular manifestations such as anterior uveitis, psoriasis and inflammatory bowel disease (IBD) (in order of decreasing prevalence) are also characteristic for axSpA.[2] Historically, end-stage patients were recognised by a characteristic stooped posture and by the presence of syndesmophytes on radiographs of the spine. Later, radiographic sacroiliitis became a crucial finding in the diagnosis and classification of patients. The modified New York criteria for ankylosing spondylitis (AS) were most frequently used in studies and drug trials.[3] Only recently it has been properly acknowledged that radiographic sacroiliitis is a rather late finding in the disease course of many patients, that MRI may show signs of inflammation much earlier than radiographs show structural damage, and that patients can also be diagnosed based on a typical clinical pattern, even in the presence of normal imaging tests.[1 4] The term axSpA comprises the whole spectrum of patients with radiographic sacroiliitis (AS or radiographic axSpA) and without radiographic sacroiliitis (non-radiographic axSpA).[4]

There is still some debate as to whether radiographic and non-radiographic axSpA should be considered as two different entities or as a continuous disease spectrum. The currently prevailing opinion is that axSpA encompasses one disease spectrum in which single patients with non-radiographic axSpA may develop radiographic changes over time.[5] However, not all patients with non-radiographic axSpA will ultimately develop radiographic sacroiliitis. Similarly, not all patients with radiographic sacroiliitis will ultimately develop syndesmophytes. In fact, radiographic sacroiliitis artificially divides the spectrum of axSpA in two groups, and it is unlikely that the sole presence of radiographic sacroiliitis is relevant for the outcome

of the disease. In addition, recent studies and trials have cast doubt on the reliability of establishing radiographic abnormalities.[6–10] Taken together, there is ample argument to use only the term axSpA in clinical practice.[11] Especially in the context of studies, it may be of value to add certain characteristics to the profile of patients, such as the presence of radiographic sacroiliitis, the presence of inflammation on MRI, the presence of arthritis, of extra-articular manifestations, to describe in detail the type of patients included.[5]

Apart from historical reasons, drug development has played a major role in distinguishing patients based on the presence of radiographic sacroiliitis: tumour necrosis factor inhibitor (TNFi) therapy was historically approved for patients with AS, and companies sought the additional regulatory approval for patients without radiographic sacroiliitis.[12–17] The newest draft guidance document of the European Medicines Agency now proposes to study patients with axSpA as one entity, which testifies of the progress in the field of axSpA.[18]

Historically, the Assessment of SpondyloArthritis international Society (ASAS) has drafted two sets of treatment recommendations, dating back to the time when TNFi were the only class of biological disease-modifying antirheumatic drugs (bDMARDs) and the concept of axSpA was not yet well established. However, it should be noted that there is no formal proof that TNFi are in fact disease modifying in axSpA. The first set included the ASAS recommendations for the use of TNFi therapy in patients with AS published first in 2003 and updated in 2006 and 2010.[19–21] In contrast to existing recommendations for the use of bDMARDs in rheumatoid arthritis (RA) and psoriatic arthritis (PsA), the ASAS recommendations on the use of TNFi in AS include specific definitions for the level of disease activity required before a TNFi can be installed.[22 23] The second set of recommendations that ASAS has drafted in collaboration with the European League Against Rheumatism (EULAR) included recommendations for the management of AS published first in 2006 and updated in 2010.[24 25] In line with a better delineation and acceptance of axSpA, in follow-up of the advent and approval of another class of bDMARDs (IL-17 inhibitors (IL-17i)), and after the publication of studies with patients covering the entire spectrum of axSpA, it was felt timely to integrate all different aspects of management into one set of recommendations and update the recommendations accordingly.[26–28] However, we have to acknowledge that the term bDMARDs is not completely correct as the disease-modifying aspect has not yet been proven in axSpA.

This document presents the 2016 ASAS-EULAR management recommendations for the management of patients with axSpA and details the process of their development.

METHODS

This was a combined project endorsed and financed by both ASAS and EULAR. One aim of this update was to aggregate the existing ASAS-EULAR management recommendations of AS and the ASAS recommendations for the management of axSpA with TNFi into one set of recommendations. The objective of this aggregated set of recommendations is to give guidance on the non-pharmacological and pharmacological management of patients with axSpA.

The 2014 updated EULAR standardised operating procedures have been applied.[29] These prescribe that the process set out in Appraisal of Guidelines for Research & Evaluation II (AGREE II) should be followed in order to design the recommendations and to write the manuscript.[29 30] The convenors formed first a task force with a steering committee. The steering committee included the convenor (DvdH), co-convenor (JB), methodologist (SR), two fellows who performed the systematic literature reviews (SLRs) (AS, AR) and three expert rheumatologists (RL, XB, FVdB). The steering committee defined the research questions for the SLRs and prepared the 1-day meeting of the task force. This task force included in addition to the steering committee 18 rheumatologists (two of them with axSpA), including three members of EMerging EUlar NETwork (EMEUNET) (AM, PM, VN-C), one healthcare professional (HD) and two patient partners (MJ, DW). The members of the task force represent 14 countries in Europe, North America and South America. All members of the task force disclosed their potential conflicts of interest before the start of the process.

Two fellows under the guidance of the methodologist performed two SLRs: one on non-pharmacological and non-biological pharmacological treatment (AR) and one on biological and targeted synthetic DMARDs (AS). These SLRs focused on the studies published after the locking date of the SLRs for the previous update, that is, 2009. The two SLRs are published in detail separately (Sepriano *et al*. Efficacy and safety of biological and targeted synthetic DMARDs: an SLR informing the 2016 update of the ASAS-EULAR recommendations for the management of axSpA. 2016, submitted for publication; Regel *et al*. Efficacy and safety of non-pharmacological and non-biological pharmacological treatment: an SLR informing the 2016 update of the ASAS-EULAR recommendations for the management of axSpA. 2016, submitted for publication). These SLRs and the current recommendations manuscript form an integral and inseparable part and should be read as such. Both SLRs addressed efficacy and safety, but because the literature on safety of specific drugs in axSpA was, as shown by the SLRs, somewhat limited, more extensive evidence collected on these drugs in SLRs for RA were also taken into account (Sepriano *et al*, 2016, submitted for publication; Regel *et al*, 2016, submitted for publication).[31] The evidence collected was presented in summary of findings (SoF) tables and included judgements about risk of bias, which was determined for every study.[32 33] SoF tables were presented to the steering committee in writing and by presentation, and served as the basis for the discussion in the full task force. When discussing the update of the recommendations, the evidence collected in the previous SLRs was also taken into consideration.[34–36]

In addition, the fellows performed an SLR on the research question whether AS Disease Activity Score (ASDAS) or Bath AS Disease Activity Index (BASDAI) should be applied to best define disease activity for the start and continuation of bDMARDs (see online supplementary material).

Based on the data obtained from the SLR, the steering committee prepared wording for the update of the overarching principles and recommendations. The overarching principles and recommendations from the 2010 update were used as a basis and were updated if considered necessary. It was decided that recommendations could only be updated if there was new evidence available that justified such an update according to the task force.

The task force met for a 1-day meeting. First, the results of the SLRs were presented to the participants. Thereafter, the updating process of the overarching principles and recommendations was done by discussion in the group. For every overarching principle and recommendation proposed, formulations were presented, discussed and voted on (informal voting). If at least 75% approved the new wording, this was accepted. If not, discussion was resumed and changes to the wording were

proposed. In the second voting round, a 67% majority was required to accept the recommendation. If this was not reached, a further round of discussion followed and completed with a vote in which a simple majority was deemed sufficient.

After the meeting, the levels of evidence (LoE) and grades of recommendation (GoR) derived from the SLRs following the standards of the Oxford Centre for Evidence Based Medicine were added to each of the recommendations.[37] In summary, level 1A refers to evidence stemming from a meta-analysis of randomised controlled trials (RCTs), level 1B corresponds to at least one RCT, level 2A means that there was at least one controlled study without randomisation, level 2B at least one type of quasi-experimental study, level 3 corresponds to descriptive studies, such as comparative studies, correlation studies or case-control studies and level 4 means from expert committee reports or opinions and/or clinical experience of respected authorities. The GoR are A, which means consistent level 1 studies, B indicating consistent level 2 or 3 studies or extrapolations from level 1 studies, grade C meaning level 4 studies or extrapolations from level 2 or 3 studies and grade D reflecting level 5 evidence or troublingly inconsistent or inconclusive studies of any level. Finally, the overarching principles and recommendations were sent to the task force members and they were asked to add the level of agreement anonymously to each of the statements. This was done by numerical rating scale (0–10) with the anchors 'do not agree at all' at 0 and 'fully agree' at 10. The average, SD and range of the level of agreement per recommendation, as well as the percentage of participants with a score of at least 8, are presented.

The exact wording of the recommendations was considered final after the end of the 1-day task force meeting. The final manuscript was drafted after the meeting, reviewed, revised and approved by all task force members, followed by final review and ratification by the EULAR Executive Committee and ASAS Executive Committee before submission to the journal.

RESULTS

It was decided to use the same terminology for DMARDs as proposed recently: conventional synthetic (cs) DMARDs for drugs such as sulfasalazine and methotrexate (MTX); targeted synthetic DMARDs for drugs such as tofacitinib and bDMARDs for drugs such as TNFi and IL-17i. bDMARDs can further be subdivided into bio-originator (bo) and biosimilar (bs) DMARDs. Only DMARDs that were approved in at least one country with an indication for axSpA were considered in the recommendations process.[38] However, all DMARDs were looked at in the SLRs.

The target-users of these recommendations are: 'All healthcare professionals taking care of patients with axSpA'. While this definition will mainly include practicing rheumatologists, it may also include medical specialists of a different discipline, general practitioners, physical therapists and other healthcare professionals, as well as medical students. These recommendations further aim at patients to be educated for informed/shared decision-making. The final target group is pharmaceutical industry in its broadest sense, national drug agencies and policy makers, as well as health insurance companies.

The recommendations describe all aspects of the management of patients with a diagnosis of axSpA. Many of these patients will also fulfil the ASAS classification criteria for axSpA.[39] The focus of these recommendations is on the musculoskeletal signs and symptoms of the disease. But when appropriate and relevant, extra-articular manifestations such as psoriasis, uveitis and IBD, as well as comorbidities including osteoporosis and cardiovascular diseases, will also be discussed. However, the actual management of these extra-articular manifestations and comorbid conditions are beyond the scope of these management recommendations. For the optimal management of these diseases, specific EULAR recommendations and respective medical specialists should be consulted.[40–42]

As the concept and term of axSpA is relatively new, the older studies in the literature are based only on patients with AS. This applies mainly to non-pharmacological treatments and to drugs that are already on the market for a long time, such as non-steroidal anti-inflammatory drugs (NSAIDs). However, the two SLRs revealed many trials that have included patients with the whole spectrum of axSpA, mainly trials with TNFi but also trials with NSAIDs and DMARDs. The task force agreed explicitly that these recommendations apply to all patients with axSpA.

Overarching principles

As in the 2010 update, the recommendations start with overarching principles, which are considered so generic and implicit in nature that they serve as a basis for the state-of-the-art management of patients with axSpA. As such, they reflect the state of practice rather than the state of science. There are in total five overarching principles; four are identical to the previous version and one new overarching principle was formulated. Only the order of the previous overarching principles 3 and 4 was switched. We present the LoA of each overarching principle in table 1.

1. axSpA is a potentially severe disease with diverse manifestations, usually requiring multidisciplinary management coordinated by the rheumatologist

This overarching principle is important, because it stresses that musculoskeletal manifestations of the disease may importantly interfere with patients' daily living and it points to the fact that patients with axSpA frequently experience extra-articular manifestations: approximately 40% of the patients experience at least one extra-articular manifestation during the course of the disease.[2 43] Some of these extra-articular manifestations require the immediate consultation of other experts, pointing to the presence of multidisciplinary networks for the best care of patients with axSpA. Some of the available (biological) drugs are efficacious for both musculoskeletal and the extra-articular manifestations, while others have effects limited to the musculoskeletal symptoms. These factors should be taken into account when choosing a drug. Since the treating rheumatologist should have extensive knowledge of the entire disease spectrum, it is crucial that the rheumatologist is the coordinator in a multidisciplinary network of care for patients with axSpA. In this network, other medical specialists and care professionals do of course also have their place.

2. The primary goal of treating the patient with axSpA is to maximise long-term health-related quality of life through control of symptoms and inflammation, prevention of progressive structural damage, preservation/normalisation of function and social participation

Management should aim at the best possible health-related quality of life. Many studies have clearly shown that patients with axSpA have a reduced quality of life in comparison to the non-diseased population.[44 45] Problems experienced by patients with axSpA can be summarised according to the International Classification of Functioning, Disability and Health (ICF) and

Table 1 2016 Update of the ASAS-EULAR recommendations for the management of axSpA

	Overarching principles	LoE	GoR	LoA (0–10)
1	axSpA is a potentially severe disease with diverse manifestations, usually requiring multidisciplinary management coordinated by the rheumatologist			9.9 (0.31) 100% ≥8
2	The primary goal of treating the patient with axSpA is to maximise health-related quality of life through control of symptoms and inflammation, prevention of progressive structural damage, preservation/normalisation of function and social participation			9.8 (0.47) 100% ≥8
3	The optimal management of patients with axSpA requires a combination of non-pharmacological and pharmacological treatment modalities			9.8 (0.45) 100% ≥8
4	Treatment of axSpA should aim at the best care and must be based on a shared decision between the patient and the rheumatologist			9.5 (0.91) 100% ≥8
5	axSpA incurs high individual, medical and societal costs, all of which should be considered in its management by the treating rheumatologist			9.3 (1.17) 97% ≥8
	Recommendations			
1	The treatment of patients with axSpA should be individualised according to the current signs and symptoms of the disease (axial, peripheral, extra-articular manifestations) and the patient characteristics including comorbidities and psychosocial factors	5	D	9.7 (0.65) 100% ≥8
2	Disease monitoring of patients with axSpA should include patient-reported outcomes, clinical findings, laboratory tests and imaging, all with the appropriate instruments and relevant to the clinical presentation. The frequency of monitoring should be decided on an individual basis depending on symptoms, severity and treatment	5	D	9.6 (0.78) 100% ≥8
3	Treatment should be guided according to a predefined treatment target	5	D	8.9 (1.45) 93% ≥8
4	Patients should be educated* about axSpA and encouraged to exercise* on a regular basis and stop smoking‡; physical therapy† should be considered	2* 5‡ 1a†	B* D‡ A†	9.6 (0.78) 100% ≥8
5	Patients suffering from pain and stiffness should use an NSAID as first-line drug treatment up to the maximum dose, taking risks and benefits into account. For patients who respond well to NSAIDs continuous use is preferred if symptomatic otherwise	1a	A	9.4 (0.94) 100% ≥8
6	Analgesics, such as paracetamol and opioid-(like) drugs, might be considered for residual pain after previously recommended treatments have failed, are contraindicated, and/or poorly tolerated	5	D	8.8 (0.94) 100% ≥8
7	Glucocorticoid injections* directed to the local site of musculoskeletal inflammation may be considered. Patients with axial disease should not receive long-term treatment with systemic glucocorticoids‡	2* 5‡	B* D‡	9.4 (0.78) 100% ≥8
8	Patients with purely axial disease should normally not be treated with csDMARDs§; sulfasalazine† may be considered in patients with peripheral arthritis	1a†	A	9.2 (0.78) 100% ≥8
9	bDMARDs should be considered in patients with persistently high disease activity despite conventional treatments (figure 1); current practice is to start with TNFi therapy	1a (TNFi); 1b (IL-17i)	A	9.6 (1.09) 93% ≥8
10	If TNFi therapy fails, switching to another TNFi* or IL-17i** therapy should be considered	2* 1b**	B* A**	9.6 (0.95) 97% ≥8
11	If a patient is in sustained remission, tapering of a bDMARD can be considered	2	B	9.1 (1.57) 97% ≥8
12	Total hip arthroplasty should be considered in patients with refractory pain or disability and radiographic evidence of structural damage, independent of age; spinal corrective osteotomy in specialised centres may be considered in patients with severe disabling deformity	4	C	9.4 (0.82) 100% ≥8
13	If a significant change in the course of the disease occurs, causes other than inflammation, such as a spinal fracture, should be considered and appropriate evaluation, including imaging, should be performed	5	D	9.9 (0.31) 97% ≥8

§1a (sulfasalazine; methotrexate); 1b (leflunomide); 4 other csDMARDs.
axSpA, axial spondyloarthritis; bDMARD, biological disease-modifying antirheumatic drug; csDMARD, conventional synthetic disease-modifying antirheumatic drug; GoR, grade of recommendation; IL-17i, interleukin-17 inhibitor; LoA, level of agreement; LoE, level of evidence; NSAIDs, non-steroidal anti-inflammatory drugs; TNFi, tumour necrosis factor inhibitor.

can be assessed using the ASAS Health Index, which is based on the ICF.[46–48] As axSpA is an inflammatory disease, suppression of inflammation by drugs has a prominent place, in order to relieve symptoms, preserve physical function and maintain quality of life. And indeed, data have accrued that suggest a direct relation between clinical disease activity and syndesmophyte formation and between disease activity and function.[49–51] Moreover, patients who have inactive disease have a better health-related quality of life.[52]

3. The optimal management of patients with axSpA requires a combination of non-pharmacological and pharmacological treatment modalities

This overarching principle is identical to number 4 in the 2010 set of recommendations.

In comparison to other chronic inflammatory rheumatic diseases such as RA and PsA, non-pharmacological management has a relatively important place in the management of patients with axSpA. While this will be highlighted in the separate recommendations, the task force wanted to draw attention to the importance of non-pharmacological treatment by formulating it as an overarching principle.

4. Treatment of axSpA should aim at the best care and must be based on a shared decision between the patient and the rheumatologist

This is an unchanged principle but is now listed as the fourth overarching principle. 'Best care' is an important concept and closely relates to overarching principle 2: 'to maximise health-related quality of life'. But 'best care' here refers to the 'best possible care' for individual patients, and still prevails when costs of treatment are taken into account, as indicated in the following fifth overarching principle.

'Shared decision-making' is the second important concept in this overarching principle and refers to the formal and informal relationship between patient and rheumatologist, that partner during all phases of their encounters, in order to collectively decide on the best possible management, given all factors that

may be relevant for such a decision. 'Shared decision' refers to the choice of a particular drug and pertains to all phases of the process: defining a treatment-goal (target), investigating potential barriers to achieve that target, choosing the best strategy to achieve the target (given the potential barriers), considering alternative strategies if the target is not reached or the treatment is not tolerated, considering tapering strategies if a target is 'sustained', etc. Shared decision-making requires sufficient education about the disease, appropriate information (ie, comprehensible risk communication) about risks and benefits of separate treatment options and the design of a feasible management plan as well as strategies to monitor treatment success. In this process of shared decision-making, rheumatologists and patients have different roles and responsibilities that ideally should merge into one management plan with full commitment from patient and care-giver, so that the likelihood of treatment success and good compliance is highest.

5. axSpA incurs high individual, medical and societal costs, all of which should be considered in its management by the treating rheumatologist

This is a new overarching principle, which has been taken from the EULAR recommendations on RA and PsA.[22 23] This overarching principle first points to the fact that there are high costs associated with the disease itself and with its treatment. This relates to the patient (individual costs) and can be seen as monetary costs, and also as burden of the disease. When assessing the financial burden for society, the direct medical costs as well as indirect costs due to work productivity loss should be taken into account. And when evaluating the cost-effectiveness of (potentially expensive) treatments, all these aspects should be considered.[53]

axSpA is a disease for which the treatment options rapidly increase. Some of them are very cheap; others are very expensive. When a choice between treatments has to be made in clinical practice, costs in its broadest sense are relevant factors. This should only be done taking 'best care' as worded in overarching principle number 4 into account. Consequently, only if the outcome for the patient is expected to be similar under either treatment, healthcare costs can drive the choice. This is an important principle in light of the fact that in many (western) countries, the pressure to reduce cost of healthcare through cuts on drug expenditure has increased significantly. Several task force members, including patients, expressed major concerns regarding this overarching principle, because of the historical—but currently untenable—premise that physicians should not be influenced by drug costs when making decisions, and because of the fear to be hindered in choosing the treatment that may provide 'best care'. Nevertheless, the vast majority (see LoA) of task force members were supportive of this principle after highlighting the fact that the principle of 'best care' (and that of shared decision-making) should always prevail. An appropriate example of the above-mentioned discussion could be the choice between a cheaper bsDMARD and a (likely) more expensive boDMARD. In this scenario, similar (efficacy and safety) outcomes can be reasonably expected, and the price of the drug may become a prevailing argument, provided that the patient is fully informed and agrees with this choice under the premise of 'shared decision-making'. Moreover, drug costs as well as costs of treatment can vary tremendously across countries, and between different regions within the same country (eg, due to price negotiations among payers). Therefore, it is strongly recommended to consider costs of treatment in the context of the local situation.

The task force is keen to point out that although no dedicated SLR on cost-effectiveness was performed, costs have been taken into account at all times during the development of these recommendations.

RECOMMENDATIONS

A total of 13 recommendations have been formulated (table 1). Two of these (#3, #11) are new from previous publications, one recommendation was split into two (old #9 into new #9, #10) and one recommendation has been deleted (old #4). The deleted recommendation dealt with the management of extra-articular manifestations and comorbidities. The task force decided that these aspects were already sufficiently covered by the overarching principles and by other recommendations. Compared with the 2010 recommendations, the new recommendations are far better formulated as recommendations. In hindsight, the 2010 recommendations represented in reality 'statements', which were based on findings of evidence in the literature and/or on expert opinion. The current recommendations are far more specific and prescribe what should be done in particular clinically relevant situations. These improvements reflect a general tendency of moving insight into recommendation development over the last decade. Moreover, LoE and GoR are now clearly added to each recommendation (table 1).

Recommendation 1

The treatment of patients with axSpA should be individualised according to the current signs and symptoms of the disease (axial, peripheral, extra-articular manifestations) and the patient characteristics including comorbidities and psychosocial factors

The content of the first recommendation is largely unchanged, and indicates the importance of personalised management in a disease with a very heterogeneous phenotype. All the factors mentioned in the body of the text may play a role in making decisions about aspects of management. It also points to the fact that group-level results of trials in patients with axSpA often suggest a certain level of homogeneity, but that individual patients with axSpA in clinical practice may deviate from this supposedly homogeneous pattern. Rheumatologists should take this principle of generalisability into consideration when treating patients with axSpA.

Recommendation 2

Disease monitoring of patients with axSpA should include patient-reported outcomes, clinical findings, laboratory tests and imaging, all with the appropriate instruments and relevant to the clinical presentation. The frequency of monitoring should be decided on an individual basis depending on symptoms, severity and treatment

Due to the heterogeneous presentation of the disease, monitoring should include a broad variety of assessments. In principle, the ASAS core set for monitoring in clinical practice is still guiding.[54] This includes questionnaires for levels of pain, disease activity (BASDAI) and physical function (Bath AS Functional Index), swollen joint counts, spinal mobility and assessment of extra-articular manifestations if appropriate.[54] Acute phase reactants now play a more prominent role in monitoring patients with axSpA than before. The ASDAS is a relatively new disease activity score, which combines patient-reported outcomes and C reactive protein (CRP) (or erythrocyte sedimentation rate) into one index.[55] It has been proven that there is a longitudinal relationship between ASDAS and subsequent syndesmophyte formation, while such a relationship between BASDAI (even if combined with CRP in the

model) and syndesmophytes was far weaker.[49] Although not (yet) included in the ASAS core set (which was defined before the development of the ASDAS), ASDAS seems a relevant measure to assess disease activity.

MRI is an imaging modality that can provide information on inflammation. Both MRI of the sacro-iliac (SI) joints and of the spine can be used for this purpose. In early disease, MRI of the SI joints may be most relevant, while in later stages especially the MRI of the spine may be informative.[28 56] However, the correlation between clinical disease activity measures and MRI inflammation is modest at best.[57–60] To date, the role (if any) of MRI in monitoring the disease remains unclear. Apart from the fact that the meaning of MRI inflammation in patients who have clinically inactive disease (they are free of symptoms) is unclear and that it is unknown if residual MRI inflammation can and should be treated, it is simply not feasible in most settings and far too expensive to repeat MRIs frequently. This explains why MRI is currently not recommended for monitoring. However, MRI can be used to define the level of present inflammation, and may add arguments to the global opinion to start or continue a particular treatment in a particular patient.

Radiographs of the SI joints are useless to monitor the disease course, but may be necessary to define if a patient is fulfilling criteria for a bDMARD start (see later). In contrast, radiographs of the spine provide important information about the presence of syndesmophytes, and about the prognosis of an individual patient, since evidences show that this is a risk factor for developing more syndesmophytes.[49 51] However, monitoring the disease by consecutive spinal radiographs is of limited value, because of the very slow rate of progression in the majority of patients. If applied, it should not be performed more frequently than once every 2 years.

Recommendation 3

Treatment should be guided according to a predefined treatment target

This is an important aspect of the treat-to-target concept and is newly added to the recommendations. For the first time in the history of SpA research, evidence has been accrued to suggest the value of 'targeting disease activity' because disease activity leads to new syndesmophytes in patients with axSpA.[49 51] As described in the overarching principles, a target should be a shared decision between patient and rheumatologist, taking all relevant situational factors into consideration. Treatment, once started, should be monitored in order to investigate if the target is reached. While amply discussed, the task force did not want to establish a preferred target (as has been done in RA and PsA). In principle, inactive disease is the ultimate goal, but depending on the phase of the disease and the treatments already used previously, it was felt that the required treatment for reaching this target (including its inherent risks) could imply an unrealistic goal. So after discussion it was decided that it is important to recommend that a target should be defined and documented, but refrain from mentioning the content of such target.

Recommendation 4

Patients should be educated about axSpA and encouraged to exercise on a regular basis and stop smoking; physical therapy should be considered

Education is an important aspect of management, it is essential for patients to make informed shared decisions and has been proven to be efficacious.[61–63] In axSpA, it is known that home exercises are efficacious and these are therefore recommended to patients.[64] However, physical therapy is proven to be more efficacious than home exercises.[64] Physiotherapy is certainly more expensive and less feasible than home exercises but may be required in some patients. Consequently, it is recommended that rheumatologists always consider if physical therapy could be beneficial for a particular patient. While quitting smoking likely has favourable health effects for every individual, it is of particular interest for patients with axSpA, since there is an established association between smoking and disease activity, inflammation on MRI and syndesmophyte formation.[65–67] In spite of these positive associations, to date there are no data showing a beneficial effect of smoking cessation on signs and symptoms of patients with axSpA.

Recommendation 5

Patients suffering from pain and stiffness should use an NSAID as first-line drug treatment up to the maximum dose, taking risks and benefits into account. For patients who respond well to NSAIDs continuous use is preferred if symptomatic otherwise

The most important aspect of this 2010 recommendation on the use of NSAIDs as first-line drug was maintained in the text of this recommendation. All task force members were still convinced of the virtues of NSAIDs administered in a full anti-inflammatory dosage. This can be based on the ASAS20 response of >70%, an ASAS40 response in >50% of the patients starting with an NSAID in early disease or 35% of patients in ASAS partial remission.[68] Important consideration however needs to be given to the potential side effects of NSAIDs, especially when administered chronically. NSAIDs should therefore only be prescribed if patients are symptomatic. If so, treatment should be advised to the maximum tolerated dose, continuously weighing the risks against the benefits. Moreover, while there is much discussion on the long-term safety of NSAIDs especially in relatively young patients, data from two studies have suggested that lack of exposure to NSAIDs is associated with an increase in mortality.[69 70] This argues against a major or important safety problem associated with the use of NSAIDs.

Given the risks of long-term NSAID use, the question about which patients require continuous NSAID treatment is valid: trial data have suggested that the continuous use of NSAIDs in patients with an elevated CRP results in reduced progression of structural damage in the spine in comparison to on-demand use only.[71 72] Similar results were found in a cohort study comparing high-dose and low-dose NSAID use.[73] However, a recent randomised trial did not confirm this effect, casting doubts on the potential structural effects of NSAIDs.[74] It was suggested during the task force discussions that the protective effects of NSAIDs may be specific for certain NSAIDs.[74] In the absence of equivocal evidence, it was finally decided to base a decision of continuous use of NSAIDs to the symptoms of the patient rather than on a possible protective effect regarding structural progression: if symptoms recur after stopping or dose reduction of an NSAID, continuous use should be advised. This was accepted by a two-third majority in the second round of voting. Whether continuous NSAID use may be beneficial in patients with risk factors for syndesmophyte progression (presence of syndesmophytes, elevated CRP, longstanding disease, spinal inflammation on MRI) remains a topic on the research agenda.[49 51 65 72 73 75–78]

Recommendation 6

Analgesics, such as paracetamol and opioid-(like) drugs, might be considered for residual pain after previously recommended treatments have failed, are contraindicated and/or poorly tolerated

This recommendation remained unchanged. It is formulated as a rather weak recommendation since formal evidence that

analgesics are efficacious in axSpA is lacking (not tested). Nevertheless, common sense justifies a statement that analgesics may relieve painful conditions, but only if positively recommended treatments for axSpA, including bDMARDs when indicated, have failed.

Recommendation 7

Glucocorticoid injections directed to the local site of musculoskeletal inflammation may be considered. Patients with axial disease should not receive long-term treatment with systemic glucocorticoids

This recommendation combines, as in the previous version, two means of glucocorticoid use: local and systemic. The formulation about the use of local injections is unchanged and indicates that the task force is still of the opinion that injections with glucocorticoids may be an option to treat arthritis and enthesitis, although direct evidence is lacking. The formulation about the use of systemic glucocorticoids has changed slightly. While systemic glucocorticoids were not specifically discouraged entirely in previous recommendations, positive data were also lacking. New data now have suggested that short-term high dose of glucocorticoids (50 mg/day) may have a very modest effect on signs and symptoms in patients with axial disease.[79] However, the task force still had the conviction that patients with axial disease should not be treated long-term with systemic glucocorticoids irrespective of the dose.

Recommendation 8

Patients with purely axial disease should normally not be treated with csDMARDs; sulfasalazine may be considered in patients with peripheral arthritis

Again this recommendation consists of two parts: the first part refers to patients with purely axial disease and the second part to patients with peripheral arthritis. The latter remained identical: sulfasalazine as a treatment option in patients with peripheral arthritis. The statement pertaining to patients with axial disease has been reworded into a real recommendation, while the previous version was rather a statement on the lack of efficacy of csDMARDs in patients with axSpA. There were no new studies on csDMARDs in axSpA. Already in the SLR informing the previous version of the recommendations, and on the basis of older studies, it had been shown that csDMARDs were not efficacious in axSpA.

The word 'normally' in the text of the recommendation created a lot of argument. Only in the third round of voting, 65% of the participants voted in favour of adding the word 'normally'. In principle, the task force was of the opinion that patients with purely axial disease should not be treated with csDMARDs. While there is evidence that sulfasalazine, MTX and leflunomide are not efficacious for axial symptoms, there may be exceptional situations in which there is no other pharmacological treatment option left for a particular patient for reasons of toxicity, contraindications or costs.[80–82] In such exceptional ('not normal') situations, a shared decision could be to try a csDMARD for a limited period of time. This policy violates the (ethical) principle of 'best care', knowing the low likelihood of treatment success, but not the principle of 'shared decision-making' since the patient should be fully informed about the low likelihood of treatment success and the likelihood of side effects, before the decision is made. This reasoning convinced the majority of the task force to accept the wording of the recommendation in such a manner that the use of csDMARDs in patients with purely axial disease can only exceptionally be defended.

Recommendation 9

bDMARDs should be considered in patients with persistently high disease activity despite conventional treatments (figure 1); current practice is to start with TNFi therapy

The previous recommendation 9 only included TNFi therapy, because no other class of bDMARDs was available. Moreover, the details about the use of TNFi therapy was discussed in the separate ASAS recommendations. Now both are integrated in the current recommendations. The first part of the recommendation remained essentially unchanged: bDMARDs (in general and not limited anymore to TNFi therapy) should be considered in patients with persistently high disease activity despite conventional treatments. These conventional treatments obviously include non-pharmacological management as well as NSAIDs. And in patients with (mainly) peripheral symptoms, 'conventional management' may also include a local glucocorticoid injection (if considered appropriate) and normally a treatment with sulfasalazine (in case of peripheral arthritis). This recommendation emphasises that a treatment 'should be considered' and the outcome of this process of consideration is dependent on an evaluation of the risks and benefits to be expected. As always, shared decision-making is key.

Figure 1 summarises the different requirements before a bDMARD could be started. The first step is the diagnosis of axSpA by a rheumatologist. Only formally fulfilling classification criteria (such as the ASAS axSpA criteria) does not suffice. A knowledgeable rheumatologist should make a diagnosis based on the full evaluation of all clinical, laboratory and imaging information, and should also exclude other potentially more likely diagnoses. While the large majority of these patients will also fulfil the ASAS axSpA criteria, the opposite is not necessarily true: solely checking and ticking boxes in order to test fulfilment of separate elements is inappropriate and obsolete.

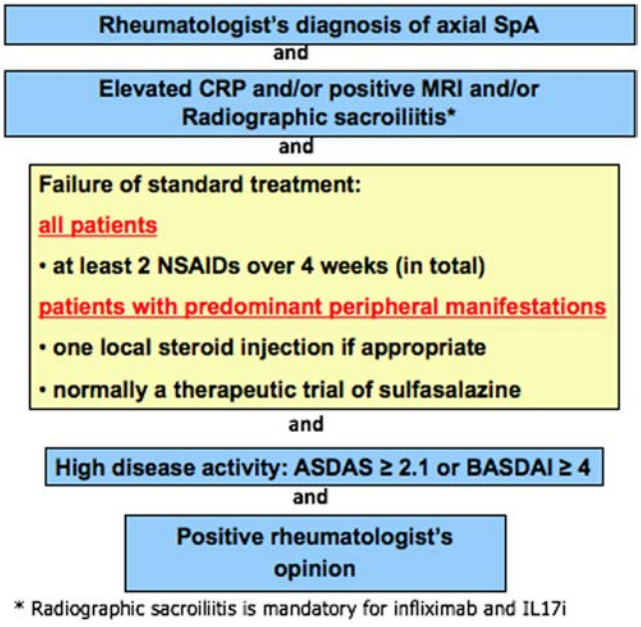

Figure 1 ASAS-EULAR recommendations for the treatment of patients with axSpA with bDMARDs. CRP, C-reactive protein; MRI, magnetic resonance imaging; NSAIDs, non-steroidal anti-inflammatory drugs; ASDAS, Ankylosing Spondylitis Disease Activity Score; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; IL17i, interleukin-17 inhibitor.

The next step is to judge if a patient fulfils 'labelling criteria': elevated CRP, the presence of inflammation on MRI of the SI joints and/or spine or the presence of radiographic sacroiliitis (defined according to the modified New York grading: at least grade 2 bilaterally or at least grade 3 unilaterally). The clarification of the content and order of this step is as follows:

TNFi therapy is approved in many countries for patients with radiographic axSpA (AS) without further limitations, and in patients with non-radiographic axSpA only if there is an elevated CRP and/or inflammation on MRI. This means that if a patient with axSpA has radiographic sacroiliitis or when this patient has either an elevated CRP or inflammation on MRI, the patient formally complies with the requirements for bDMARD therapy mentioned in the label of the respective drugs. While not brought up as a limitative factor, the task force was of the opinion that many studies have now suggested that also patients with radiographic axSpA who have an increased CRP have the highest likelihood of treatment success.[83 84] In addition, recent observational studies, as well as re-evaluations of clinical trials, have cast doubts on the reliability of the finding of radiographic sacroiliitis by (untrained) single evaluators.[6 7] Elaborating on this principle, one may argue that—albeit formally justifiable—a sole finding of radiographic sacroiliitis in a patient without further indication of objective disease activity may be too meagre to justify proper bDMARD treatment in the spirit of 'best possible care' as defined in overarching principle number 4. Therefore, the task force decided to start with 'elevated CRP' as being the strongest predictor of a good response to TNFi therapy, both in patients with radiographic axSpA and non-radiographic axSpA.[15 85] In addition, inflammation on MRI appeared to be second-best predictor of response to TNFi therapy, again irrespective of the presence of radiographic sacroiliitis.[13 15 17] The task force hopes that rheumatologists will take CRP and (when available) MRI into consideration when deciding about the appropriateness of starting a bDMARD, irrespective of whether radiographic sacroiliitis is present or not.[13 15 17 85] Radiographic sacroiliitis is not a predictor of response: a study stratified on radiographic sacroiliitis has shown that patients with radiographic and non-radiographic sacroiliitis have similar response rates.[28] But there is one proviso here: while figure 1 pertains to treatment with bDMARDs, currently the use of IL-17i therapy and of infliximab in patients with non-radiographic axSpA is not approved by the agencies and therefore for IL-17i therapy and infliximab radiographic sacroiliitis is mandatory.

Step 3 refers to the failure of standard treatment as explained above. A treatment with sulfasalazine should be evaluated after 3 months of treatment reaching a dose of 3 g/day if tolerated. This is different in comparison to the 2010 ASAS recommendations, as in those recommendations MTX was also advocated as a possible treatment for patients with peripheral symptoms. As there are no data proving the efficacy of MTX and there are with regard to sulfasalazine, this was changed back to sulfasalazine in accordance with earlier recommendations.[20 21]

Step 4 is to define the level of disease activity. Historically, active disease has been defined by a BASDAI level of at least 4. But ASDAS is a better index than BASDAI (see below), and active disease can also be defined by ASDAS of at least 2.1.[86] ASDAS is placed first, as it is the preferred measure. This decision was based on data from the SLR of the fellows and on expert opinion (see online supplementary material). The BASDAI is a fully patient-reported outcome, while the ASDAS is a combination of patient-reported outcomes and CRP. BASDAI and physicians' opinion on disease activity only correlate weakly, while ASDAS correlates far better with both patients' and physicians' level of disease activity.[55 87] Another argument is that increased ASDAS may lead to syndesmophyte formation, while this has not been proven for BASDAI alone (BASDAI works only if combined with CRP).[49] Moreover, a high BASDAI appeared to be a predictor for stopping TNFi therapy, while a high ASDAS was a predictor for continuation of TNFi, which can be seen as a surrogate outcome for efficacy.[83] Frequently, there is concordance between a BASDAI ≥4 and ASDAS ≥2.1, but in discordant cases an elevated ASDAS was more predictive of a good response than an elevated BASDAI.[88 89] Finally, the ASDAS cut-offs for disease activity states and response criteria were based on a thorough validation process, while the BASDAI cut-offs were arbitrarily chosen.[86]

In addition to the level of high disease activity, the rheumatologist should be convinced that in a particular patient there is a favourable benefit/risk profile before a treatment with a bDMARD is started. In order to construct this profile intuitively, the rheumatologist can take 'positive factors' such as inflammation on MRI, into consideration, but should also weigh potential contraindications such as risk for side effects, or compliance. Ultimately, only a shared decision between patient and rheumatologist will result in the start of a bDMARD.

The second part of recommendation 9 refers to 'current practice', in which it is normal to start with TNFi therapy. TNFis registered for the indication of axSpA are (in alphabetical order) adalimumab, certolizumab pegol, etanercept, golimumab and infliximab. The wording for this recommendation was borrowed from previous EULAR recommendations for RA at the time that TNFis were already on the market for a long time; there was extensive experience with the use of TNFi; TNFi were also used in clinical practice in a wide variety of patients; registry data suggested positive long-term safety.[22] This is exactly how the situation is in axSpA in 2016. For the first time, there is a different class of bDMARDs on the market with a different mode of action: an IL-17 pathway inhibition. Currently, only secukinumab is approved, but several other agents are far in their development. To date, only trial data on IL-17i in radiographic axSpA are available and data in patients with non-radiographic axSpA are still lacking. So it is obvious that the body of experience with TNFi in axSpA on efficacy, safety and variety of indications greatly outweighs that with IL-17 pathway inhibition, both in terms of volume and time of follow-up. This is why the task force has decided to recommend TNFi as the first bDMARD, use the wording 'current practice' to justify that choice and implicitly give endorsement to this practice. Moreover, the use of IL-17i therapy should be avoided in patients with active IBD, as secukinumab in comparison to placebo was not efficacious in Crohn's disease and resulted in more adverse events.[90] Secukinumab has proven efficacy for the treatment of psoriasis.[91] Apart from IL-17i therapy, there is no other non-TNFi bDMARD on the market. Various IL-6is have been tried in well-designed trials but were proven not efficacious.

Several TNFis have been approved for axSpA. All, except infliximab, have indications for both radiographic and non-radiographic axSpA. Their efficacy with regard to musculoskeletal signs and symptoms seems very comparable, although no head-to-head comparisons are available. However, there seems to be a difference in efficacy with regard to extra-articular manifestations. Monoclonal antibodies (infliximab, adalimumab, certolizumab, golimumab) are efficacious in the treatment of IBD and in preventing the recurrence of uveitis (no data on golimumab) and, whereas etanercept has shown contradictory results

for uveitis and no efficacy in IBD.[92–101] Etanercept seems to be less efficacious for psoriatic skin involvement than other TNFi, although no head-to-head comparisons are available.[23]

In this entire document, we refer to both boDMARDs as well as bsDMARDs when we mention TNFi therapy. The price of a bDMARD should be taken into account when choosing a particular drug. The choice is very much dependent on local situations, and general recommendations cannot be made, but given the similar expected safety and efficacy with regard to alleviating musculoskeletal symptoms, cost is potentially an important consideration in making a choice between a boDMARD and a bsDMARD. In many countries and regions within countries, this choice is increasingly determined by payers based on cost considerations rather than by individual rheumatologists and their patients.

Finally, figure 2 clarifies when and how should efficacy of bDMARDs be evaluated and in which circumstances it is recommended to continue. First, the wording has changed from 'stopping' a bDMARD in the previous versions of the ASAS recommendations to 'continuation' in the current recommendations. The response should be defined by the same outcome used to initiate: either ASDAS or BASDAI. For ASDAS, a clinically important improvement of ≥1.1 is required, while this is ≥2.0 for BASDAI. Importantly, such an evaluation should coincide with the positive opinion from the rheumatologist, who will take all potential risks and benefits into consideration, before deciding together with the patient whether treatment with a bDMARD should be continued.

Recommendation 10

If TNFi therapy fails, switching to another TNFi or an anti-IL-17 therapy should be considered

With the advent of a second class of bDMARDs available, there is a potential choice after failure of TNFi therapy. Data suggest that a second TNFi (after failure of the first TNFi) can still be efficacious, although the level of efficacy may be lower than with the first TNFi.[102] IL-17i therapy has proven efficacy in patients who had failed a TNFi but this was also less than in TNFi-naïve patients.[26 27] In patients with a primary non-response to the first TNFi, it may be more rational to switch to another class of drugs, that is, an IL-17i. However, before doing so, it is important to reconsider if the indication for the start of the first TNFi was indeed correct. Rather than drug failure, primary failure can also be the consequence of an incorrect diagnosis, in which no clinical efficacy can be expected. The task force was of the opinion that true primary failure is an infrequent observation in correctly diagnosed patients with axSpA with active disease.

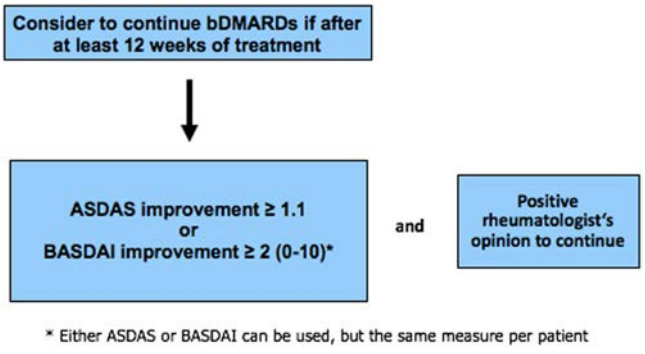

Figure 2 ASAS-EULAR recommendations for the continuation of bDMARDs. ASDAS, Ankylosing Spondylitis Disease Activity Score; axSpA, axial spondyloarthritis; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; bDMARD, biological disease modifying antirheumatic drug.

Toxicity to a TNFi may also be a reason to switch directly to an IL-17i. Data proving whether a TNFi is efficacious in patients who have failed IL-17i therapy are still lacking. Therefore, evidence-based guidance cannot be provided, but the task force felt it is reasonable to assume that a TNFi in this situation makes sense. It is important to formally investigate the efficacy of a TNFi after failure of an IL-17i (research agenda).

Figure 3 summarises all the various phases of treatment in a graphical representation.

Recommendation 11

If a patient is in sustained remission, tapering of a bDMARD can be considered

This recommendation is a completely new one. Since the SLRs in 2009 new data have become available that suggest the possibility of successful tapering of bDMARDs and acceptable efficacy after restart.[103 104] However, complete discontinuation of bDMARDs seems to lead to a high percentage of patients that experience flares.[105 106] Given the high costs of long-term bDMARD use, it is considered appropriate to slowly taper bDMARDs in patients who are in sustained remission. Although remission is not defined here, ASDAS inactive disease is a clinical remission-like definition, which could be used. Currently, it is unclear what the definition of 'sustained' should be, but the task force was of the opinion that this should be at least 6 months, possibly longer. Data should be collected that provide insight on predictors of a flare after tapering treatment. It is, for instance, important to know if residual inflammation on MRI may predict a flare or if there is an association between the length of time in remission and likelihood of flare. In principle, tapering can be done by either dose reduction or increasing the interval ('spacing'). Again it is unclear if one method is better than the other, but 'spacing' seems to be the most practical approach. Although tapering can theoretically be continued until zero (discontinuation), it is recommended to do this only very slowly and assuring a sufficient period of time remaining in remission after the previous step of tapering. Shared decision-making is pivotal in tapering. This opinion was specifically expressed by the patients since they fear that the need for cost reduction will outweigh principles of 'best care' as the most important driving factor. Needless to say that—for the quality of life of patients with axSpA—principles of 'best care' and 'shared decision-making' should outweigh cost considerations, but the latter remain significant.

Recommendation 12

Total hip arthroplasty should be considered in patients with refractory pain or disability and radiographic evidence of structural damage, independent of age; spinal corrective osteotomy in specialised centres may be considered in patients with severe disabling deformity

The old recommendation on surgery consisted of the above aspects on total hip arthroplasty and corrective osteotomy, which remained unchanged for the current recommendation. However, a third item, referring to the consultation of a spinal surgeon in case of an acute vertebral fracture, was deleted. It was broadly felt that this item is already sufficiently covered by the last recommendation. Hip involvement is a frequent problem in patients with axSpA.[107] In case of symptoms and a compatible radiograph with destruction, patients at any age should be considered candidates for a total hip arthroplasty. Especially in young patients, cementless prostheses are preferred. Corrective spine osteotomy

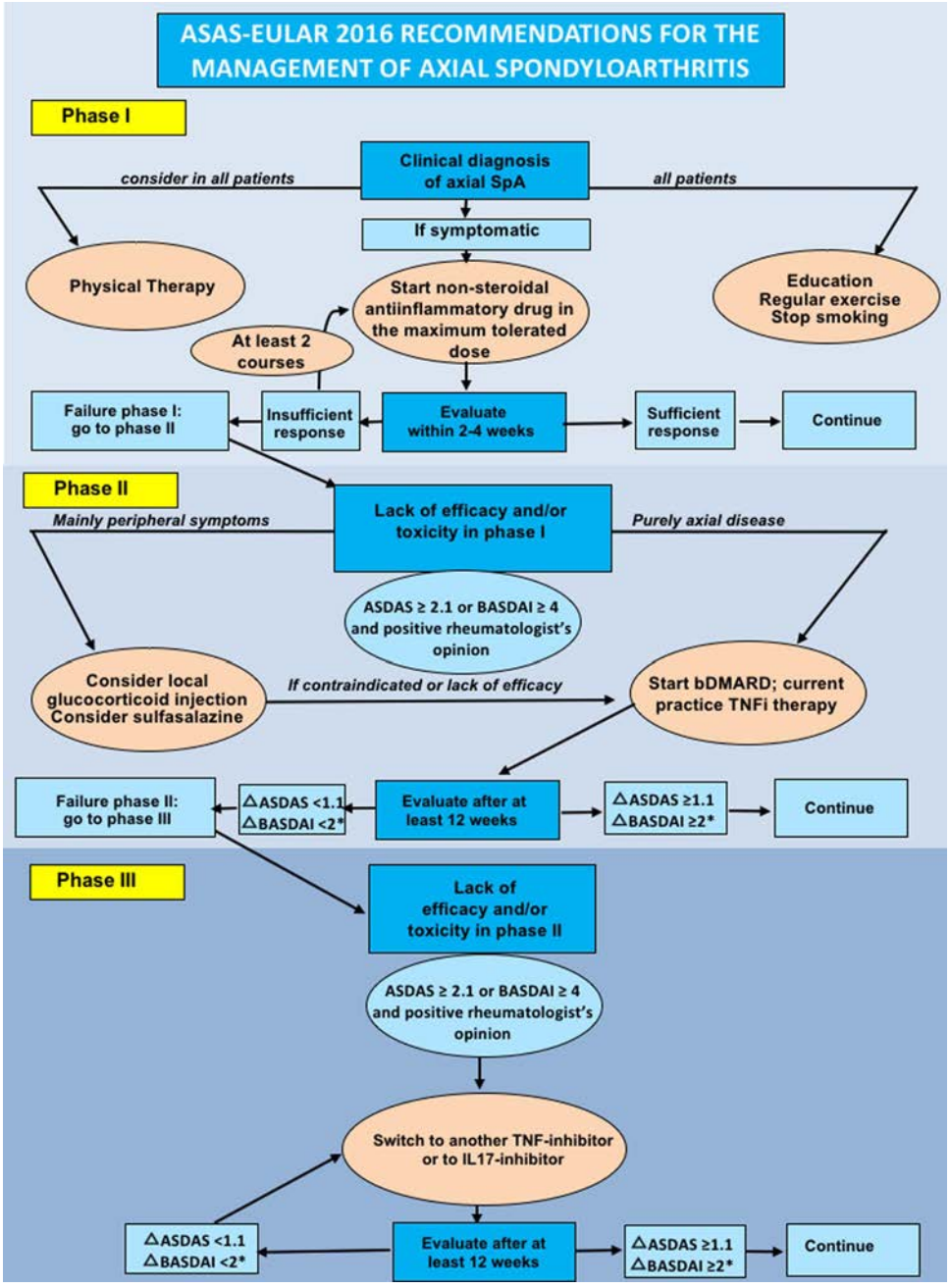

Figure 3 Algorithm based on the ASAS-EULAR recommendations for the management of axial spondyloarthritis. ASDAS, Ankylosing Spondylitis Disease Activity Score; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; bDMARD, biological disease-modifying antirheumatic drug; TNFi, tumor necrosis factor inhibitor; IL17-inhibitor, interleukin-17 inhibitor. *Either BASDAI or ASDAS, but the same outcome per patient.

is available only in specialised centres, and patients with severe deformities could consult a specialised spinal surgeon to discuss risks and benefits of this procedure.[108]

Recommendation 13

If a significant change in the course of the disease occurs, causes other than inflammation, such as a spinal fracture, should be considered and appropriate evaluation, including imaging, should be performed

The final recommendation was kept unchanged. Frequently, axial symptoms in patients with axSpA are caused by inflammation, but other causes should always be considered. This is especially important if a patient is not responding to pharmacological treatment and if there is a major, frequently sudden, change in the course of the disease. In this case, a spinal fracture should be suspected, since these are more prevalent than often expected.[109] They may occur with neurological symptoms but most frequently are without neurological symptoms and can even occur without preceding trauma. In case of suspicion, proper imaging such as MRI and/or CT scanning should be performed, and an experienced spinal surgeon may need to be consulted.[110]

DISCUSSION

The 2010 ASAS-EULAR recommendations on the management of AS and the 2010 ASAS recommendation on the use of TNFi in axSpA have been updated and aggregated into one set of management recommendations intended for patients with axSpA. The integrated set is more 'user friendly' and clearer to users than two separate sets. There are two major novelties: (1)

unlike the previous sets, these recommendations apply to patients with radiographic axSpA (AS) and to all patients with axSpA, irrespective of the presence of radiographic sacroiliitis; (2) these recommendations include a new class of bDMARDs, IL-17 pathway inhibiting therapy, which recently has become available for the treatment of patients with (radiographic) axSpA. Both aspects are integrated into figure 1 explaining requirements to start a bDMARD. As a first step, there is emphasis on the fact that a proper diagnosis is key, that such a diagnosis should be made by an expert rheumatologist and that classification criteria do not suffice to make a diagnosis. On the contrary, a proper diagnosis of axSpA includes a credible pattern of axSpA and exclusion of more likely diagnoses.

Thereafter, the various aspects that are mentioned in the labelling of bDMARDs are combined. All TNFis except infliximab have been approved for the treatment of patients with AS (radiographic sacroiliitis) and for patients with non-radiographic axSpA. But in this latter group, the presence of an elevated CRP or inflammation on MRI is mandatory. By combining this into one step as a requirement in addition to a diagnosis of axSpA, we have integrated two separate lines of drug registration (bDMARDs for AS and bDMARDs for non-radiographic axSpA) into one workable definition with profound predictive validity: while increased CRP is formally not required to indicate a patient with AS for a treatment with a bDMARD, ample evidence suggests that elevated CRP (and to a lesser extent: inflammation on MRI) predisposes to clinical efficacy, both in radiographic and non-radiographic axSpA.

It may even be questioned if patients with radiographic sacroiliitis only (without syndesmophytes), normal CRP and no inflammation on MRI are good candidates for bDMARD therapy. Given the lack of reliability of assessing SI joints for radiographic sacroillitis, misdiagnosis could be an important aspect in this group of patients and more information on the efficacy of bDMARDs in these patients is warranted.[6 7]

It needs to be stressed that this formulation formally does not apply to IL-17i therapy, which has been approved for axSpA with radiographic sacroiliitis only.[26 27]

Another new aspect is the use of ASDAS to assess the level of disease activity, the response to bDMARDs and the decision on continuation of the bDMARD. Taking several aspects as discussed into account, the ASDAS is likely to be the preferred assessment. Although the task force has decided to include a treat-to-target principle and has formulated one recommendation on the definition of a target, it was considered too early to give a recommendation on the content of the target. A task force that is updating the current treat-to-target recommendations for SpA will further work on this aspect.

Although a lot of attention is paid to the use of bDMARDs, it is important to stress that non-pharmacological management remains an important aspect of management in patients with axSpA. This applies to all phases of the disease, and is irrespective of the pharmacological treatment. In addition, NSAIDs continue to be the first-line drug in axSpA.

For the first time, cost considerations received a prominent place in the axSpA recommendations. The task force considers this an important aspect, given the extreme drug costs for individual patients and society, and feels a responsibility to help minimising total health care expenditures for patients with axSpA. However, here lies also a clear responsibility for the pharmaceutical industry.[111] But it is clearly stated in this document that this should not go at the cost of access to 'best possible care'. In case of similar efficacy and safety, the cheapest treatment option can be chosen. Tapering of a bDMARD is also recommended as an option, but again under the condition of maximising health-related quality of life.

For an easier understanding and presentation, the recommendations are presented in table 1 and figures 1–3. However, we like to underline these cannot be read and interpreted without the accompanying text. Furthermore, the text of the current manuscript cannot be well understood without the accompanying SLRs, which form an integrated whole (Sepriano *et al*, 2016, submitted for publication; Regel *et al*, 2016, submitted for publication). Even the SLRs of the previous recommendations need to be consulted in order to be informed about the complete body of evidence published in the literature.[34–36] The SLRs also give information on the quality of the publications, for example, by presenting the risk of bias estimates.

The American College of Rheumatology (ACR) and SpondyloArthritis Research & Treatment Network (SPARTAN) have published recommendations for the treatment of AS and non-radiographic axSpA in 2015.[112] While these have been developed according to the Grading of Recommendations Assessment, Development and Evaluation (GRADE) methodology, and our recommendations have applied the Oxford LoE to assess the evidence of the literature, the overall recommendations are very similar. Differences are mainly in those areas where strong evidence is lacking (eg, corrective osteotomy, injections with glucocortiocids). The presentation, although, is fundamentally different. The ACR-SPARTAN recommendations are grouped for various stages and presentations of the disease (eg, patients with AS with active disease, with stable disease, with various extra-articular conditions), while the ASAS-EULAR recommendations are more condensed and integrated. The ACR-SPARTAN set of recommendations comprises 38 separate recommendations and the ASAS-EULAR set comprises 13 recommendations. A few of the unique aspects of the ASAS-EULAR recommendations are: treatment according to a target, the explicit conditions in which a bDMARD should be started, tapering of a bDMARD, the use of IL-17i, taking aspects of costs into account and treating axSpA as one continuum of the disease.

The 2016 ASAS-EULAR recommendations for the management of axSpA provide in a single set of recommendations guidance for the management of patients from the whole spectrum of the disease, including radiographic and non-radiographic axSpA, and address the whole disease management, including non-pharmacological and pharmacological treatment. While this aspect can be seen as a facilitator of these recommendations, a potential barrier is that it implies acceptance of the concept of axSpA. There are clear signs confirming that this is the world-spread movement, but still some challenges remain. Efforts shall be made towards the implementation of these recommendations, namely through dissemination across national societies, websites and presentations made in congresses, as well as in educational sessions to physicians. Both ASAS and EULAR will lead these efforts, and support implementation efforts at a national level, preferably involving all the stakeholders, namely patient groups, national rheumatologist societies and policy makers.

This was the first update since 2010 and this relatively long period could be explained by an absence of new treatment options until recently. The next update will be undertaken when there are sufficient new data on existing treatments or when data on new treatment options will become available. Until then, we hope that the current recommendations will be useful for healthcare professionals taking care of patients with axSpA, for patients themselves, for the pharmaceutical industry and for payers.

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EXTENDED REPORT

Short-term changes on MRI predict long-term changes on radiography in rheumatoid arthritis: an analysis by an OMERACT Task Force of pooled data from four randomised controlled trials

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ABSTRACT

Objective In rheumatoid arthritis (RA), MRI provides earlier detection of structural damage than radiography (X-ray) and more sensitive detection of intra-articular inflammation than clinical examination. This analysis was designed to evaluate the ability of early MRI findings to predict subsequent structural damage by X-ray.

Methods Pooled data from four randomised controlled trials (RCTs) involving 1022 RA hands and wrists in early and established RA were analysed. X-rays were scored using van der Heijde-modified or Genant-modified Sharp methods. MRIs were scored using Outcome Measures in Rheumatology (OMERACT) RA MRI Score (RAMRIS). Data were analysed at the patient level using multivariable logistic regression and receiver operating characteristic curve analyses.

Results Progression of MRI erosion scores at Weeks 12 and 24 predicted progression of X-ray erosions at Weeks 24 and 52, with areas under the curve (AUCs) of 0.64 and 0.74, respectively. 12-week and 24-week changes in MRI osteitis scores were similarly predictive of 24-week and 52-week X-ray erosion progressions; pooled AUCs were 0.78 and 0.77, respectively. MRI changes in synovitis at Weeks 12 and 24 also predicted progression of X-ray joint damage (erosion and joint-space narrowing) at Weeks 24 and 52 (AUCs=0.72 and 0.65, respectively).

Conclusions Early changes in joint damage and inflammation detected with MRI predict changes in joint damage evident on subsequent X-rays. These findings support the use of MRI as a valid method for monitoring structural damage in short-duration RCTs.

INTRODUCTION

Radiography has been the standard for assessing structural damage in rheumatoid arthritis (RA) randomised controlled trials (RCTs) for many years. Recently, however, discriminating differences in the rates of progression of X-ray damage between treatment arms has become more challenging. The most important reason for this has been recognition that exposing subjects with active RA to placebo for longer than 12 weeks is unethical.[1] Accordingly, current US Food and Drug Administration (FDA) guidance states that trials of >12 weeks should include an active comparator as the control or make provisions for rescue therapy.[2] This poses a major obstacle to using X-ray in RCTs, because 24 weeks is typically necessary for radiographic demonstration of inhibition of structural progression, and longer treatment duration and larger numbers are necessary to resolve differences between active comparators. A method that more reliably detects structural progression within a 3-month time frame would therefore be helpful.

MRI has demonstrated criterion validity for osteitis and synovitis with histology and construct validity for erosions when compared with CT.[3 4] Numerous studies have demonstrated MRI to be more sensitive than X-ray in detecting joint damage and to detect synovitis and osteitis more sensitively than clinical examination does. Consequently, there has been a rapid increase in the use of MRI in RA RCTs over the past decade.[4] A recent report by the imaging subcommittee of the American College of Rheumatology (ACR) Clinical Trials Task Force[5] concluded that MRI met the Outcome Measures in Rheumatology (OMERACT) validation filter for 'truth, discrimination and feasibility'.[6 7] It concluded that 'among all of the currently available imaging modalities that have been validated with supportive data, MRI best serves the purpose of achieving sensitive ascertainment of structural damage in RCTs while also providing objective measures of inflammatory predictors of damage'. The report proposed analysing recently completed RCTs that included both MRI and X-ray assessments to evaluate the predictive validity of MRI.

Accordingly, under the auspices of OMERACT, a task force of the members of the imaging subcommittee of the ACR Clinical Trials Task Force obtained and pooled data from four RCTs that included both serial MRIs (baseline to 12 and/or 24 weeks) and X-rays (baseline to 24 and/or 52 weeks) to evaluate the ability of MRI to predict long-term structural damage on X-rays at the individual patient level using a statistical meta-analysis approach. The overall prediction performance for the patient population was evaluated by receiver operating characteristic (ROC) analysis.

METHODS

Data from four placebo RCTs (table 1) in patients with active RA were included, in which 1022 hands

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Table 1 Imaging schedule for included trials

Trial	Baseline	Week 12	Week 24	Week 52	Rescue treatment information
A	MRI, X-ray	MRI	MRI, X-ray	N/A	Available
B	MRI, X-ray	MRI	MRI, X-ray	MRI, X-ray	N/A
C	MRI, X-ray	MRI	MRI, X-ray	MRI, X-ray	N/A
D	MRI, X-ray	N/A	MRI*, X-ray	X-ray	Available

*Only erosion scores available.
N/A, not available.

and wrists had both MRI and X-ray erosion scores at baseline. Information included RCT design, MRI protocols and baseline MRI and X-ray scores. Individual patient identification, study identification and treatment assignments remained blinded. To maintain confidentiality, the RCTs are referred to as Trials A–D. Measurement schedules are summarised in table 1. Additional methodological details are provided in the online supplementary material. Multivariable logistic regression analysis coupled with a non-parametric spline method was performed to assess the ability of

1. baseline MRI erosion scores and changes from baseline to Weeks 12 or 24 to predict X-ray progression (increase >0.5 in X-ray erosion scores from baseline to Weeks 24 or 52),
2. baseline MRI osteitis scores and changes from baseline to Weeks 12 or 24 to predict X-ray progression (increase >0.5 in X-ray erosion scores from baseline to Weeks 24 or 52),
3. Baseline MRI synovitis scores and changes from baseline to Weeks 12 or 24 to predict X-ray progression (increases >0.5 in X-ray total modified Sharp scores from baseline to Weeks 24 or 52).

Specifically, the regression included three dummy variables indicating the four studies and five basis functions, MRI_0, Δ_{MRI}, Δ^2_{MRI}, $(\Delta_{MRI}-0.5)_+$, $(\Delta_{MRI})_+$ and $(\Delta_{MRI}+0.5)_+$, as independent variables, where MRI_0 and Δ_{MRI} were the baseline MRI measure and short-term change in MRI measure (erosion, osteitis or synovitis score), respectively, and x_+ represented the positive part of x. The dummy variables representing four RCTs accounted for different progression rates among patients enrolled in each trial. The association between baseline and short-term changes in MRI and longer-term X-ray progression was characterised by the estimated linear combination of the aforementioned basis functions. The ROC curves of the estimated linear combination and area under the curve (AUC) measurements were derived to determine the discriminative power of early changes in MRI endpoints for detecting subsequent structural progression by X-ray (AUC 0.5–0.7=poor, 0.7–0.8=acceptable, 0.8–0.9=excellent, >0.9=outstanding discrimination[5]). All statistical analyses were performed using R-3.2.2 (The R foundation of Statistical Computing).

RESULTS

Table 2 shows baseline X-ray and MRI scores of included patients from the four trials. The association between 12-week change in MRI erosion score and 24-week change in X-ray erosion score was examined in Trials A, B and C; Week 12 MRI data were not available for Trial D. After excluding patients with missing information, the proportions of patients with X-ray erosion progression >0.5 Sharp units at Week 24 in Trials A, B, C and the pooled cohort were 5.7% (10/166), 7.5% (69/855), 4.0% (22/534) and 6.1% (101/1555), respectively. ROC curve analysis of the prediction of X-ray progression at Week 24 based on a logistic regression model of baseline MRI erosion score and 12-week MRI progression in erosion score showed an AUC of 0.64 (95% CIs 0.54 to 0.75) (figure 1). Since we were interested in the predictive value of MRI beyond that due to varying progression rates across trials, we also performed a logistic regression with trial indicators as the only independent variables, and the AUC for this was only 0.51 (95% CI 0.41 to 0.62). Adjusted for trial indicators, the predictiveness of 12-week MRI changes combined with baseline MRI erosion scores was statistically significantly greater than that using the trial indicator alone (p=0.031). The results by trial are shown in table 3.

The association between 24-week change in MRI erosion score and 52-week change in X-ray erosion score was examined using data from Trials B, C and D, as Trial A did not include Week 52 X-ray data (table 1). The proportions of patients with X-ray erosion progression at Week 52 were 9.0% (79/799), 4.3% (22/494), 7.8% (31/364) and 7.4% (132/1657) in Trials B, C, D and the pooled cohort, respectively. The AUC for predicting X-ray erosion progression at Week 52 based on MRI erosion scores at baseline and change at Week 24 was 0.74 (95% CI 0.66 to 0.82) (figure 1), which is considered acceptable.[5] If the logistic regression model considered only the trial as a variable, the AUC was poor (0.55; 95% CI 0.48 to 0.62). Adjusted for the trials, the predictiveness of 24-week change combined with baseline MRI erosion scores was highly statistically significantly greater than that using the trial indicator alone (p<0.001). The results by trial are shown in table 3.

Table 2 Baseline X-ray and MRI scores

Trial	X-ray Erosion	X-ray Total	MRI Erosion	MRI Osteitis	MRI Synovitis
A (n=185)	3.25 (3.68), 2.00 (0.75, 4.00)	5.36 (6.52), 3.00 (1.00, 7.00)	13.63 (12.44), 10.00 (4.50, 20.00)	7.23 (8.06), 4.50 (1.00, 10.50)	7.82 (4.60), 7.00 (4.50, 11.00)
B (n=1272)	3.50 (6.29), 1.00 (0.50, 3.50)	6.52 (12.40), 1.50 (0.50, 6.00)	22.17 (22.96), 14.50 (10.50, 23.50)	10.02 (11.21), 6.00 (2.50, 13.50)	10.14 (6.80), 9.00 (5.00, 13.50)
C (n=888)	5.44 (8.97), 1.50 (0.00, 7.00)	12.06 (18.07), 3.00 (0.50, 16.50)	23.50 (24.71), 14.75 (6.88, 30.12)	4.98 (7.54), 2.00 (0.00, 6.63)	7.15 (5.26), 6.50 (3.50, 9.50)
D (n=450)	5.90 (7.07), 3.50 (1.00, 8.50)	12.42 (15.19), 6.50 (1.50, 18.50)	18.72 (18.17), 12.50 (5.25, 26.31)	N/A	N/A
Pooled (n=2795)	4.47 (7.17), 1.50 (0.50, 3.25)	9.05 (14.62), 2.50 (0.50, 11.00)	19.42 (20.03), 13.12 (6.50, 25.00)	7.76 (9.59), 4.25 (1.00, 11.00)	8.59 (5.92), 7.50 (4.50, 11.50)

Values are mean (SD), median (upper, lower quartiles).
n, all hands, including those with missing measurements at baseline or follow-up; N/A, not available.

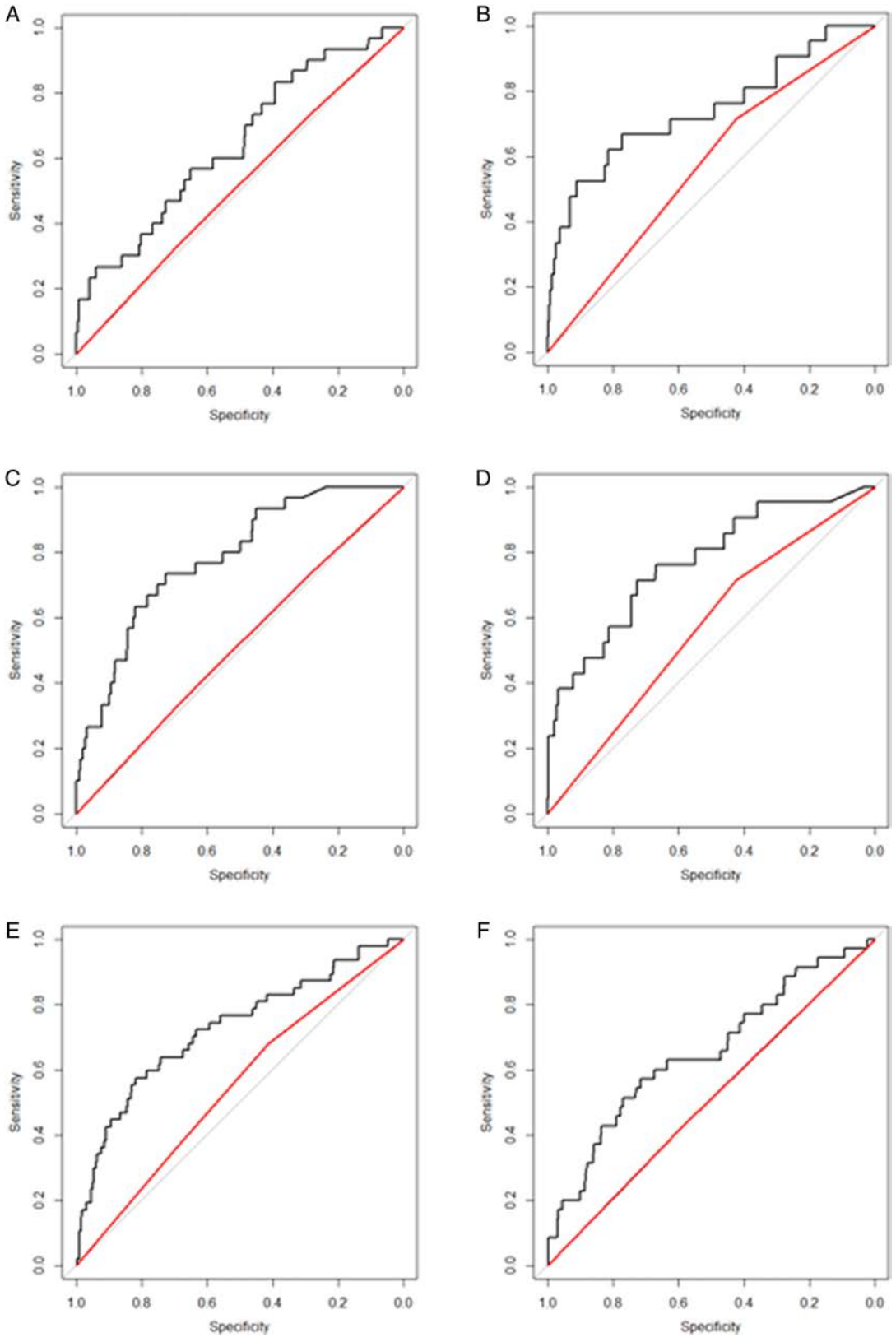

Figure 1 Receiver operating characteristic curves for predicting 24-week (A, C and E) and 52-week (B, D and F) changes in X-ray erosion (A–D) or total modified Sharp (E and F) scores using 12-week (A, C and E) or 24-week (B, D and F) MRI changes in erosion (A and B), osteitis (C and D) and synovitis (E and F) scores based on pooled trial data. Red line: only trial information; black line: trial and MRI information (baseline scores and 12-week or 24-week change scores); grey line: theoretical absence of discrimination.

Table 3 AUC (95% CI) values based on ROC curve analysis for individual trials

	Trial A	Trial B	Trial C	Trial D
12-week MRI erosion versus 24-week X-ray erosion	0.60 (0.44 to 0.77) n=169 hands	0.67 (0.46 to 0.88) n=218 hands	0.65 (0.51 to 0.78) n=153 hands	N/A
24-week MRI erosion versus 52-week X-ray erosion	0.77 (0.62 to 0.93) n=208 hands	N/A	0.70 (0.44 to 0.95) n=148 hands	0.73 (0.62 to 0.85) n=387 hands
12-week MRI osteitis versus 24-week X-ray erosion	0.78 (0.63 to 0.93) n=169 hands	0.82 (0.71 to 0.94) n=218 hands	0.51 (0.24 to 0.78) n=153 hands	N/A
24-week MRI osteitis versus 52-week X-ray erosion	N/A	0.77 (0.64 to 0.90) n=208 hands	0.67 (0.38 to 0.96) n=148 hands	N/A
12-week MRI synovitis versus 24-week X-ray total	0.70 (0.56 to 0.84) n=169 hands	0.69 (0.54 to 0.84) n=218 hands	0.76 (0.65 to 0.88) n=153 hands	N/A
24-week MRI synovitis versus 52-week X-ray total	N/A	0.66 (0.52 to 0.80) n=208 hands	0.65 (0.50 to 0.79) n=148 hands	N/A

AUC, area under the curve; N/A, not available; ROC, receiver operating characteristic.

The association between 12-week change in MRI osteitis score and 24-week change in X-ray erosion score was examined in Trials A, B and C; Trial D did not include osteitis scores. ROC analysis of the prediction of X-ray erosion progression at Week 24 based on 12-week MRI progression in osteitis showed a near excellent AUC of 0.78 (95% CI 0.70 to 0.86) (figure 1). As a reference, if only trial indicators were included as the predictors, the AUC of the logistic regression was very poor (0.51; 95% CI 0.41 to 0.62) suggesting that trial indicators alone are not predictive of X-ray erosion progression. Adjusted for the trials, the predictiveness of 12-week change and baseline MRI osteitis scores was highly statistically significantly greater than that using the trial indicator alone ($p<0.001$). The association between 24-week change in MRI osteitis score and 52-week change in X-ray erosion score was examined in Trials B and C; Trial A lacked Week 52 X-ray data and Trial D lacked osteitis scores. The AUC for predicting X-ray erosion progression based on MRI osteitis scores at baseline and change at Week 24 was also near excellent (0.77; 95% CI0 .66 to 0.88) (figure 1) and again significantly greater ($p<0.001$) than that observed if only the trial indicator was considered in the regression model (0.57; 95% CI 0.47 to 0.67).

The association between 12-week change in MRI synovitis score and 24-week change in X-ray total modified Sharp score was examined in Trials A, B and C, as Trial D did not include synovitis scores. At Week 24, 9.7% (17/159), 9.7% (90/834), 8.6% (48/508) and 9.4% (155/1501) of hands demonstrated X-ray progression in Trials A, B, C and the combined cohort, respectively. The AUC for predicting X-ray progression by MRI was acceptable (0.72; 95% CI 0.64 to 0.81) (figure 1) and significantly greater ($p<0.001$) than that observed if only the trial was considered (0.55; 95% CI 0.47 to 0.63).

The association between 24-week change in MRI synovitis score and 52-week change in X-ray total modified Sharp score was examined in Trials B and C; Trial A did not include Week 52 X-ray data and Trial D did not include synovitis scores. At Week 52, 12.0% (105/773), 9.7% (50/466) and 11.1% (155/1239) of hands demonstrated X-ray progression in Trials B, C and the pooled cohort, respectively. The AUC of the ROC curve of MRI scores at baseline, Week 24 MRI changes and trial data predicting X-ray progression at 52 weeks was 0.65 (95% CI 0.55 to 0.75) (figure 1), compared with 0.51 (95% CI 0.42 to 0.59, $p=0.063$) if only the trial was considered in the regression model.

DISCUSSION

This analysis shows that changes in joint damage and inflammation detected with MRI as early as 12 weeks predict changes in joint damage evident on subsequent X-rays. The current analysis of pooled data from four RCTs that included both MRI and X-ray demonstrated that progression of MRI erosion scores at Weeks 12 and 24 predict progression of X-ray erosions at Weeks 24 and 52. Twelve-week and 24-week changes in MRI osteitis scores and synovitis scores were similarly predictive of 24-week and 52-week X-ray erosion progressions. These findings corroborate those of Baker *et al*[8] who further showed that MRI could allow a large reduction in the number of patients needed to assess structural damage in RA RCTs relative to that required with X-ray.[9]

MRI has been used in 13 multicentre, placebo RCTs reported until now,[10–22] involving 10 different biological therapies. Nine RCTs[11 13–18 21 22] included follow-up intervals ≤12–16 weeks, and in seven of the nine, MRI demonstrated statistically significant inhibition of progression of bone erosions with active treatment compared with placebo within that time frame[14 15 17 18] or showed a lack of inhibition consistent with later X-ray data within the trial[16 22] or in subsequent trials.[23] Two of the nine RCTs were underpowered, but did show numerical suppression of erosion progression on early MRI (one RCT included only 20–21 patients per arm, and in contrast to the other RCTs, used only a single reader;[13] the second RCT included 28–35 patients per arm and showed numerical suppression of MRI erosion relative to placebo at 4 and 12 weeks and statistically significant suppression by 24 weeks).[21]

Two of the nine RCTs discussed above[17 21] and an active-comparator trial[24] included MRI follow-up intervals of 4 weeks or less. Two of these trials demonstrated statistically significant suppression of synovitis and osteitis with MRI after only 2 weeks of active therapy, using 30–32[24] and 30–31[17] patients per arm, respectively. Both trials also showed inhibition of erosion with MRI at later time points. The third study[21] was underpowered for RA MRI Score(RAMRIS), as noted above, but showed numerical decreases in osteitis, synovitis as well as in erosion progression with treatment compared with placebo at 4 weeks.

There were a number of limitations to this analysis. Some trial data sets could not be included because they did not have earlier MRI followed by later X-ray outcomes. Of the three studies referred to above with MRI follow-up intervals <12 weeks, one[17] did not include X-ray and the other[24] used

0.2T rather than 1.5T MRI, so we were unable to examine whether very early MRI inflammation measures would be predictive of X-ray structural outcomes. Another limitation was that all but one of the four data sets rescued placebo patients with active therapy by 24 weeks, confounding analyses based on X-ray data over longer time intervals. This is, however, an issue for all modern RCTs given current restrictions on the duration of placebo treatment. If by 24 weeks the most rapidly progressing patients in the placebo arm of a trial have received rescue treatment, X-rays acquired at 24 weeks will underestimate the true placebo progression rate and thus the effect size of treatment. This limitation highlights why a method, such as MRI, that is sensitive enough to discriminate treatment effect within only 12 weeks, that is, before rescue treatment, is needed. Similarly, in this analysis, 24-week X-rays of patients rescued prior to 24 weeks will categorise some 12-week MRI progressors incorrectly as false positives and artificially reduce the AUC. Which patients received rescue therapy was known in two of the four RCTs analysed. However, removing these patients from analysis in one trial (A) did not significantly change the results (data not shown).

While the non-parametric spline fitting method used in this analysis is a flexible non-parametric approach, the resulting model may not have been optimal, and higher AUCs for the MRI measures could potentially have been attained by including, for example, additional information about the individual patients and more flexible basis functions of the MRI measures. Nevertheless, the estimated predictive value of MRI measures summarised by the AUCs of the ROC curves offers a conservative estimate of the true predictive value.

Lastly, we did not have access to MRI cartilage loss or MRI joint-space narrowing scores for any of the trials included in this analysis. However, the validity of assessing cartilage loss and joint-space narrowing with MRI has been well documented,[25–28] and six RA RCTs have included MRI scoring of cartilage loss[14 17 18 22 26] or joint-space narrowing;[20] all have demonstrated good responsiveness.

In summary, on the basis of this analysis and previous studies, we conclude that MRI can detect progression of structural damage in RA RCTs as soon as 3 months and discriminate inhibition of progression of joint damage within this time frame in placebo-controlled trials with approximately 30–70 patients per treatment arm. We therefore recommend MRI as an imaging modality to assess inflammation and joint damage in short-duration RCTs in RA to reduce the number of patients and trial duration required to demonstrate inhibition of structural damage.

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Ethics approval This was a post hoc blinded analysis and ethical approval was obtained for the four included studies. All studies were conducted in accordance with the ethical principles of the Declaration of Helsinki.

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EXTENDED REPORT

Filgotinib (GLPG0634/GS-6034), an oral JAK1 selective inhibitor, is effective in combination with methotrexate (MTX) in patients with active rheumatoid arthritis and insufficient response to MTX: results from a randomised, dose-finding study (DARWIN 1)

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ABSTRACT

Objectives To evaluate the efficacy and safety of different doses and regimens of filgotinib, an oral Janus kinase 1 inhibitor, as add-on treatment to methotrexate (MTX) in patients with active rheumatoid arthritis (RA) and inadequate response to MTX.

Methods In this 24-week phase IIb study, patients with moderate-to-severe active RA receiving a stable dose of MTX were randomised (1:1:1:1:1:1:1) to receive placebo or 50, 100 or 200 mg filgotinib, administered once daily or twice daily. Primary end point was the percentage of patients achieving a week 12 American College of Rheumatology (ACR)20 response.

Results Overall, 594 patients were randomised and treated. At week 12, significantly more patients receiving filgotinib 100 mg once daily or 200 mg daily (both regimens) achieved an ACR20 response versus placebo. For other key end points at week 12 (ACR50, ACR-N, Disease Activity Score based on 28 joints and C reactive protein value, Clinical Disease Activity Index, Simplified Disease Activity Index and Health Assessment Questionnaire-Disability Index), differences in favour of 100 or 200 mg filgotinib daily were seen versus placebo; responses were maintained or improved through to week 24. Rapid onset of action and dose-dependent responses were observed for most efficacy end points and were associated with an increased haemoglobin concentration. No significant differences between once-daily and twice-daily regimens were seen. Treatment-emergent adverse event rates were similar in placebo and filgotinib groups. Serious infections occurred in one and five patients in the placebo and filgotinib groups, respectively. No tuberculosis or opportunistic infections were reported.

Conclusions Filgotinib as add-on to MTX improved the signs and symptoms of active RA over 24 weeks and was associated with a rapid onset of action. Filgotinib was generally well tolerated.

Trial registration number: NCT01888874.

INTRODUCTION

Current rheumatoid arthritis (RA) guidelines advise treat-to-target strategies, with a focus on patient involvement in treatment decisions.[1 2] With the emergence of novel and effective therapeutic agents for the treatment of RA, patients and physicians are able to consider factors alongside efficacy and safety, including the rapidity with which agents reduce pain and inflammation and the convenience of administration. Since conventional disease-modifying antirheumatic drugs (cDMARDs) are often slow acting, and biological DMARDs (bDMARDs) are limited to intravenous or subcutaneous use, and also have the potential for immunogenicity (responsible both for immune-related side effects and loss of efficacy),[3] there remains a need for novel, rapidly acting agents that can be orally administered.[4 5] In addition to improved convenience for patients, such agents may reduce the need for glucocorticoid-bridging therapy.

The Janus kinase (JAK) receptor JAK1 is implicated in the RA disease process through its role in cytokine signalling. For example, the pro-inflammatory cytokine interleukin-6, which is known to play a major role in RA pathogenesis, acts through a JAK1/JAK2 heterodimer-mediated signalling cascade.[6 7] By contrast, other signal transduction pathways can function independently of JAK1, such as erythropoietin signalling in erythrocyte precursors, which exclusively uses a JAK2 homodimer. JAK inhibitors are low-molecular-weight products that can be administered orally. The pan-JAK inhibitor tofacitinib has been approved by the US Food and Drug Administration for use in patients with moderately to severely active RA as a second-line agent after methotrexate (MTX), and other JAK inhibitors are in development.[8 9] Filgotinib (GLPG0634/GS-6034) is a potent and selective inhibitor of JAK1,[10–12] currently under investigation for the treatment of RA and inflammatory bowel disease. Pharmacokinetic–pharmacodynamic studies of filgotinib and its active metabolite indicate that both moieties contribute to pharmacodynamic effects, resulting in a relatively long duration of JAK1 inhibition,[13] suggesting that filgotinib has the potential to be active not only in twice-daily dosing but also in a once-daily regimen. The efficacy and safety of filgotinib in patients with RA has previously been investigated in two 4-week phase IIa

studies;[14–16] results from these studies informed the design of this phase IIb dose-finding study.

DARWIN 1 was designed to evaluate the efficacy and safety of different doses of filgotinib, administered as once-daily or twice-daily regimens, as add-on treatment to MTX, in patients with moderate-to-severe active RA and an inadequate response to MTX.

METHODS

Study design and treatments

This was a 24-week, multicentre, randomised, double-blind, placebo-controlled, phase IIb, dose-finding study of oral filgotinib, administered as add-on treatment to patients' stable dose of MTX (ClinicalTrials.gov identifier: NCT01888874). The study was conducted at 106 centres in 21 countries in four predefined geographical regions.

Eligible patients were randomly assigned to treatment using a computerised IXRS system (S-Clinica, 6, Chaussée de Boondael, 1050 Brussels, Belgium) to receive placebo twice-daily or three daily dose levels of filgotinib—50, 100 or 200 mg—administered twice daily or once daily, in a 1:1:1:1:1:1:1 ratio, stratified by geographical region and previous use of bDMARDs. At each study visit, numbered kits containing study medication were dispensed via the IXRS system. Patients, investigators, study coordinators, the sponsor and study team were blinded to treatment assignment. At week 12, patients on placebo who had not achieved a 20% improvement in swollen joint count based on 66 joints (SJC66) and tender joint count based on 68 joints (TJC68) were reassigned to receive filgotinib 100 mg once daily or 50 mg twice daily; patients who had not achieved this target who were receiving filgotinib 50 mg once daily were reassigned to receive filgotinib 100 mg once daily, and patients on filgotinib 25 mg twice daily received filgotinib 50 mg twice daily, continuing on their new dose until week 24.

Patients

Enrolled patients were ≥18 years of age with a diagnosis of RA for ≥6 months prior to screening, met the 2010 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) criteria for RA and ACR functional class I–III, had ≥6/66 SJC and ≥8/68 TJC, a screening serum C reactive protein (CRP) ≥0.7×upper limit of laboratory normal range (ULN) (changed from ≥1.5×ULN in May 2014 to facilitate recruitment), had been receiving MTX for ≥6 months and on a stable dose (15–25 mg/week, oral or parenteral) 4 weeks prior to screening, and if receiving oral glucocorticoids (≤10 mg/day) or non-steroidal anti-inflammatory drugs were on a stable dose for ≥4 and ≥2 weeks, respectively, prior to baseline. Females of childbearing potential were required to be using a medically acceptable means of contraception. Details of laboratory-defined inclusion criteria are listed in the online supplementary materials.

Patients were excluded if they were receiving current therapy with any DMARD other than MTX, or if they were receiving or had previous RA treatment with a bDMARD. The only exception to this was if a biological agent had been received in a single clinical study >6 months prior to enrolment and if the drug was effective. Patients were also excluded if they had ever used a JAK inhibitor, a cytotoxic agent other than MTX or had received intra-articular or parenteral glucocorticoids within four weeks of screening. Further details of the exclusion criteria, including a list of infections that precluded enrolment in the study, are listed in the online supplementary materials.

Outcomes and assessments

Efficacy assessments were performed at screening (joint counts and Patient's Global Assessment of Disease Activity only), baseline and at weeks 1, 2, 4, 8, 12, 16, 20 and 24. The primary efficacy end point was the percentage of patients achieving an ACR20 response at week 12. Key secondary end points were the percentages of patients achieving ACR20, ACR50, ACR70 and ACR-N responses, Disease Activity Score based on 28 joints and CRP value (DAS28 (CRP), including remission and low-disease activity (LDA)/remission), EULAR response and ACR/EULAR remission, Clinical Disease Activity Index (CDAI) and Simplified Disease Activity Index (SDAI) at every visit from baseline to week 24. Health-related quality of life (HRQoL) was evaluated to week 24 using the Health Assessment Questionnaire-Disability Index (HAQ-DI).

Safety variables included adverse events (AEs) throughout the study period; vital signs (at each visit); physical examinations (at screening, baseline, week 12 and week 24); and 12-lead ECG (at screening, week 12 and week 24). Haematology and clinical chemistry laboratory assessments were performed at each visit. The National Institute of Health Common Terminology Criteria for Adverse Events (CTCAE) V.3.0 was used to describe laboratory changes during the study.

Sample sizes and statistical analyses

All randomised patients who received at least one dose of study drug were included in the intent-to-treat (ITT) and safety populations. Patients who discontinued the study prior to week 12 were treated as non-responders for the primary analysis, and those who switched treatments at week 12 were handled as discontinuations and data were imputed from week 12 onwards.

Efficacy data were analysed using non-responder imputation (NRI) for the ITT population and confirmed using last observation carried forward (LOCF) and observed case imputations in the ITT population; NRI and LOCF imputations were used for efficacy data in the per-protocol population.

The primary analysis was conducted using a logistic regression model including treatment, geographical region and previous use of bDMARDs as covariates. Continuous parameters were analysed using analysis of covariance. Time-to-first response (ACR20/50/70) was analysed using Kaplan-Meier survival techniques, with treatment groups compared with placebo using a Cox proportional hazard regression model. Treatment versus placebo comparisons were carried out for each dose group versus placebo using Hommel's closed-testing correction procedure to adjust for multiplicity. Differences between the once-daily and twice-daily regimens were analysed exploratively.

A sample size of n=85 per study group (N=595) was estimated to provide 90% power to detect minimum 28–30% treatment difference versus placebo, assuming a 20–40% placebo ACR20 response at week 12.

RESULTS

The study was initiated in July 2013 and completed in May 2015. Of the 1255 patients screened, 599 were randomised and 594 received at least one dose of study drug and were included in the ITT and safety populations. At week 12, 66 non-responders were re-randomised to 100 mg daily dose of filgotinib (figure 1). The overall treatment discontinuation rate was low (n=61, 10.3%), and there was no significant difference in the number of patients who discontinued between the filgotinib and placebo groups. In addition, dropout rates did not increase with increasing doses of filgotinib or over time (weeks 0–12 vs weeks 12–24).

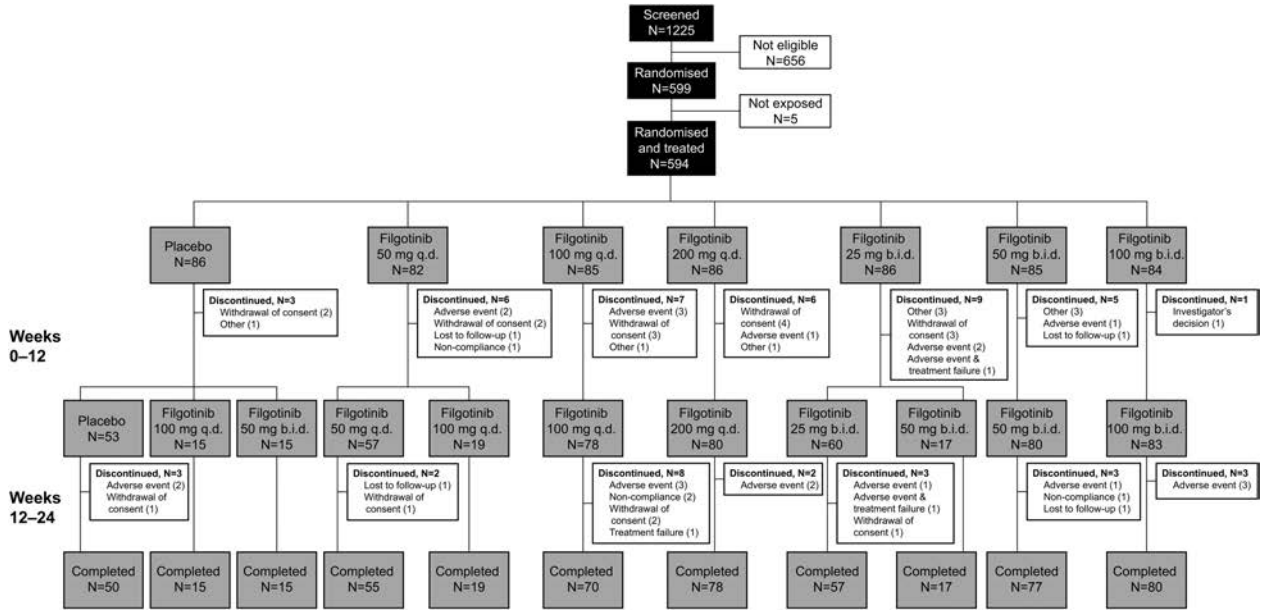

Figure 1 Patient disposition. Patients were randomised across 106 sites in 21 countries (Argentina, Australia, Belgium, Bulgaria, Chile, Columbia, Czech Republic, France, Germany, Guatemala, Hungary, Israel, Latvia, Mexico, Moldova, New Zealand, Poland, Russian Federation, Spain, Ukraine and the USA). b.i.d., twice daily; q.d., once daily.

Baseline patient demographics and disease characteristics were well balanced (table 1), apart from a trend towards (non-significant, p=0.0555) lower mean CRP in the placebo group.

Efficacy

Primary efficacy analysis

The primary end point of the study was met: at week 12, statistically significantly more patients achieved an ACR20 response compared with placebo (44% (38/86)) in the filgotinib 100 mg once-daily (64% (54/85), p=0.0435), 200 mg once-daily (69% (59/86), p=0.0068) and 100 mg twice-daily (79% (66/84), p<0.0001) dose groups (figure 2A). Raw ACR20 data for each time point are presented in online supplementary table S1.

Secondary efficacy analyses

A dose–response was observed for all three ACR parameters (ACR20, ACR50 and ACR70; figure 2), and no statistically significant difference was found between the filgotinib once-daily and twice-daily regimens. An early onset of response was observed for ACR20 (from week 2 in the filgotinib 200 mg once-daily and 100 mg twice-daily dose groups (figure 2A)) and ACR50 (from week 2 in the filgotinib 100 mg once-daily and 100 mg twice-daily dose groups (figure 2B)).

At week 24, the percentage of patients meeting ACR20 response criteria was also significantly higher compared with placebo in the 100 mg once-daily, 200 mg once-daily, 50 mg twice-daily and 100 mg twice-daily dose groups (A). An increase in the ACR20 response over time was observed that appeared to plateau at week 8 in the majority of filgotinib treatment groups and was maintained up to week 24. The percentage of ACR50 responders was statistically significantly higher compared with placebo across all filgotinib dose groups and regimens at weeks 12; this response was maintained or improved to week 24 (figure 2B) and 100 mg twice-daily dose groups compared with placebo at week 12; this response was improved or maintained at week 24, such that a significant response was observed across all filgotinib dose groups and regimens at week 24 (figure 2C).

Statistically significant improvements compared with placebo were observed after 1 week of treatment in the filgotinib 200 mg daily dose group for some components of the ACR index (TJC and serum CRP) (data not shown). ACR20/50/70 responses improved up to week 24 in non-responders who switched to 100 mg daily filgotinib at week 12 (see online supplementary table S2).

At both weeks 12 and 24, disease activity (CDAI) had decreased to a significant extent versus placebo in all dose groups, with the exception of the lowest dose of filgotinib at week 12. An effect was observed early, with significant reductions versus placebo noted by week 2 in the 100 mg once-daily and 100 mg twice-daily dose groups (figure 2D). Similarly, at both weeks 12 and 24, the mean decrease in DAS28 (CRP) was statistically significantly greater across all filgotinib dose groups and regimens compared with placebo (figure 2E). An early onset of effect was observed in DAS28 (CRP) (from week 1 in the 100 mg once-daily, 200 mg once-daily and 100 mg twice-daily dose groups) (figure 2E). Both indices of disease activity showed a dose–response relationship and no statistical differences were noted between the once-daily and twice-daily regimens. For HAQ-DI, significant improvements versus placebo were noted as early as week 2 for filgotinib 200 mg daily. By week 12, these improvements were also noted in the 100 mg once-daily group, and by week 24, significant improvements compared with placebo were observed across all filgotinib groups (figure 2F). Raw data for each of the secondary efficacy end points illustrated in figure 2 are presented in online supplementary table S1.

As detailed in table 2, a dose–response relationship was observed for all other efficacy variables. There were too few patients in each dose group who had previously received and responded to a biological agent to make valid comparisons of the efficacy of filgotinib in this patient population versus patients who were naive to biological treatments.

Safety

Adverse events

Treatment-emergent adverse events (TEAEs) were reported at similar frequencies across all dose groups and treatment

Table 1 Baseline patient demographics, disease characteristics and treatment history (safety population)

		Filgotinib once-daily dose groups			Filgotinib twice-daily dose groups		
	Placebo (N=86)	50 mg (N=82)	100 mg (N=85)	200 mg (N=86)	2×25 mg (N=86)	2×50 mg (N=85)	2×100 mg (N=84)
Patient demographics							
Age, mean (SE), years	52 (1.4)	53 (1.5)	52 (1.4)	55 (1.3)	52 (1.4)	55 (1.3)	54 (1.3)
Female, n (%)	70 (81.4)	69 (84.1)	65 (76.5)	74 (86.0)	68 (79.1)	65 (76.5)	70 (83.3)
Disease characteristics							
Duration of RA, mean (SE), years	8 (0.8)	7 (0.6)	8 (0.7)	9 (0.9)	9 (0.8)	8 (0.7)	10 (1.0)
Anti-CCP positive, n (%)	72 (83.7)	64 (78.0)	60 (70.6)	69 (80.2)	70 (82.4)	70 (82.4)	68 (81.0)
RF positive, n (%)	65 (76.5)	64 (78.0)	57 (67.1)	65 (75.6)	66 (76.7)	64 (75.3)	65 (77.4)
DAS28 (CRP), mean (SE)	5.98 (0.088)	6.08 (0.093)	6.14 (0.091)	6.22 (0.088)	6.05 (0.086)	6.10 (0.098)	6.14 (0.090)
CDAI, mean (SE)	42 (1.2)	41 (1.2)	43 (1.3)	43 (1.3)	41 (1.2)	42 (1.3)	42 (1.2)
SDAI, mean (SE)	44 (1.3)	44 (1.3)	45 (1.4)	46 (1.3)	44 (1.3)	45 (1.4)	45 (1.3)
ACR components							
CRP, mean (SE), mg/L	16.25 (1.567)	27.71 (3.235)	24.54 (2.849)	27.10 (2.780)	26.01 (3.142)	24.60 (2.627)	26.86 (2.729)
TJC68, mean (SE)	24.98 (1.345)	24.91 (1.499)	25.32 (1.490)	28.84 (1.650)	25.43 (1.420)	27.16 (1.546)	25.95 (1.525)
SJC66, mean (SE)	16.13 (0.8990)	17.02 (1.116)	16.31 (0.9387)	17.36 (0.958)	15.66 (0.8839)	17.53 (1.124)	16.36 (0.9372)
HAQ-DI total score, mean (SE)	1.692 (0.0576)	1.705 (0.0690)	1.700 (0.0687)	1.764 (0.0606)	1.696 (0.0515)	1.779 (0.0611)	1.775 (0.0707)
Patient's global assessment, mean (SE)	64.2 (1.96)	68.2 (2.23)	67.6 (2.09)	68.7 (2.09)	64.3 (1.95)	65.7 (1.92)	66.6 (2.20)
Investigator's global assessment, mean (SE)	66.5 (1.62)	66.2 (1.55)	66.4 (1.67)	65.8 (1.79)	63.4 (1.59)	66.6 (1.71)	64.6 (1.72)
Patient's pain (VAS), mean (SE)	65.7 (2.16)	66.9 (2.20)	65.4 (2.41)	67.0 (2.16)	65.7 (2.23)	67.8 (2.12)	67.2 (2.19)
Treatments							
Methotrexate dose, mean (SE), mg/week	16.5 (0.46)	16.4 (0.45)	16.6 (0.44)	17.3 (0.47)	17.5 (0.53)	16.7 (0.45)	17.3 (0.43)
Methotrexate duration, mean (SE), years	5 (0.4)	5 (0.5)	6 (0.6)	5 (0.6)	5 (0.6)	5 (0.6)	4 (0.5)
Corticosteroids, n (%)	50 (58.1)	48 (58.5)	47 (55.3)	49 (57.0)	51 (59.3)	57 (67.1)	50 (59.5)
Previous bDMARDs, n (%)	8 (9.3)	6 (7.3)	6 (7.1)	11 (12.8)	6 (7.0)	6 (7.1)	7 (8.3)

No significant differences for all parameters apart from a trend towards (non-significant, p=0.0555) lower mean CRP in the placebo group.
ACR, American College of Rheumatology; bDMARD, biological disease-modifying antirheumatic drugs; CCP, cyclic citrullinated peptide; CDAI, Clinical Disease Activity Index; DAS28 (CRP), Disease Activity Score based on 28 joints and C reactive protein value; HAQ-DI, Health Assessment Questionnaire-Disability Index; N, number of patients per treatment group; n, number of patients per category; RA, rheumatoid arthritis; RF, rheumatoid factor; SDAI, Simple Disease Activity Index; SJC66, swollen joint count based on 66 joints; TJC68, tender joint count based on 68 joints; VAS, visual analogue score.

regimens (table 3). Fifteen patients had ≥1 serious TEAE (table 3), and of these, one patient (in the filgotinib 100 mg twice-daily group) died due to pneumonia and septic shock; this was the only death in the study and was considered by the investigator as possibly treatment related. Two patients had serious cardiovascular events: one patient (with a history of myocardial infarction and cardiac failure) experienced unstable angina and subsequent myocardial infarction and one patient experienced an ischaemic cerebral infarction; these events were not considered to be treatment related. Serious TE infections were reported in one patient receiving placebo and five patients receiving filgotinib (see table 3 for details). TEAEs considered related to study treatment occurred more frequently in the filgotinib groups (20.9%) compared with placebo (10.7%). Few patients in any group discontinued due to TEAE (table 3); infections led to discontinuation in one patient receiving placebo and five patients receiving filgotinib. Herpes zoster infections were observed in five patients, one receiving placebo and four receiving filgotinib; all of these cases resolved without complications. No cases of tuberculosis (TB), opportunistic infections, lymphoma or cancer were reported throughout the study.

Haematology

Data for haematology parameters are presented in online supplementary table S3. Up to week 12, dose-dependent increases were observed in mean haemoglobin concentrations in all filgotinib groups, but appeared to plateau thereafter (figure 3A). Overall, no decreases in mean absolute lymphocyte counts were observed, although there were individual fluctuations. Dose-dependent decreases in neutrophil counts were seen in all filgotinib groups (figure 3B). These stabilised at week 4, with the exception of the filgotinib 100 mg twice-daily group, in which a further decrease was seen from weeks 16 to 24. Non-responders who switched to filgotinib 100 mg also experienced a reduction in mean neutrophil count from week 12 (data not shown). Dose-dependent decreases in mean absolute platelet count were observed in the filgotinib treatment groups up to week 4, following which counts appeared to plateau, with some fluctuations (figure 3C). There were no dose-dependent changes in mean natural killer (NK) cell counts over time. The number of CTCAE grade 3 or 4 laboratory abnormalities was low, and in most cases did not lead to study discontinuation (table 3).

Clinical chemistry

Up to week 4, dose-dependent increases in mean creatinine concentrations in filgotinib groups were observed, which plateaued in most treatment groups thereafter (data not shown). A mean increase of 6.1 µmol/L (11.5%) from baseline value of 58.9 µmol/L was observed in the filgotinib 100 mg twice-daily group. No CTCAE grade 3 or grade 4 abnormally high alanine transaminase (ALT) values were observed. One patient in the filgotinib 100 mg once-daily group had a CTCAE grade 3 aspartate transaminase (AST) value (table 3), not considered to be related to study medication; this subject had AST grade 2 abnormality at baseline and discontinued the study. Up to week 4, dose-dependent increases in both high-density lipoprotein

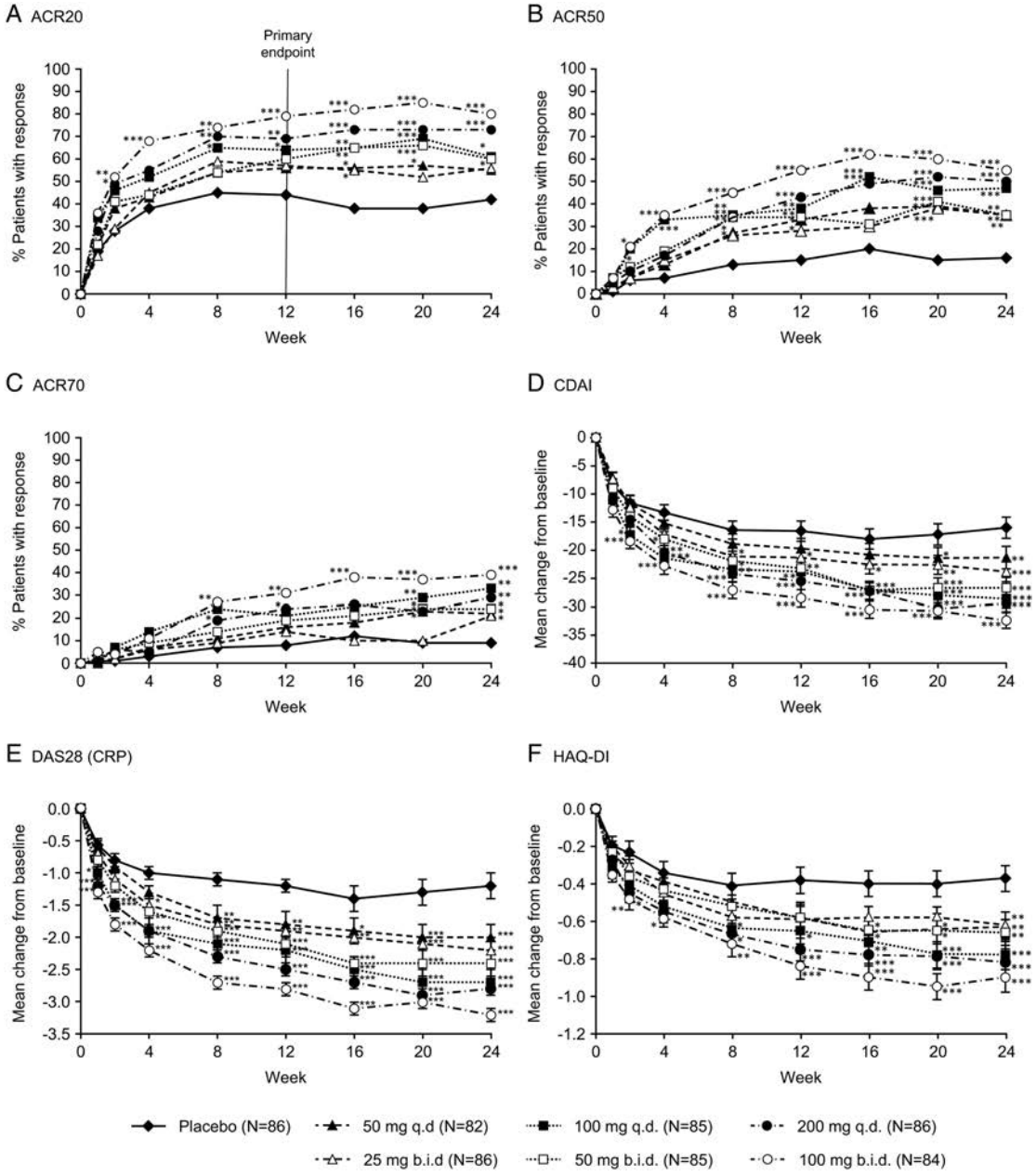

Figure 2 Efficacy end points: the percentage of patients achieving an improvement in American College of Rheumatology (ACR) of (A) 20% (ACR20), (B) 50% (ACR50) or (C) 70% (ACR70) over time though 24 weeks; (D) mean change from baseline in Clinical Disease Activity Index (CDAI) over time; (E) mean change from baseline in Disease Activity Score based on 28 joints and C reactive protein value (DAS28) (CRP) over time; (F) mean change from baseline in Health Assessment Questionnaire-Disability Index (HAQ-DI) over time. The vertical line at 12 weeks in (A) indicates the primary efficacy time point (non-responder imputation (NRI) (intent-to-treat population)). Patients who switched at week 12 were handled as if they discontinued at week 12 and were imputed using NRI (A–C) or last observation carried forward (D and E). *p<0.05; **p<0.01; ***p<0.001. b.i.d., twice daily; N, number of subjects per group; q.d., once daily.

(HDL) and low-density lipoprotein (LDL) cholesterol were observed in all filgotinib groups, which stabilised thereafter. The LDL:HDL ratio decreased over this period, indicating a greater proportional increase in HDL versus LDL.

DISCUSSION

In this study, clinical efficacy in patients treated with filgotinib added to a stable dose of background MTX was evident in a dose-dependent manner, with an early onset of action. By week 12, statistically significantly higher proportions of patients who received 100 mg once daily, or 200 mg daily, regardless of the dose regimen used, achieved ACR20 response, compared with placebo. This response was maintained at week 24. Baseline imbalances in CRP level between the active treatment groups and placebo were explored in a logistic regression model: the discrepancy in baseline levels of inflammation did not influence the primary end point. At week 12, dose-dependent, statistically significant beneficial effects were also seen across the majority of secondary end points, which were maintained or exceeded at week 24, as illustrated by improving responses between weeks 12 and 24 in two major relevant outcomes, ACR70 and DAS28 (CRP) remission.[17 18] The remission rates observed for CDAI also support a clinical benefit of filgotinib that is independent of CRP levels. A fast onset of effect was observed for ACR20/50/

Table 2 Efficacy assessments and disease activity assessments at weeks 12 and 24 (NRI (ITT population) and LOCF (ITT population))

		Filgotinib once-daily dose groups			Filgotinib twice-daily dose groups		
Time point	**Placebo (N=86)**	**50 mg (N=82)**	**100 mg (N=85)**	**200 mg (N=86)**	**2×25 mg (N=86)**	**2×50 mg (N=85)**	**2×100 mg (N=84)**
ACR20†							
Week 12, n (%)	38 (44.2)	46 (56.1)	54 (63.5)*	59 (68.6)**	49 (57.0)	51 (60.0)	66 (78.6)***
Week 24, n (%)	36 (41.9)	45 (54.9)*	52 (61.2)***	63 (73.3)	48 (55.8)	51 (60.0)*	67 (79.8)***
ACR50†							
Week 12, n (%)	13 (15.1)	27 (32.9)*	32 (37.6)**	37 (43.0)***	24 (27.9)*	29 (34.1)*	46 (54.8)***
Week 24, n (%)	14 (16.3)	29 (35.4)**	40 (47.1)***	43 (50.0)***	30 (34.9)**	30 (35.3)**	46 (54.8)***
ACR70†							
Week 12, n (%)	7 (8.1)	13 (15.9)	18 (21.2)	21 (24.4)*	12 (14.0)	16 (18.8)	26 (31.0)**
Week 24, n (%)	8 (9.3)	18 (22.0)*	28 (32.9)**	25 (29.1)**	18 (20.9)*	20 (23.5)*	33 (39.3)***
ACR-N‡							
Week 12, mean (SE)	23.09 (2.911)	34.03 (3.335)*	39.87 (3.449)***	42.10 (3.277)***	34.12 (3.144)*	35.86 (3.290)**	51.17 (3.379)***
Week 24, mean (SE)	22.06 (2.846)	37.13 (3.582)**	50.86 (3.645)***	50.40 (3.291)***	38.56 (3.384)***	40.50 (3.299)***	58.69 (3.204)***
CRP‡							
Week 12, mean (SE), mg/L	2.67 (2.219)	−13.15 (2.890)*	−13.57 (2.771)***	−17.24 (3.322)***	−10.26 (2.873)*	−12.97 (2.277)**	−20.54 (2.665)***
Week 24, mean (SE), mg/L	2.00 (1.776)	−15.22 (3.316)**	−14.89 (2.712)***	−15.57 (4.112)**	−11.68 (3.020)*	−11.96 (2.488)*	−20.82 (2.264)***
Change from baseline in TJC68‡							
Week 12, mean (SE) change	−9.2 (1.35)	−12.2 (1.34)*	−14.1 (1.33)**	−17.6 (1.33)***	−14.2 (1.37)**	−15.0 (1.37)**	−18.0 (1.31)***
Week 24, mean (SE) change	−8.9 (1.43)	−12.7 (1.42)*	−17.1 (1.32)***	−20.6 (1.49)***	−15.9 (1.51)***	−18.1 (1.44)***	−21.4 (1.38)***
Change from baseline in SJC66‡							
Week 12, mean (SE) change	−7.6 (0.89)	−8.5 (1.01)	−9.8 (0.97)	−11.0 (0.95)*	−8.8 (0.87)	−11.0 (1.10)	−12.2 (0.84)***
Week 24, mean (SE) change	−7.3 (1.00)	−9.2 (1.05)	−12.6 (0.91)***	−13.2 (0.87)***	−10.2 (0.93)**	−12.9 (1.29)***	−13.8 (0.85)***
Change from baseline in HAQ-DI							
Week 12, mean (SE) change	−0.383 (0.0691)	−0.577 (0.789)	−0.653 (0.0728)*	−0.753 (0.0648)***	−0.590 (0.0659)	−0.584 (0.0677)	−0.840 (0.0726)***
Week 24, mean (SE) change	−0.365 (0.0671)	−0.633 (0.0795)**	−0.783 (0.0761)***	−0.818 (0.0675)***	−0.618 (0.0660)**	−0.659 (0.0702)**	−0.903 (0.0813)***
Change from baseline in DAS28 (CRP)‡							
Week 12, mean (SE) decrease	−1.19 (0.148)	−1.75 (0.152)**	−2.23 (0.151)***	−2.47 (0.136)***	−1.88 (0.145)**	−2.10 (0.161)***	−2.84 (0.146)***
Week 24, mean (SE) decrease	−1.18 (0.163)	−1.98 (0.179)***	−2.70 (0.156)***	−2.80 (0.139)***	−2.19 (0.157)***	−2.40 (0.175)***	−3.23 (0.138)***
DAS28 (CRP) LDA‡							
Week 12, n (%)	6 (7.0)	10 (12.2)	10 (11.8)	13 (15.1)	11 (12.8)	9 (10.6)	12 (14.3)
Week 24, n (%)	8 (9.3)	10 (12.2)	12 (14.1)	22 (25.6)	14 (16.3)	12 (14.1)	20 (23.8)
DAS28 (CRP) remission‡							
Week 12, n (%)	6 (7.0)	10 (12.2)	19 (22.4)*	19 (22.1)*	13 (15.1)	15 (17.6)	30 (35.7)***
Week 24, n (%)	8 (9.3)	17 (20.7)*	31 (36.5)***	22 (25.6)*	20 (23.3)*	20 (23.5)*	34 (40.5)***
DAS28 (CRP) remission/LDA‡							
Week 12, n (%)	12 (14.0)	20 (24.4)	29 (34.1)**	32 (37.2)**	24 (27.9)	24 (28.2)*	41 (50.0)***
Week 24, n (%)	16 (18.6)	27 (32.9)*	43 (50.6)***	44 (51.2)***	34 (39.5)**	32 (37.6)*	54 (64.3)***
DAS 28 (CRP) EULAR response‡							

Continued

Table 2 Continued

Time point	Placebo (N=86)	Filgotinib once-daily dose groups			Filgotinib twice-daily dose groups		
		50 mg (N=82)	100 mg (N=85)	200 mg (N=86)	2×25 mg (N=86)	2×50 mg (N=85)	2×100 mg (N=84)
Week 12, n (%)							
Moderate	39 (45)	36 (44)	41 (48)	47 (55)	38 (44)	48 (56)	36 (43)
Good	12 (14)	19 (23)	29 (34)**	32 (37)***	24 (28)*	24 (28)**	42 (50)***
Week 24, n (%)							
Moderate	29 (34)	29 (35)	32 (38)	33 (38)	32 (37)	42 (49)	26 (31)
Good	16 (19)	26 (32)	43 (51)***	44 (51)***	34 (40)**	31 (36)***	54 (64)***
ACR/EULAR remission†							
Week 12, n (%)	3 (3.5)	3 (3.7)	3 (3.5)	5 (5.8)	4 (4.7)	4 (4.7)	8 (9.5)
Week 24, n (%)	1 (1.2)	9 (11.0)	7 (8.2)	10 (11.6)	5 (5.8)	3 (3.5)	16 (19.0)*
Change from baseline in SDAI‡							
Week 12, mean (SE) decrease	−16.3 (1.84)	−21.0 (1.84)*	−25.2 (1.69)***	−27.2 (1.55)***	−22.3 (1.71)*	−24.5 (1.87)***	−30.6 (1.57)***
Week 24, mean (SE) decrease	−15.8 (2.00)	−22.8 (2.07)**	−30.1 (1.66)***	−31.0 (1.62)***	−24.9 (1.85)***	−27.9 (2.00)***	−34.4 (1.47)***
SDAI LDA‡							
Week 12, n (%)	8 (9.3)	19 (23.2)	22 (25.9)	23 (26.7)	19 (22.1)	18 (21.2)	27 (32.1)
Week 24, n (%)	17 (19.8)	17 (20.7)	32 (37.6)	29 (33.7)	29 (33.7)	27 (31.7)	34 (40.5)
SDAI remission†							
Week 12, n (%)	4 (4.7)	6 (7.3)	6 (7.1)	10 (11.6)	7 (8.1)	8 (9.4)	14 (16.7)
Week 24, n (%)	1 (1.2)	13 (15.9)*	13 (15.3)*	12 (14.0)*	10 (11.6)*	12 (14.1)*	16 (19.0)*
Change from baseline in CDAI‡							
Week 12, mean (SE) decrease	−16.6 (1.84)	−19.7 (1.77)	−23.8 (1.66)**	−25.5 (1.50)***	−21.3 (1.65)*	−23.2 (1.81)**	−28.5 (1.49)***
Week 24, mean (SE) decrease	−16.0 (1.95)	−21.3 (1.97)**	−28.6 (1.63)***	−29.4 (1.50)***	−23.8 (1.75)***	−26.7 (1.90)***	−32.4 (1.39)***
CDAI LDA‡							
Week 12, n (%)	13 (15.1)	20 (24.4)	20 (23.5)	23 (26.7)	16 (18.6)	19 (22.4)	27 (32.1)
Week 24, n (%)	16 (18.6)	15 (18.3)	24 (28.2)	28 (32.6)	27 (31.4)	25 (29.4)	30 (35.7)
CDAI remission†							
Week 12, n (%)	2 (2.3)	6 (7.3)	7 (8.2)	9 (10.5)	9 (10.5)	7 (8.2)	15 (17.9)*
Week 24, n (%)	2 (2.3)	15 (18.3)*	18 (21.2)**	13 (15.1)*	11 (12.8)*	13 (15.3)*	16 (19.0)**

*p<0.05; **p<0.01; ***p<0.001.
†NRI (ITT population).
‡LOCF (ITT population).
ACR, American College of Rheumatology; ACR-N, American College of Rheumatology N% improvement; CDAI, Clinical Disease Activity Index; DAS28 (CRP), Disease Activity Score based on 28 joints and C reactive protein value; EULAR, European League Against Rheumatism; HAQ-DI, Health Assessment Questionnaire-Disability Index; ITT, intent-to-treat; LDA, low-disease activity; LOCF, last observation carried forward; N, number of patients per group; n, number of patients with response/change; NRI, non-responder imputation; SDAI, Simplified Disease Activity Index; SJC66, swollen joint count based on 66 joints; TJC68, tender joint count based on 68 joints.

Table 3 Summary of absolute numbers and proportions of patients in each treatment group who experienced TEAEs and laboratory abnormalities over the course of the study

		Continued once-daily groups			Continued twice-daily groups			Non-responders* switching to 100 mg/day			
Patients with	Continued placebo (N=56)	50 mg (N=63)	100 mg (N=85)	200 mg (N=86)	2×25 mg (N=69)	2×50 mg (N=85)	2×100 mg (N=84)	Placebo to 100 mg (N=15)	Placebo to 2×50 mg (N=15)	50–100 mg (N=19)	2×25 mg to 2×50 mg (N=17)
TEAE, n (%)	32 (57.1)	33 (52.4)	37 (43.5)	50 (58.1)	37 (53.6)	46 (54.1)	45 (53.6)	7 (46.7)	7 (46.7)	9 (47.4)	12 (70.6)
Serious TEAE, n (%)	4 (7.1)	0 (0)	4 (4.7)	2 (2.3)	1 (1.4)	0 (0)	3 (3.6)	0 (0)	0 (0)	0 (0)	1 (5.9)
SAE leading to death, n (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.2)	0 (0)	0 (0)	0 (0)	0 (0)
Serious TE infection, n (%)	1 (1.8)†	0 (0)	3 (3.5)‡	1 (1.2)§	0 (0)	0 (0)	1 (1.2)¶	0 (0)	0 (0)	0 (0)	0 (0)
Related TEAE, n (%)	6 (10.7)	13 (20.6)	11 (12.9)	21 (24.4)	14 (20.3)	19 (22.4)	21 (25.0)	2 (13.3)	0 (0)	2 (10.5)	2 (11.8)
Related TEAE infection, n (%)	1 (1.8)	4 (6.3)	4 (4.7)	7 (8.1)	5 (7.2)	7 (8.2)	7 (8.3)	0 (0)	0 (0)	0 (0)	0 (0)
Herpes zoster infection	1	0	0	1	1	0	2	0	0	0	0
TEAE leading to permanent discontinuation of study treatment, n (%)	2 (3.6)	2 (3.2)	5 (5.9)**	3 (3.5)	5 (7.2)	2 (2.4)	3 (3.6)	0 (0)	0 (0)	0 (0)	0 (0)
TE laboratory abnormalities, n (%)											
Decreased haemoglobin, g/dL											
Grade 1 (10, LLN)	11 (19.36)	13 (20.6)	10 (11.8)	11 (12.8)	11 (15.9)	13 (15.3)	13 (15.5)	6 (40.0)	4 (26.7)	3 (15.8)	4 (23.5)
Grade 2 (<10–8)	4 (7.1)	2 (3.2)	7 (8.3)	2 (2.3)	2 (2.3)	4 (4.7)	1 (1.2)	0 (0)	0 (0)	2 (10.5)	1 (5.9)
Grade 3 (<8.0–6.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.2)	0 (0)	0 (0)	0 (0)	0 (0)
Grade 4 (<6.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Decreased lymphocytes, $\times 10^9$/L											
Grade 1 (0.8, LLN)	1 (1.8)	2 (3.2)	0 (0)	3 (3.5)	3 (4.3)	1 (1.2)	2 (2.4)	0 (0)	1 (6.7)	1 (5.3)	0 (0)
Grade 2 (<0.8–0.5)	3 (5.4)	6 (9.5)	4 (4.7)	5 (5.8)	2 (2.9)	4 (4.7)	4 (4.87)	0 (0)	0 (0)	1 (5.3)	3 (17.6)
Grade 3 (<0.5–0.2)	1 (1.8)	1 (1.6)	2 (2.4)	0 (0)	1 (1.4)	1 (1.2)	0 (0)	0 (0)	0 (0)	0 (0)	1 (5.9)
Grade 4 (<0.2)	0 (0)	0 (0)	1 (1.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Decreased neutrophils, $\times 10^9$/L											
Grade 1 (1.5, LLN)	1 (1.8)	1 (1.6)	1 (1.2)	4 (4.7)	3 (4.3)	1 (1.2)	1 (1.2)	0 (0)	0 (0)	0 (0)	0 (0)
Grade 2 (<1.5–1.0)	3 (5.4)	0 (0)	0 (0)	3 (3.5)	2 (2.9)	1 (1.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Grade 3 (<1.0–0.5)	0 (0)	0 (0)	0 (0)	1 (1.2)	0 (0)	0 (0)	0 (0)	1 (6.7)	0 (0)	0 (0)	0 (0)
Grade 4 (<0.5)	0 (0)	0 (0)	1 (1.2)	0 (0)	0 (0)	0 (0)	1 (1.2)	0 (0)	0 (0)	0 (0)	0 (0)
Decreased platelets, $\times 10^9$/L											
Grade 1 (75, LLN)	1 (1.8)	0 (0)	2 (2.4)	3 (3.5)	0 (0)	1 (1.2)	2 (2.4)	0 (0)	0 (0)	1 (5.3)	0 (0)
Grade 2 (<75–50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Grade 3 (<50–25)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Grade 4 (<25)	0 (0)	0 (0)	1 (1.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NK cells (CD16CD56), $\times 10^9$/L											
Decrease to <LLN	3 (5.4)	5 (7.9)	5 (5.9)	12 (14.0)	7 (10.1)	5 (5.9)	6 (7.1)	2 (13.3)	2 (13.3)	0 (0)	3 (17.6)
Increase to >ULN	2 (3.6)	0 (0)	3 (3.5)	3 (3.5)	1 (1.4)	0 (0)	2 (2.4)	0 (0)	0 (0)	0 (0)	1 (5.9)
Elevated creatinine µmol/L											
Grade 1 (1–1.5×ULN)	0 (0)	1 (1.6)	2 (2.4)	1 (1.2)	0 (0)	2 (2.4)	1 (1.2)	0 (0)	0 (0)	0 (0)	0 (0)
Grade 2 (1.5–3×ULN)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Grade 3 (3–6×ULN)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Grade 4 (>6×ULN)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Elevated ALT											
Grade 1 (1–2.5×ULN)	3 (5.4)	6 (9.5)	9 (10.6)	10 (11.6)	10 (14.5)	7 (8.2)	7 (8.3)	0 (0)	3 (20.0)	1 (5.3)	0 (0)
Grade 2 (2.5–5×ULN)	1 (1.8)	0 (0)	1 (1.2)	2 (2.4)	0 (0)	0 (0)	1 (1.2)	0 (0)	0 (0)	0 (0)	0 (0)

Continued

Table 3 Continued

Patients with	Continued placebo (N=56)	Continued once-daily groups			Continued twice-daily groups			Non-responders* switching to 100 mg/day			
		50 mg (N=63)	100 mg (N=85)	200 mg (N=86)	2×25 mg (N=69)	2×50 mg (N=85)	2×100 mg (N=84)	Placebo to 100 mg (N=15)	Placebo to 2×50 mg (N=15)	50–100 mg (N=19)	2×25 mg to 2×50 mg (N=17)
Grade 3 (5–20×ULN)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Grade 4 (>20×ULN)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0	0 (0)	0 (0)	0 (0)	0 (0)
Elevated AST											
Grade 1 (1–2.5×ULN)	1 (1.8)	5 (7.9)	8 (9.4)	10 (11.6)	6 (8.7)	9 (10.6)	9 (10.7)	1 (6.7)	0 (0)	1 (5.3)	3 (17.6)
Grade 2 (2.5–5×ULN)	0 (0)	0 (0)	0 (0)	2 (2.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Grade 3 (5–20×ULN)	0 (0)	0 (0)	1 (1.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Grade 4 (>20×ULN)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Ratio LDL/HDL											
Increase to >ULN	5 (8.9)	9 (14.3)	10 (11.8)	6 (7.0)	3 (4.3)	5 (5.9)	2 (2.4)	0 (0)	0 (0)	3 (15.8)	3 (17.6)

†Appendicitis.
‡Pneumonia, diabetic gangrene, subcutaneous abscess.
§Erysipelas.
¶Intervertebral discitis+pneumonia+septic shock.
*Non-responders defined as patients who had not achieved a 20% improvement in swollen joint count based on 66 joints (SJC66) and tender joint count based on 68 joints (TJC68) by Week 12. Patients on placebo were reassigned to receive filgotinib 100 mg once daily or 50 mg twice daily; patients who were receiving filgotinib 50 mg once daily were reassigned to receive filgotinib 100 mg once daily, and patients on filgotinib 25 mg twice daily received filgotinib 50 mg twice daily, continuing on their new dose until week 24.
**One subject had a pretreatment AE (decreased lymphocyte count) that was ongoing throughout the study, for which the study medication was permanently discontinued. This AE was not taken into account in this table as it was not a TEAE.
AE, adverse event; ALT, alanine transaminase; AST, aspartate transaminase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LLN, lower limit of normal; N, number of patients per group; n, number of patients with event; NK, natural killer; SAE, serious adverse event; TEAE, treatment-emergent adverse event; ULN, upper limit of normal.

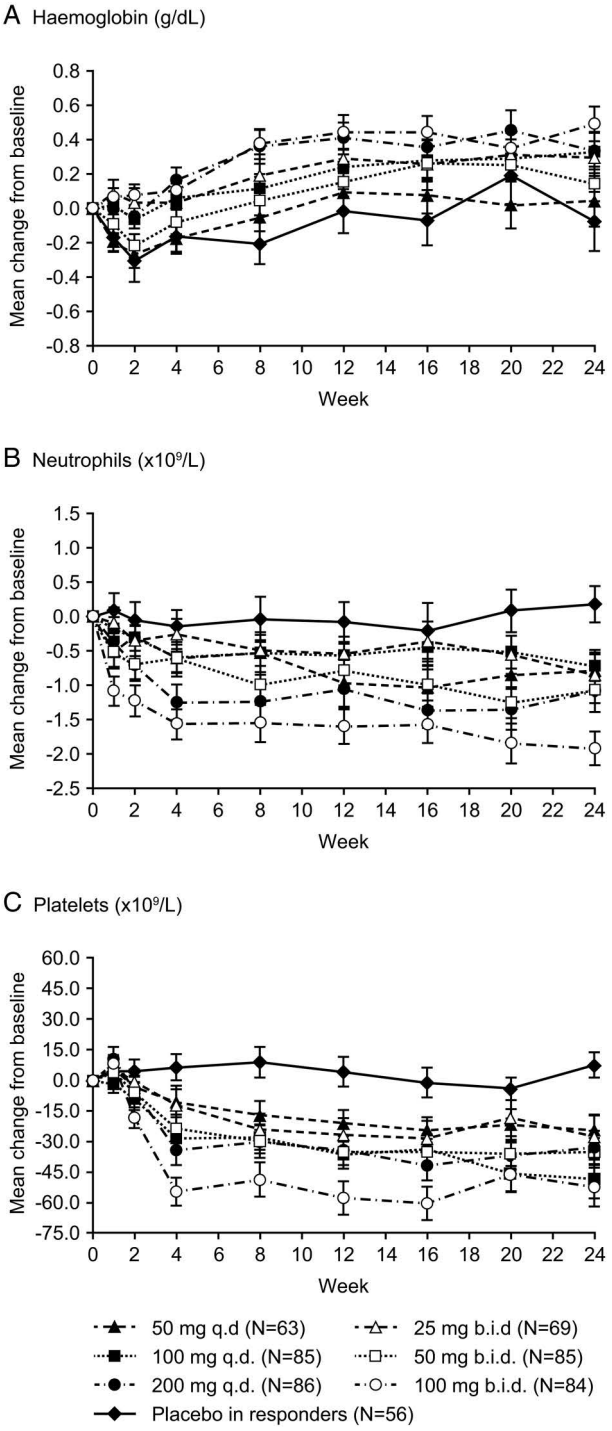

Figure 3 Mean (SE) change from baseline over time in patients considered to be responders (achieved a 20% improvement in swollen joint count based on 66 joints and tender joint count based on 68 joints) and who continued on the same treatment throughout the study for (A) haemoglobin (g/dL), (B) neutrophils (×10^9/L) and (C) platelets (×10^9/L). b.i.d., twice daily; N, number of subjects per group; q.d., once daily.

70 and DAS28 (CRP) responses, along with improvements in HRQoL (HAQ-DI); in addition to the convenience of oral administration, rapid action may facilitate effective treat-to-target strategies without the need for bridging glucocorticoids. The filgotinib doses studied and the similar efficacy noted between the once-daily and twice-daily dosing regimens

are in line with the previously reported pharmacokinetic and pharmacodynamic effects of filgotinib and its major metabolite, both of which selectively inhibit JAK1.[13] Although there was a numerical trend towards better efficacy results with the 200 mg dose given as 100 mg twice daily versus 200 mg once daily, this trend did not extend to the next (lower) dose level of 100 mg, where the reverse trend was observed, such that the once-daily schedule generally performed better than the split dose. In terms of safety, there were no major differences in terms of AEs between the once-daily and twice-daily regimens.

Harmful complications would be expected if any member of the JAK family is completely inhibited, as exemplified by the relationship between JAK3 deficiency and severe combined immunodeficiency.[19] However, with small-molecule inhibitors selective for particular JAK enzymes, the heterodimeric pairing of enzymes and the unique pharmacological profile of a given small molecule makes AEs difficult to predict.[20 21] In the current study, filgotinib was well tolerated at all doses evaluated. Although infections were the most frequent AE, few were serious AEs and overall were infrequent; few AEs led to discontinuation. Importantly, no cases of TB or opportunistic infections were reported. Careful monitoring and management of infections will be required in future studies of filgotinib. Small, dose-dependent changes in mean laboratory values were observed, including increases in mean haemoglobin and decreases in mean neutrophil counts; however, the latter were without clinical consequence. No reductions in absolute lymphocyte counts were observed, and there were no dose-dependent changes in mean NK cell counts. The dose-dependent increase in mean haemoglobin can be attributed to the decrease in inflammation resulting from a therapeutic effect and the lack of any associated JAK2 inhibitory effect.[22] A dose-dependent decline in platelet counts was observed; however, platelet counts plateaued at week 4 and remained relatively stable thereafter. This observation contrasts with the dose-dependent platelet count increase seen in the 24-week phase IIb study of the JAK1/2 inhibitor baricitinib in patients receiving MTX.[20] Small increases in mean creatinine concentration were not associated with clinical consequences and the effect of filgotinib co-administered with MTX on liver parameters was minimal. Although dose-dependent increases in both HDL and LDL cholesterol were observed in all filgotinib groups, the LDL: HDL ratio fell. This is in contrast to results seen with some RA treatments that preferentially increase LDL, thereby worsening the atherogenic index.[21 23]

The chief limitation of the study was its short (24 weeks) duration, hampering definite judgement of longer maintenance of efficacy and eventual side effects. Furthermore, radiographic assessments were not included in the study design, so the impact of filgotinib on the structure of bones and joints could not be evaluated.

In conclusion, the results of this phase IIb study of filgotinib, added to a stable background dose of MTX, demonstrate clinically relevant dose-dependent improvements in the signs and symptoms of active RA. At a daily dose of 200 mg filgotinib, these improvements were initiated rapidly and were sustained throughout 24 weeks of treatment, regardless of whether a once-daily or twice-daily dosing regimen was used. These robust data support the future development of filgotinib for the treatment of active RA in patients receiving MTX treatment.

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Patient consent Obtained.

Ethics approval The study was conducted in accordance with the ethical principles of the Declaration of Helsinki, Good Clinical Practice, International Council for Harmonisation guidelines, and all applicable national and local laws and regulatory requirements.

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EXTENDED REPORT

Filgotinib (GLPG0634/GS-6034), an oral selective JAK1 inhibitor, is effective as monotherapy in patients with active rheumatoid arthritis: results from a randomised, dose-finding study (DARWIN 2)

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ABSTRACT

Objectives To evaluate the efficacy and safety of different doses of filgotinib, an oral Janus kinase 1 inhibitor, as monotherapy in patients with active rheumatoid arthritis (RA) and previous inadequate response to methotrexate (MTX).

Methods In this 24-week phase IIb study, patients with moderately to severely active RA were randomised (1:1:1:1) to receive 50, 100 or 200 mg filgotinib once daily, or placebo, after a ≥4-week washout from MTX. The primary end point was the percentage of patients achieving an American College of Rheumatology (ACR) 20 response at week 12.

Results Overall, 283 patients were randomised and treated. At week 12, significantly more patients receiving filgotinib at any dose achieved ACR20 responses versus placebo (≥65% vs 29%, p<0.001). For other key end points at week 12 (ACR50, ACR70, ACR-N, Disease Activity Score based on 28 joints and C reactive protein, Clinical Disease Activity Index, Simplified Disease Activity Index and Health Assessment Questionnaire-Disability Index) significant differences from baseline in favour of filgotinib 100 and 200 mg versus placebo were seen; responses were maintained or improved through week 24. Rapid onset of action was observed for most efficacy end points. Dose-dependent increases in haemoglobin were observed. The percentage of patients with treatment-emergent adverse events (TEAE) was similar in the placebo and filgotinib groups (~40%). Eight patients on filgotinib and one on placebo had a serious TEAE, and four patients, all of whom received filgotinib, experienced a serious infection. No tuberculosis or opportunistic infections were reported.

Conclusions Over 24 weeks, filgotinib as monotherapy was efficacious in treating the signs and symptoms of active RA, with a rapid onset of action. Filgotinib was generally well tolerated.

Trial registration number NCT01894516.

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INTRODUCTION

The disease-modifying antirheumatic drug (DMARD) methotrexate (MTX) is a cornerstone of rheumatoid arthritis (RA) treatment, improving outcomes in many patients.[1] However, even with optimal dosing only around 50% of patients respond adequately to MTX.[2 3]

Janus kinase (JAK) inhibitors are low molecular weight, orally available products that can impact intracellular molecules involved in the signalling of various cytokines, growth factors and hormones, such as pro-inflammatory cytokine interleukin-6.[4] The JAK1/JAK3, JAK2 inhibitor tofacitinib has been approved by the Food and Drug Administration for second-line use after MTX in patients with moderately to severely active RA.[5] A number of other JAK inhibitors are currently in development for the management of RA, with differing in vitro specificities towards the various members of the JAK family.[6]

Filgotinib (GLPG0634/GS-6034) is a potent and selective inhibitor of JAK1,[7] which is currently under investigation for the treatment of RA and inflammatory bowel disease.[7–10] The efficacy and safety of filgotinib in patients with RA has previously been investigated in two short-term phase IIa studies, as add-on treatment to MTX, which suggested that filgotinib has the potential to be effective when administered as a once-daily dosing regimen.[11–13] In pharmacokinetic-pharmacodynamic studies, filgotinib was shown to have a terminal half-life ($t_{1/2}$) of 5–11 hours with an active metabolite that has a $t_{1/2}$ of 21–27 hours; both moieties contribute to the pharmacodynamic effects and together provide a relatively long duration of JAK1 inhibition,[10] supporting further investigation of a once-daily dosing regimen.

The DARWIN 2 study reported here was conducted to evaluate the efficacy and safety of varying once-daily doses of filgotinib, administered as monotherapy in patients with moderately to severely active RA, who had an inadequate response to previous MTX treatment.

METHODS

Study design and treatments

This was a 24-week, multicentre, randomised, double-blind, phase IIb, dose-finding study of orally administered filgotinib as monotherapy (ClinicalTrials.gov identifier: NCT01894516). The study was conducted at 59 centres in 18 countries (Argentina, Austria, Bulgaria, Chile, Columbia, Germany, Guatemala, Hungary, Latvia, Mexico, Moldova, New Zealand, Poland, Romania, Russian Federation, Spain, Ukraine and the USA) across four predefined geographic regions. Eligible patients were randomly assigned to treatment using a computerised interactive voice and web response system

(IXRS) (S-Clinica, 6, Chaussée de Boondael, 1050 Brussels, Belgium), to receive placebo or 1 of 3 filgotinib regimens (50, 100 or 200 mg), each as two capsules administered once daily in the morning), in a 1:1:1:1 ratio, stratified by region and previous use of biological DMARDs (bDMARDs). At each study visit, numbered kits containing medication were dispensed via the IXRS system. Patients, investigators, study coordinators, the sponsor and study team were blinded to treatment assignment. At week 12, all patients in the placebo group, and patients in the filgotinib 50 mg group who had not achieved at least a 20% improvement in swollen joint count based on 66 joints (SJC66) and tender joint count based on 68 joints (TJC68), were reassigned to receive filgotinib 100 mg and continued on this dose until week 24.

The study was conducted in accordance with the ethical principles based on the Declaration of Helsinki, Good Clinical Practice, International Council for Harmonisation guidelines and all applicable national and local laws and regulatory requirements.

Patients

Enrolled patients were ≥18 years of age with a diagnosis of RA for ≥6 months prior to screening and met the 2010 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) criteria for RA and ACR functional class I–III, had ≥6/SJC66 and ≥8/TJC68, a screening serum C reactive protein (CRP) ≥0.7×upper limit of laboratory normal range (ULN) (changed in May 2014 from 1.5×ULN to facilitate recruitment), had shown an inadequate response to MTX (in the opinion of the treating physician) and agreed to be washed out from MTX for ≥4 weeks before or during screening. Patients receiving oral glucocorticoids (≤10 mg/day) or non-steroidal anti-inflammatory drugs were on a stable dose for ≥4 and ≥2 weeks, respectively, prior to baseline. Enrolled patients were also required to be using a medically acceptable means of contraception. Details of laboratory-defined inclusion criteria are listed in the online supplementary materials.

Patients were excluded if they were receiving current therapy with any DMARD (with the exception of antimalarials), or had previous RA treatment with a bDMARD. The only exception to this was if the biological agent had been received in a single clinical study more than 6 months prior to enrolment and if the drug had been effective. Patients were also excluded if they had ever used any kind of JAK inhibitor, had previously used a cytotoxic agent other than MTX or had received intra-articular or parenteral corticosteroid injection within 4 weeks of screening. Patients who were pregnant were excluded, as were patients who were immunocompromised and, in the opinion of the investigator, participation in the study would pose an unacceptable risk. Further details of the exclusion criteria, including a list of infections that precluded enrolment in the study, are listed in the online supplementary materials. All patients provided written informed consent prior to study participation.

Outcomes and assessments

Efficacy and disease activity assessments were performed at screening (joint counts and Patient's Global Assessment of Disease Activity), baseline and at weeks 1, 2, 4, 8, 12, 16, 20 and 24. The primary efficacy end point was the percentage of patients achieving an ACR20 response at week 12. Key secondary end points were the percentages of patients achieving ACR20, ACR50, ACR70 and ACR-N responses, Disease Activity Score based on 28 joints and CRP value (DAS28 (CRP)), including remission and Low Disease Activity (LDA)/remission), EULAR response, ACR/EULAR remission, Clinical Disease Activity Index (CDAI) and Simplified Disease Activity Index (SDAI) compared with placebo at every visit from baseline to week 24. Health-related quality of life (HRQoL) was assessed with the Health Assessment Questionnaire-Disability Index up to week 24.

Safety variables included adverse events (AEs) throughout the study period; vital signs (at each visit); physical examinations (at screening, baseline, week 12 and week 24) and 12-lead ECG (at screening, week 12 and week 24). Haematology and clinical chemistry laboratory assessments were performed at each visit. The National Institutes of Health Common Terminology Criteria for Adverse Events (CTCAE) V.3.0 was used to describe laboratory changes during the study.

Sample sizes and statistical analyses

All randomised patients who received at least one dose of study drug were included in the intent-to-treat (ITT) and safety populations. All patients in the placebo group and patients who were considered non-responders on filgotinib 50 mg once daily and were switched to 100 mg once daily at week 12, were handled as discontinuations and data were imputed from week 12 onwards. Efficacy data were analysed using non-responder imputation (NRI) for the ITT population and confirmed using last observation carried forward (LOCF) and observed case imputations in the ITT population, and NRI and LOCF imputations in the per-protocol population.

The primary analysis was conducted using a logistic regression model including treatment, region and previous use of bDMARDs as covariates. Continuous parameters were analysed using analysis of covariance. Time-to-first response (ACR20/50/70) was analysed using Kaplan-Meier survival techniques, with treatment groups compared with placebo using a Cox proportional hazard regression model. Treatment versus placebo comparisons were carried out for each dose group using Hommel's closed-testing correction procedure to adjust for multiplicity.

For the safety analysis, two periods were analysed: the period up to week 12 including the four original groups, and the full 24-week period including five treatment groups (two switched groups and three continued groups).

A sample size of 280 patients (70 patients in each arm) was estimated to provide 90% power to detect a minimum 28%–30% treatment effect versus placebo, assuming a 15%–40% placebo ACR20 response at week 12.

RESULTS

The study was initiated in October 2013 and completed in May 2015. Of the 625 patients screened, 287 were randomised to receive treatment, and 283 received at least one dose of study drug and were included in the ITT and safety populations (figure 1). At week 12, all patients in the placebo group (n=65) and 15 non-responding patients in the filgotinib 50 mg group were re-allocated to filgotinib 100 mg. The overall treatment discontinuation rate was low (n=26, 9.2%) and there were no significant differences in discontinuation rate between filgotinib and placebo groups, with no dose-response relationship apparent with respect to discontinuation rates. Demographic and baseline disease characteristics were well balanced between the different treatment groups (table 1), apart from a numerically higher mean CRP in the placebo group (not statistically significant, p=0.0865). Overall, 167/283 (59%) patients received concomitant systemic corticosteroid treatment and 12/283 (4.2%) patients received concomitant antimalarial treatment during the study.

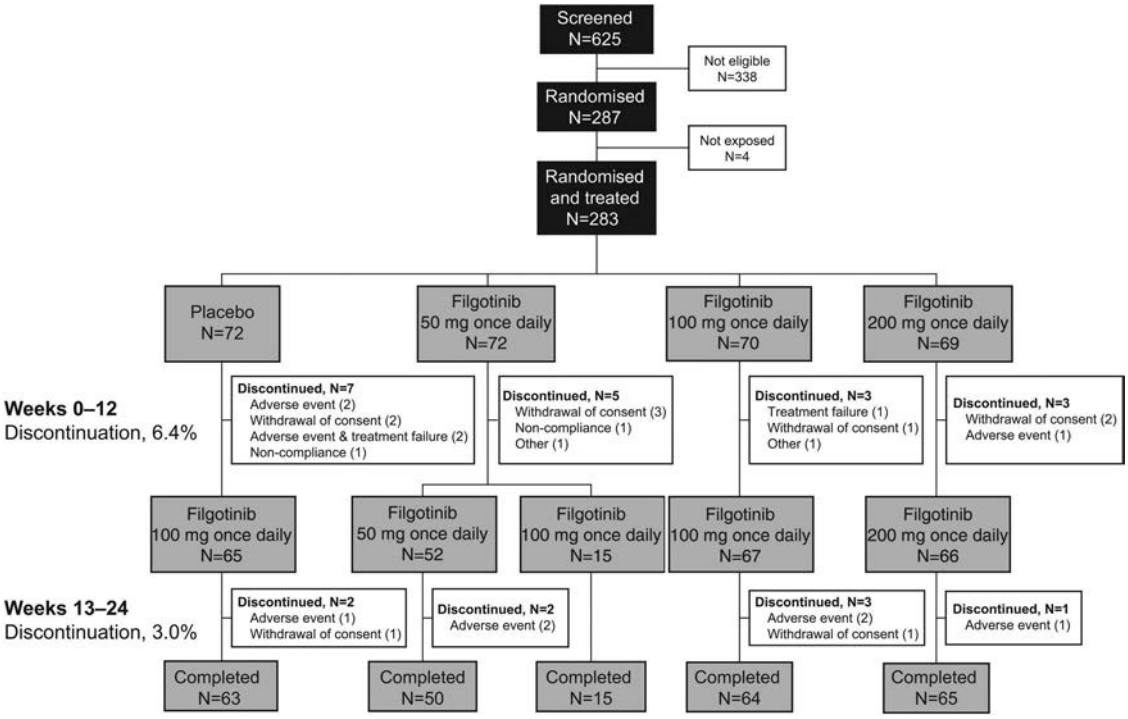

Figure 1 Patient disposition. The study was conducted at 59 centres in 18 countries (Argentina, Austria, Bulgaria, Chile, Columbia, Germany, Guatemala, Hungary, Latvia, Mexico, Moldova, New Zealand, Poland, Romania, Russian Federation, Spain, Ukraine and the USA).

Efficacy

Primary efficacy analysis

The primary end point of the study was met: at week 12, a statistically significantly higher proportion of patients in all the filgotinib treatment groups (50 mg, 67% (48/72); 100 mg, 66% (46/70); 200 mg, 73% (50/69)) achieved an ACR20 response compared with placebo (29% (21/72)) (all $p<0.0001$; figure 2A). Raw ACR20 data for each time point are presented in online supplementary table S1.

Secondary efficacy analyses

At week 12, a statistically significantly greater proportion of patients had an ACR50 response in all filgotinib dose groups compared with placebo ($p<0.05$; figure 2B). In the 100 and 200 mg groups, patients were more likely to achieve ACR70 compared with placebo (figure 2C). These responses persisted (ACR50) or even improved (ACR70) up to week 24. The ACR20 response appeared to plateau around week 8 and was maintained throughout the rest of the study to week 24 (figure 2A).

An early onset of response was observed for ACR20 (statistically significantly higher at week 1 in the filgotinib 200 mg group figure 2A), ACR50 (from week 2 in the filgotinib 200 mg once-daily dose group) and ACR70 (week 4 in the filgotinib 200 mg once-daily dose group). Patients randomised to placebo and those on filgotinib 50 mg who were considered non-responders and were switched to filgotinib 100 mg at week 12 showed increased ACR20/50/70 responses at week 24, similar to the week 12 responses seen in patients randomised to receive filgotinib 100 mg from baseline (see online supplementary figure S1).

At week 12, there was a significantly greater mean decrease in DAS28 (CRP) for all filgotinib groups, compared with placebo, which was maintained in the 50 mg dose group and showed a small improvement in the higher dose groups at week 24 (figure 2D). These improvements in DAS28 (CRP) versus placebo were evident at week 1 in all filgotinib groups. A similar pattern in terms of disease activity was observed for CDAI (figure 2E). Improvements in HRQoL were noted as early as week 2 for the filgotinib 200 mg group; by week 12, significant improvements from baseline were noted in all active treatment groups (figure 2F). Raw data for each of the secondary efficacy end points illustrated in figure 2 are presented in online supplementary table S1.

There were trends in favour of the active treatment across various definitions of disease remission and responses to treatment; statistical significance versus placebo was observed at week 12 for all filgotinib dose groups for ACR-N, DAS28 (CRP) EULAR 'good' responses and SDAI (table 2). Higher doses of filgotinib were generally associated with the most substantial reductions in disease activity and the highest disease remission rates (table 2).

There were too few patients in each dose group who had previously received and responded to a biological agent to make valid comparisons of the efficacy of filgotinib in this patient population, versus patients who were naïve to biological treatments.

Safety

Adverse events

Treatment-emergent AEs (TEAEs) were reported at similar frequencies across all groups from baseline to week 12 (the period during which placebo could be compared directly with active treatment) and to week 24 (table 3). Of nine serious TEAEs occurring up to week 24, there were four serious infections. Overall, three serious TEAEs occurred in the filgotinib 200 mg group (back pain, osteoarthritis, pneumonia), two occurred in the filgotinib 100 mg group (cellulitis, vertigo), two in the filgotinib 50 mg group (gastroenteritis, humerus fracture) and two in the placebo group (RA worsening (prior to switching treatment), and chronic pyelonephritis) (table 3). A greater proportion of TEAEs were considered related to study treatment in the

Table 1 Baseline patient demographics, disease characteristics and treatment history

	Placebo N=72	Filgotinib once daily 50 mg N=72	100 mg N=70	200 mg N=69
Patient demographics				
Age, mean (SE), years	52 (1.4)	52 (1.6)	53 (1.4)	52 (1.4)
Female, n (%)	56 (77.8)	62 (86.1)	53 (75.7)	60 (87.0)
Disease characteristics				
Duration of RA, mean (SE), years	10 (0.8)	9 (0.8)	9 (0.8)	9 (1.0)
Anti-CCP positive, n (%)	58 (80.6)	56 (77.8)	54 (77.1)	57 (82.6)
RF positive, n (%)	57 (79.2)	53 (73.6)	51 (72.9)	50 (72.5)
DAS28 (CRP), mean (SE)	6.22 (0.099)	6.03 (0.105)	6.18 (0.101)	6.09 (0.102)
CDAI, mean (SE)	42 (1.3)	41 (1.5)	44 (1.5)	42 (1.4)
SDAI, mean (SE)	46 (1.5)	44 (1.6)	47 (1.7)	44 (1.5)
ACR components				
CRP, mean (SE), mg/L	35.26 (4.434)	24.67 (3.257)	25.55 (4.247)	23.16 (2.492)
TJC68, mean (SE)	25.23 (1.480)	25.58 (1.620)	27.20 (1.770)	26.24 (1.506)
TJC28, mean (SE)	15.85 (0.715)	15.30 (0.790)	16.52 (0.820)	16.58 (0.750)
SJC66, mean (SE)	15.98 (0.853)	16.97 (1.074)	18.65 (1.418)	15.74 (1.047)
SJC28, mean (SE)	12.18 (0.596)	12.55 (0.710)	13.19 (0.760)	11.58 (0.654)
HAQ-DI total score, mean	1.80 (0.058)	1.84 (0.068)	1.81 (0.068)	1.80 (0.063)
Patient's global assessment, mean (SE)	71.1 (2.02)	68.6 (2.41)	71.5 (2.23)	68.9 (2.07)
Investigator's global assessment, mean (SE)	70.4 (1.73)	68.2 (1.73)	72.0 (1.59)	67.7 (1.86)
Patient's pain, mean (SE)	71.6 (2.37)	71.0 (2.38)	72.6 (1.85)	68.1 (2.35)
Treatments				
Corticosteroids, n (%)	45 (62.5)	47 (65.3)	50 (71.4)	47 (68.1)
Duration of corticosteroid use, mean (SE), years	4.59 (0.791)	5.31 (0.771)	3.39 (0.528)	5.41 (0.757)
Previous biological DMARD use, n (%)	3 (4.2)	7 (9.7)	4 (5.7)	5 (7.2)
Previous conventional DMARD use*, n (%)	71 (98.6)	70 (97.2)	68 (97.1)	67 (97.1)
MTX, n (%)	60 (83.3)	61 (84.7)	59 (84.3)	58 (84.1)
MTX-sodium, n (%)	5 (6.9)	4 (5.6)	7 (10.0)	6 (8.7)
Duration of prior MTX use, mean (SE), years	4.86 (0.656)	4.44 (0.557)	3.55 (0.464)	3.85 (0.435)

*Patients had MTX washed out for ≥4 weeks before or during screening.
ACR, American College of Rheumatology; CCP, cyclic citrullinated peptide; CDAI, Clinical Disease Activity Index; CRP, C reactive protein; DAS28, Disease Activity Score based on 28 joints; DMARD, disease-modifying antirheumatic drug; HAQ-DI, Health Assessment Questionnaire-Disability Index; MTX, methotrexate; N, number of patients per treatment group; n, number of patients per category; RA, rheumatoid arthritis; RF, rheumatoid factor; SDAI, Simple Disease Activity Index; SJC28, swollen joint count based on 28 joints; SJC66, swollen joint count based on 66 joints; TJC28, tender joint count based on 28 joints; TCJ68, tender joint count based on 68 joints.

filgotinib groups compared with placebo (table 3). Occurrences of TEAEs leading to discontinuation were similarly rare in all treatment groups, with a greater proportion of TEAEs leading to discontinuations in the placebo group (table 3). There was one case of herpes zoster during the study, which occurred during weeks 12–24 of the study in a patient receiving filgotinib 50 mg once daily; the infection resolved after 10 days. No cases of TB, opportunistic infections, lymphoma or cancer were reported throughout the 24-week treatment period.

Haematology

Up to week 12, increases in mean haemoglobin concentrations were observed in the filgotinib 100 and 200 mg groups (up to 0.39 g/dL, a 3.4% increase in the 200 mg group). These remained stable to week 24. In patients who were switched to filgotinib 100 mg at week 12, there were increases in mean haemoglobin concentration between weeks 12 and 24 (table 4, see online supplementary figure S2). Decreases in mean neutrophil counts were seen in filgotinib groups through to week 4 that appeared to plateau thereafter and remained stable until week 24, with the exception of an overall decrease to week 24 in patients who were switched from filgotinib 50 to 100 mg (see online supplementary figure S2). Two patients experienced CTCAE grade 3 abnormally low neutrophil counts during filgotinib treatment (neither with concomitant infections); both continued their filgotinib treatment. Overall, more increases than decreases were observed in mean absolute lymphocyte counts, and there were no apparent correlations between treatment groups and mean lymphocyte counts over time (table 4). There were no decreases from baseline in lymphocyte subsets, including mean natural killer (NK) cell counts. Three patients on filgotinib treatment experienced CTCAE grade 3 abnormally low lymphocyte counts (table 3). One patient had an abnormally low lymphocyte count at baseline and the other two had a coinciding mild infection (urinary tract and pharyngitis). Five patients discontinued the study due to lymphopenia (two subjects receiving placebo and three subjects receiving filgotinib), as per protocol stopping rule of precautionary discontinuation for two sequential lymphocyte counts $<0.75\times10^9$ cells/L. Absolute platelet counts decreased in all filgotinib groups by week 4, but thereafter the counts remained stable up to week 24 (decreases from baseline of 44.7 and 22.3 $\times10^9$/L, respectively, in the filgotinib 100 and 200 mg groups) (table 4).

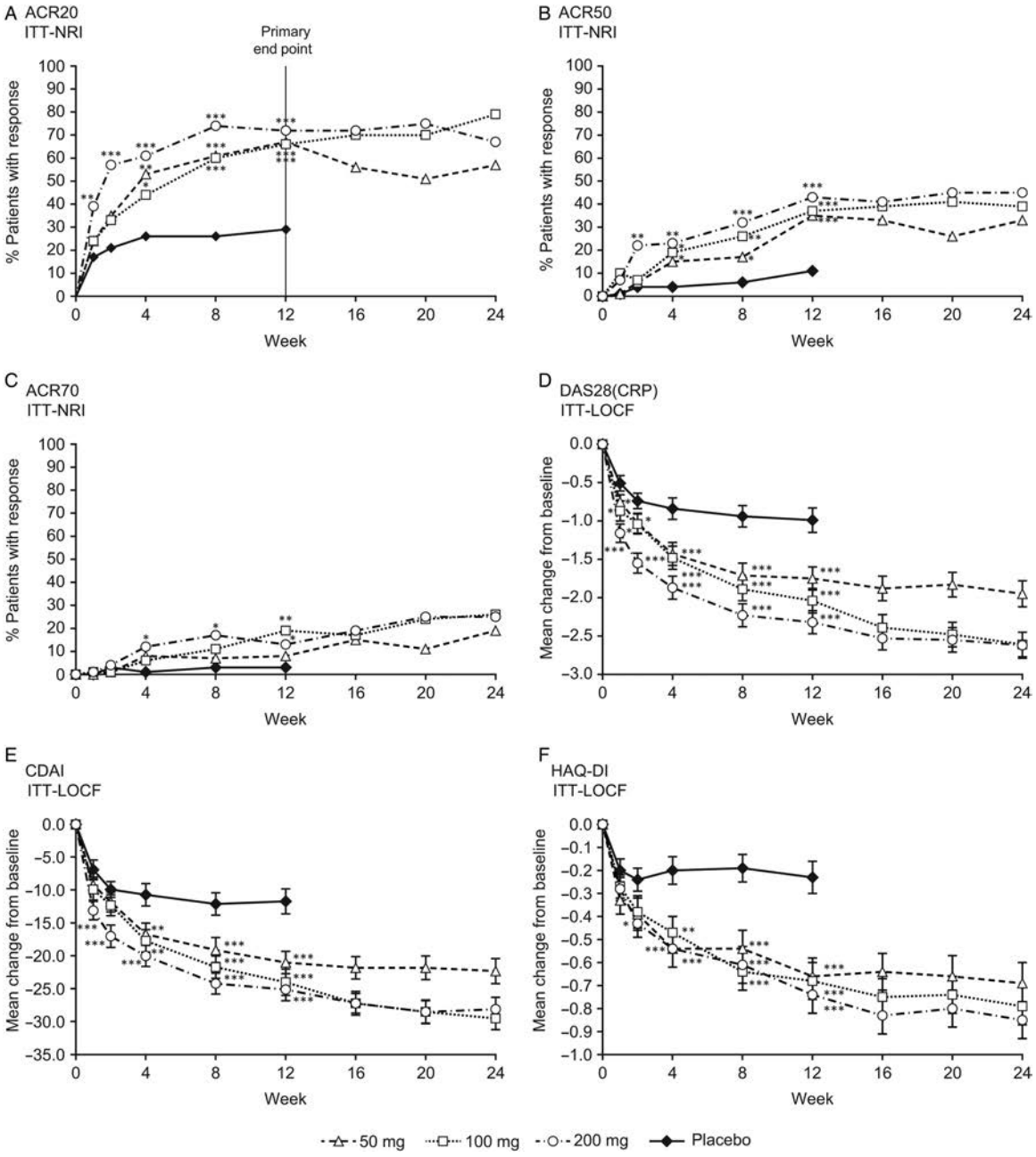

Figure 2 Efficacy end points. The percentage of patients achieving an improvement in American College of Rheumatology (ACR) of (A) 20% (ACR20); (B) 50% (ACR50); (C) 70% (ACR70) over time though 24 weeks; (D) mean change from baseline in DAS28 (CRP) over time; (E) mean change from baseline CDAI over time; (F) mean change from baseline in HAQ-DI over time. The vertical line at 12 weeks in 2A indicates the primary efficacy time point (NRI (ITT population)). Patients who switched treatment at week 12 are treated as if they discontinued treatment at week 12. *p<0.05; **p<0.01; ***p<0.001. CDAI, Clinical Disease Activity Index; CRP, C reactive protein; DAS, Disease Activity Score; HAQ-DI, Health Assessment Questionnaire-Disability Index; ITT, intent-to-treat; LOCF, last observation carried forward; NRI, non-responder imputation.

Clinical chemistry

Up to week 4, dose-dependent increases were observed in mean creatinine concentrations with filgotinib (see online supplementary figure S2); these subsequently plateaued in the filgotinib 100 and 200 mg groups and remained stable until week 24, with overall increases of up to 3.5 µmol/L in the filgotinib 200 mg group. Mean lipase concentrations remained stable throughout the study in all treatment groups, although for the filgotinib 200 mg group a small increase from baseline was observed in mean concentrations at all time points (increase of 2.9 U/L from 26.4 U/L at baseline in the filgotinib 200 mg once-daily group as of week 24). Mean alanine transaminase (ALT) and aspartate transaminase (AST) concentrations remained stable throughout the study in all treatment groups (table 4) with the exception of two high values (one in the continued filgotinib 100 mg group and one in the group switching from placebo to filgotinib 100 mg) (data not shown). One patient in the filgotinib 200 mg group had a CTCAE grade 3 abnormally high total cholesterol value. One patient had CTCAE grade 3 increases in both ALT and AST, one patient had a CTCAE grade 3 increase in AST (table 3); no patients discontinued their study treatment because of ALT or AST elevations. Mean levels of total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol increased in the filgotinib

Table 2 Efficacy assessments at weeks 12 and 24 (NRI (ITT population) and LOCF (ITT population))

		Filgotinib once daily		
	Placebo N=72	50 mg N=72	100 mg N=70	200 mg N=69
ACR20†				
W12, n (%)	21 (29.2)	48 (66.7)***	46 (65.7)***	50 (72.5)***
W24, n (%)	–	41 (56.9)	55 (78.6)	46 (66.7)
ACR50†				
W12, n (%)	8 (11.1)	25 (34.7)***	26 (37.1)***	30 (43.5)***
W24, n (%)	–	24 (33.3)	27 (38.6)	31 (44.9)
ACR70†				
W12, n (%)	2 (2.8)	6 (8.3)	13 (18.6)**	9 (13.0)*
W24, n (%)	–	14 (19.4)	18 (25.7)	17 (24.6)
ACR-N‡				
W12, mean change (SE)	16.28 (2.723)	35.03 (3.178)***	38.35 (3.533)***	41.00 (3.477)***
W24, mean change (SE)	–	38.75 (3.748)	46.32 (3.295)	46.78 (3.648)
CRP‡				
W12, mean change (SE), mg/L	−8.71 (4.273)	−4.43 (4.741)	−12.25 (4.516)*	−14.85 (2.680)**
W24, mean change (SE), mg/L	–	−9.16 (3.119)	−14.81 (4.211)	−15.17 (2.515)
TJC68‡				
W12, mean change (SE)	−5.8 (1.48)	−12.7 (1.38)***	−15.1 (1.53)***	−17.4 (1.48)***
W24, mean change (SE)	–	−13.9 (1.48)	−20.3 (1.64)	−19.1 (1.55)
TJC28‡				
W12, mean change (SE)	−4.1 (0.88)	−7.6 (0.80)***	−8.8 (0.95)***	−10.7 (0.86)***
W24, mean change (SE)	–	−8.0 (0.80)	−12.0 (0.85)	−11.5 (0.89)
SJC66‡				
W12, mean change (SE)	−4.1 (1.22)	−9.3 (1.00)***	−11.4 (1.20)***	−10.5 (0.98)***
W24, mean change (SE)	–	−10.2 (1.12)	−13.8 (1.20)	−11.9 (0.95)
SJC28‡				
W12, mean change (SE)	−3.7 (0.78)	−7.2 (0.72)***	−8.1 (0.79)***	−7.4 (0.66)***
W24, mean change (SE)		−7.6 (0.76)	−9.6 (0.68)	−8.6 (0.64)
DAS28 (CRP)‡				
W12, mean change (SE)	−0.99 (0.162)	−1.75 (0.145)***	−2.04 (0.162)***	−2.32 (0.155)***
W24, mean change (SE)	–	−1.95 (0.168)	−2.61 (0.163)	−2.62 (0.165)
DAS28 (CRP) LDA‡				
W12, n (%)	5 (7)	8 (11)	9 (13)	19 (28)
W24, n (%)	–	12 (17)	20 (29)	13 (19)
DAS28 (CRP) remission†				
W12, n (%)	5 (6.9)	9 (12.5)	10 (14.3)	12 (17.4)
W24, n (%)	–	14 (19.4)	15 (21.4)	17 (24.6)
DAS28 (CRP) remission/LDA†				
W12, n (%)	10 (13.9)	17 (23.6)	19 (27.1)	31 (44.9)***
W24, n (%)	–	25 (34.7)	35 (50.0)	29 (42.0)
DAS28 (CRP) EULAR response‡§				
W12, n (%)				
Moderate	27 (38)	33 (46)	37 (53)	28 (41)
Good	10 (14)	17 (24)*	19 (27)***	31 (45)***
W24, n (%)				
Moderate	–	26 (36)	29 (41)	30 (43)
Good	–	26 (36)	35 (50)	32 (46)
ACR/EULAR remission†				
W12, n (%)	1 (1.4)	1 (1.4)	3 (4.3)	3 (4.3)
W24, n (%)	–	6 (8.3)	6 (8.6)	6 (8.7)
SDAI‡				
N	71	70	70	68
W12, mean change (SE)	−12.6 (1.98)	−21.4 (1.80)***	−25.3 (1.99)***	−26.5 (1.75)***
W24, mean change (SE)	–	−23.2 (1.94)	−31.0 (1.77)	−29.6 (1.86)

Continued

Table 2 Continued

	Placebo N=72	Filgotinib once daily 50 mg N=72	100 mg N=70	200 mg N=69
SDAI LDA‡				
W12, n (%)	7 (10)	20 (28)	14 (20)	23 (33)
W24, n (%)	–	21 (29)	26 (37)	26 (38)
SDAI remission†				
W12, n (%)	2 (2.8)	2 (2.8)	5 (7.1)	5 (7.2)
W24, n (%)	–	8 (11.1)	8 (11.4)	8 (11.6)
CDAI‡				
W12, mean change (SE)	−11.7 (1.88)	−21.0 (1.72)	−24.0 (1.97)	−25.1 (1.74)
W24, mean change (SE)	–	−22.3 (1.86)	−29.5 (1.69)	−28.1 (1.82)
CDAI LDA‡				
W12, n (%)	8 (11)	21 (29)	16 (23)	21 (30)
W24, n (%)	–	20 (28)	25 (36)	23 (33)
CDAI remission‡				
W12, n (%)	2 (2.8)	2 (2.8)	4 (5.7)	6 (8.7)
W24, n (%)	–	9 (12.5)	8 (11.4)	9 (13.0)
HAQ-DI‡				
W12, mean change (SE)	−0.226 (0.07)	−0.661 (0.08)***	−0.677 (0.08)***	−0.739 (0.08)***
W24, mean change (SE)		−0.690 (0.09)	−0.786 (0.08)	−0.850 (0.08)

*p<0.05; **p<0.01; ***p<0.001. Percentage responders was calculated based on the total number of subjects per group with a response (yes/no) at that time.
†NRI (ITT population).
‡LOCF (ITT population).
§Good: DAS28 (CRP) of ≤3.2 and improvement of >1.2; moderate: DAS28 (CRP) of >3.2 and improvement of >1.2 or DAS28 (CRP) of ≤5.1 and improvement of >0.6–1.2.
ACR, American College of Rheumatology; ACR-N, ACR N% improvement; CDAI, Clinical Disease Activity Index; CRP, C reactive protein; DAS-28, Disease Activity Score based on 28 joints and C reactive protein; HAQ-DI, Health Assessment Questionnaire-Disability Index; EULAR, European League Against Rheumatism; ITT, intent-to-treat; LDA, Low Disease Activity; LOCF, last observation carried forward; N, number of patients per group; n, number of patients with response/change; NRI, non-responder imputation; SDAI, Simplified Disease Activity Index; SJC28, swollen joint count based on 28 joints; SJC66, swollen joint count based on 66 joints; TJC28, tender joint count based on 28 joints; TCJ68, tender joint count based on 68 joints.

100 and 200 mg groups up to week 4, but plateaued and remained stable to week 24 (see online supplementary figure S2). The LDL:HDL ratio fell slightly over the study period indicating that proportional increases in HDL cholesterol were greater than those in LDL cholesterol. There were increases in mean triglycerides in filgotinib groups with no apparent dose relationship (data not shown).

DISCUSSION

In this study, clinical efficacy in patients treated with filgotinib monotherapy provided statistically significant, dose-dependent improvements in the signs and symptoms of active RA, and clinical improvements were evident soon after active treatment had begun. Clinical improvements were paralleled by rapid improvements in HRQoL.

Consistent with previous studies,[13] filgotinib was well tolerated at all the doses evaluated, with similar proportions of patients experiencing TEAEs in the placebo and filgotinib treatment groups. Serious AEs and those leading to study discontinuation were relatively low: of the 283 patients treated overall in this study, 9 had a serious TEAE (8 receiving filgotinib) and 11 subjects had ≥1 TEAE leading to permanent study discontinuation. There were four serious infections occurring in subjects receiving filgotinib and this risk warrants further evaluation in future clinical trials. Increases in mean haemoglobin were observed in filgotinib treatment groups, along with reductions in neutrophils, but these were mostly considered by investigators as without clinical consequence and did not lead to study discontinuation. In agreement with the short-term phase IIa studies of filgotinib, no reduction in lymphocyte or NK cell counts was observed[8] and a small decline in platelet counts plateaued at week 4 but remained stable thereafter. Although small (up to 3.5 µmol/L in the highest dose group) increases in creatinine were observed with filgotinib, effects on the liver were minimal. AST and ALT levels were stable throughout the study. In line with other studies, increases in both HDL and LDL cholesterol were seen with filgotinib treatment, however, the LDL:HDL ratio fell during the study, thereby indicating greater increases in HDL compared with LDL. Throughout the study, CTCAE grade 3 or 4 laboratory abnormalities were infrequent (11 events reported overall), and in most cases did not lead to study discontinuation.

Among the limitations of the study, the duration of the placebo control period was only 12 weeks because of the ethical implications inherent in continuing patients with active RA on placebo for a longer duration. As the therapeutic options of RA continue to evolve, from an ethical perspective, future studies would ideally use an active comparator instead of placebo to evaluate the efficacy and safety of novel compounds, such that no patient with moderately to severely active RA is left without potentially efficacious medication. Although the placebo period ended at 12 weeks, the study remained double blind to dose through to 24 weeks. The relatively short (24 weeks) duration of the study also limits interpretation of side effects. Radiographic assessments were not included in the study design, so the impact of filgotinib on the physical structure of bones and joints could not be evaluated. In conclusion, the results of this phase IIb study of filgotinib without background MTX treatment demonstrate improvements in the signs and symptoms of active RA, with an early onset, sustained effect and an acceptable safety

Table 3 Summary of TEAEs in all patients, baseline–week 12, patients who continued on the same treatment from baseline to week 24 and patients who switched treatment group, week 12–week 24

	Baseline–week 12				Continued treatment Baseline–week 24*			Switchers Week 12–week 24†	
Patients with:	**Placebo (N=72)**	**Filgotinib 50 mg (N=72)**	**Filgotinib 100 mg (N=70)**	**Filgotinib 200 mg (N=69)**	**Filgotinib 50 mg (N=57)**	**Filgotinib 100 mg (N=70)**	**Filgotinib 200 mg (N=69)**	**Placebo to filgotinib 100 mg (N=65)**	**Filgotinib 50 mg to 100 mg (N=15)**
TEAE, n (%)	28 (38.9)	29 (40.3)	23 (32.9)	30 (43.5)	30 (52.6)	31 (44.3)	35 (50.7)	10 (15.4)	4 (26.7)
Serious TEAE, n (%)	1 (1.4)	1 (1.4)	0	3 (4.3)	2 (3.5)	2 (2.9)	3 (4.3)	1 (1.5)	0
Serious TE infection, n (%)‡	0	1 (1.4)	0 (0)	1 (1.4)	1 (2)	1 (1)	1 (1)	1 (1.5)	0 (0)
Death, n (%)	0	0	0	0	0	0	0	0	0
Related TEAE, n (%)	7 (9.7)	11 (15.3)	7 (10.0)	9 (13.0)	14 (24.6)	12 (17.1)	12 (17.4)	5 (7.7)	1 (6.7)
TEAE leading to permanent discontinuation of study treatment, n (%)	4 (5.6)	1 (1.4)	0 (0)	1 (1.4)	2 (3.5)	2 (2.9)	2 (2.9)	1 (1.5)	0
TE laboratory abnormalities, n (%)									
Decreased haemoglobin, g/dL									
Grade 1 (10, LLN)	18 (25)	15 (20.8)	1 (10)	6 (8.7)	12 (21.1)	9 (12.9)	8 (11.6)	19 (26.4)	5 (33.3)
Grade 2 (<10–8)	1 (1.4)	3 (4.2)	2 (2.9)	1 (1.4)	4 (7.0)	4 (5.7)	1 (1.4)	1 (1.4)	1 (6.7)
Grade 3 (<8–6.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Grade 4 (<6.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Decreased neutrophils, $\times 10^9$/L									
Grade 1 (1.5, LLN)	1 (1.4)	0 (0)	0 (0)	2 (2.9)	2 (3.5)	2 (2.9)	3 (4.3)	2 (2.8)	0 (0)
Grade 2 (<1.5–1.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Grade 3 (<1.0–0.5)	0 (0)	0 (0)	1 (1.4)	1 (1.4)	0 (0)	1 (1.4)	1 (1.4)	0 (0)	0 (0)
Grade 4 (<0.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Decreased lymphocytes, $\times 10^9$/L									
Grade 1 (0.8, LLN)	1 (1.4)	1 (1.4)	2 (2.9)	2 (2.9)	1 (1.8)	2 (2.9)	1 (1.4)	1 (1.4)	0 (0)
Grade 2 (<0.8–0.5)	5 (6.9)	2 (2.8)	2 (2.9)	1 (1.4)	2 (3.5)	5 (7.1)	6 (8.7)	5 (6.9)	2 (13.3)
Grade 3 (<0.5–0.2)	0 (0)	1 (1.4)	0 (0)	0 (0)	1 (1.8)	1 (1.4)	0 (0)	1 (1.5)	0 (0)
Grade 4 (<0.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Decreased platelets, $\times 10^9$/L									
Grade 1 (75, LLN)	0 (0)	0 (0)	0 (0)	1 (1.4)	0 (0)	1 (1.4)	1 (1.4)	0 (0)	0 (0)
Grade 2 (<75–50)	0 (0)	0 (0)	1 (1.4)	0 (0)	0 (0)	1 (1.4)	0 (0)	0 (0)	0 (0)
Grade 3 (<50–25)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Grade 4 (<25)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NK cells (CD16–CD56), $\times 10^9$/L									
Decrease to <LLN	6 (8.3)	4 (5.6)	3 (4.3)	3 (4.3)	4 (7.0)	4 (5.7)	5 (7.2)	6 (8.3)	0 (0)
Increase to >ULN	0 (0)	1 (1.4)	0 (0)	2 (2.9)	2 (3.5)	1 (1.4)	5 (7.2)	2 (2.8)	0 (0)
Elevated creatinine, μmol/L									
Grade 1 (1–1.5×ULN)	0 (0)	1 (1.4)	0 (0)	3 (4.3)	2 (3.5)	0 (0)	3 (4.3)	1 (1.4)	0 (0)
Grade 2 (1.5–3×ULN)	0 (0)	0 (0)	1 (1.4)	1 (1.4)	0 (0)	1 (1.4)	1 (1.4)	0 (0)	0 (0)

Continued

Table 3 Continued

Patients with:	Baseline–week 12				Continued treatment Baseline–week 24*			Switchers Week 12–week 24†	
	Placebo (N=72)	Filgotinib 50 mg (N=72)	Filgotinib 100 mg (N=70)	Filgotinib 200 mg (N=69)	Filgotinib 50 mg (N=57)	Filgotinib 100 mg (N=70)	Filgotinib 200 mg (N=69)	Placebo to filgotinib 100 mg (N=65)	Filgotinib 50 mg to 100 mg (N=15)
Grade 3 (3–6×ULN)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Grade 4 (>6×ULN)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Elevated ALT									
Grade 1 (1–2.5×ULN)	0 (0)	1 (1.4)	5 (7.1)	2 (2.9)	1 (1.4)	8 (11.4)	4 (5.8)	1 (1.4)	1 (6.7)
Grade 2 (2.5–5×ULN)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.4)	0 (0)	0 (0)	0 (0)
Grade 3 (5–20×ULN)	0 (0)	1 (1.4)	0 (0)	0 (0)	1 (1.8)	0 (0)	0 (0)	0 (0)	0 (0)
Grade 4 (>20×ULN)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Elevated AST									
Grade 1 (1–2.5×ULN)	1 (1.4)	4 (5.6)	1 (1.4)	1 (1.4)	4 (7.0)	4 (5.7)	3 (4.3)	3 (4.2)	0 (0)
Grade 2 (2.5–5×ULN)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Grade 3 (5–20×ULN)	0 (0)	1 (1.4)	0 (0)	0 (0)	1 (1.8)	1 (1.4)	0 (0)	0 (0)	0 (0)
Grade 4 (>20×ULN)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Ratio LDL/HDL increase to >ULN	4 (5.6)	12 (16.7)	10 (14.3)	4 (5.8)	10 (17.5)	11 (15.7)	6 (8.7)	5 (6.9)	3 (20.0)

*Patients receiving continued treatment on the same dose of filgotinib.
†Patients in the placebo group, and patients without an ACR20 response in the 50 mg filgotinib group, were switched to the 100 mg dose at week 12.
‡Four serious infections: one in the filgotinib 200 mg group (pneumonia), one in the filgotinib 100 mg group (cellulitis), one in the filgotinib 50 mg group (gastroenteritis) and one in the group that was switched from placebo to filgotinib 100 mg (chronic pyelonephritis).
ACR, American College of Rheumatology; ALT, alanine transaminase; AST, aspartate transaminase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LLN, lower limit of normal; N, number of patients per group; n, number of patients with event; NK, natural killer; SAE, serious adverse event; TE, treatment-emergent; TEAE, treatment-emergent adverse event; ULN, upper limit of normal.

Table 4 Summary of laboratory data at weeks 12 and 24 (data represented are mean change from baseline±SE)

		Filgotinib once daily			
Haemoglobin, g/dL	Placebo to filgotinib 100 mg N=72	50 mg continued N=57	50 mg switching to 100 mg N=15	100 mg continued N=70	200 mg continued N=69
W12	0.02 (0.11)	0.26 (0.11)	−0.18 (0.12)	0.31 (0.12)	0.39 (0.11)
W24	0.34 (0.12)	0.20 (0.16)	0.69 (0.30)	0.28 (0.13)	0.24 (0.11)
Lymphocytes, $\times10^9$/L					
W12	0.20 (0.10)	−0.11 (0.10)	0.17 (0.17)	0.21 (0.10)	0.03 (0.11)
W24	0.10 (0.09)	−0.10 (0.14)	0.36 (0.13)	0.10 (0.10)	0.09 (0.12)
Neutrophils, $\times10^9$/L					
W12	−0.22 (0.28)	−0.16 (0.42)	−0.87 (0.71)	−1.22 (0.30)	−1.25 (0.29)
W24	−0.66 (0.33)	−0.56 (0.44)	−2.34 (0.48)	−1.04 (0.32)	−1.43 (0.30)
Platelets, $\times10^9$/L					
W12	8.2 (6.7)	−10.3 (10.0)	−14.3 (11.2)	−30.4 (7.3)	−27.9 (8.4)
W24	−31.8 (6.8)	−26.1 (9.6)	−28.6 (10.5)	−44.7 (7.9)	−22.3 (7.7)
Creatinine, μmol/L					
W12	0.16 (0.95)	4.04 (1.42)	0.02 (1.57)	1.8 (1.10)	3.8 (1.10)
W24	4.57 (1.16)	1.61 (1.09)	5.24 (1.78)	1.97 (1.14)	3.49 (1.21)
ALT, U/L					
W12	−0.8 (0.91)	−1.6 (1.18)	−0.9 (1.05)	1.3 (1.48)	−1.1 (1.43)
W24	0.9 (1.05)	−0.2 (1.89)	3.2 (2.33)	0.4 (1.74)	−1.3 (1.59)
AST, U/L					
W12	−0.70 (0.73)	−0.5 (0.93)	0.8 (1.00)	1.9 (0.97)	1.1 (1.05)
W24	2.1 (0.86)	−0.5 (1.19)	2.6 (1.72)	1.5 (1.00)	1.4 (1.29)
LDL cholesterol, mmol/L					
W12	−0.01 (0.06)	0.09 (0.09)	0.11 (0.13)	0.28 (0.10)	0.35 (0.07)
W24	0.10 (0.07)	0.00 (0.11)	0.14 (0.17)	0.31 (0.10)	0.38 (0.07)
HDL cholesterol, mmol/L					
W12	0.03 (0.04)	0.06 (0.04)	0.150 (0.06)	0.19 (0.03)	0.19 (0.04)
W24	0.16 (0.04)	0.17 (0.05)	0.30 (0.07)	0.18 (0.03)	0.19 (0.04)

ALT, alanine transaminase; AST, aspartate transaminase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; N, number of patients per group.

profile. These encouraging data support the future development of filgotinib monotherapy for treatment of patients who have had an inadequate response to MTX treatment.

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OPEN ACCESS

EXTENDED REPORT

A randomised phase IIb study of mavrilimumab, a novel GM–CSF receptor alpha monoclonal antibody, in the treatment of rheumatoid arthritis

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ABSTRACT

Objectives Despite the therapeutic value of current rheumatoid arthritis (RA) treatments, agents with alternative modes of action are required. Mavrilimumab, a fully human monoclonal antibody targeting the granulocyte–macrophage colony-stimulating factor receptor-α, was evaluated in patients with moderate-to-severe RA.

Methods In a phase IIb study (NCT01706926), patients with inadequate response to ≥1 synthetic disease-modifying antirheumatic drug(s), Disease Activity Score 28 (DAS28)–C reactive protein (CRP)/erythrocyte sedimentation rate ≥3.2, ≥4 swollen joints despite methotrexate (MTX) were randomised 1:1:1:1 to subcutaneous mavrilimumab (150, 100, 30 mg), or placebo every other week (eow), plus MTX for 24 weeks. Coprimary outcomes were DAS28–CRP change from baseline to week 12 and American College of Rheumatology (ACR) 20 response rate (week 24).

Results 326 patients were randomised (150 mg, n=79; 100 mg, n=85; 30 mg, n=81; placebo, n=81); 305 completed the study (September 2012–June 2013). Mavrilimumab treatment significantly reduced DAS28–CRP scores from baseline compared with placebo (change from baseline (SE); 150 mg: −1.90 (0.14), 100 mg: −1.64 (0.13), 30 mg: −1.37 (0.14), placebo: −0.68 (0.14); p<0.001; all dosages compared with placebo).

Significantly more mavrilimumab-treated patients achieved ACR20 compared with placebo (week 24: 73.4%, 61.2%, 50.6% vs 24.7%, respectively (p<0.001)). Adverse events were reported in 43 (54.4%), 36 (42.4%), 41 (50.6%) and 38 (46.9%) patients in the mavrilimumab 150, 100, 30 mg eow and placebo groups, respectively. No treatment-related safety signals were identified.

Conclusions Mavrilimumab significantly decreased RA disease activity, with clinically meaningful responses observed 1 week after treatment initiation, representing a novel mechanism of action with persuasive therapeutic potential.

Trial registration number NCT01706926; results.

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INTRODUCTION

Biological therapies have improved disease control and patient outcomes in rheumatoid arthritis (RA). However, approximately 50% of patients do not achieve low disease activity criteria within 12 months of antitumour necrosis factor-α treatment,[1] while approximately 80% of patients do not achieve Disease Activity Score 28 (DAS28)–erythrocyte sedimentation rate (ESR)<2.6.[2] It is possible that biologics targeting novel signalling pathways may prove beneficial in RA, including in these patients.

Recently, granulocyte–macrophage colony-stimulating factor (GM–CSF), a proinflammatory multifunctional cytokine, has emerged as a novel and important therapeutic target in autoimmune/inflammatory diseases.[3] In RA pathogenesis, GM–CSF plays a key role through activation, differentiation and survival of macrophages, dendritic cells and neutrophils.[4–6] In addition, GM–CSF is now well recognised as an effector T helper 1/17 cell cytokine.[3 7] Elevated concentrations of GM–CSF and its receptor have been observed in tissue and synovial fluid of patients with RA,[8–10] and recombinant GM–CSF administration exacerbates RA disease activity.[11] Moreover, signalling through the GM–CSF receptor-α subunit (GM–CSFR-α) has been shown to have a role in animal models of arthritis[10 12] and modulation of pain pathways.[13] Inhibition of the GM–CSF pathway reduces macrophage and/or neutrophil numbers in inflammatory lesions.[14] This treatment approach may hold promise in RA and other diseases characterised by the activation of the monocyte–macrophage pathway. In humans, full inhibition of GM–CSF signalling, via emergence of GM–CSF neutralising polyclonal autoantibodies, has been associated with the development of foamy alveolar macrophages, and, clinically, with a lung disorder, pulmonary alveolar proteinosis (PAP).[15]

Mavrilimumab, a fully human monoclonal antibody which blocks the GM–CSF receptor, is the first biologic in clinical development to target this pathway. Clinical studies demonstrated the pharmacokinetics, pharmacodynamics and safety/tolerability of mavrilimumab, and provided evidence of efficacy.[16–20] In this longer 24-week phase IIb study, we evaluated the therapeutic potential of GM–CSF antagonism in patients with moderate-to-severe, adult-onset RA by comparing the efficacy and

safety/tolerability of subcutaneous mavrilimumab, at dosages of up to 150 mg every other week (eow) plus methotrexate (MTX), with that of placebo.

METHODS

Study design

This phase IIb, randomised, double-blind, parallel-group, placebo-controlled study (EARTH EXPLORER 1; NCT01706926) was conducted in 48 specialist sites (14 countries; Europe, South America, South Africa) (see online supplementary table S1). Population pharmacokinetic efficacy modelling and stochastic clinical trial simulations facilitated selection of the optimal dose range for the study.

Due to the theoretical risk associated with GM–CSF inhibition and data from non-clinical (animal toxicology) studies of mavrilimumab,[21] standardised pulmonary monitoring with independent expert adjudication was undertaken.[22]

The study was conducted in accordance with the principles of the Declaration of Helsinki and the International Conference on Harmonisation Guidance for Good Clinical Practice and approved by appropriate institutional review boards or independent ethics committees at each site.

Patients

Patients were 18–80 years with moderate-to-severe, adult-onset RA,[23] DAS28–C reactive protein (CRP) ≥3.2 at screening and DAS28–ESR ≥3.2 at day 1,[24] and ≥4 swollen joints at screening and day 1, and were receiving stable dosages of MTX (7.5–25.0 mg/week). Patients were required to have received treatment with ≥1 traditional disease-modifying antirheumatic drug (DMARD) prior to screening. Previous treatment with any biological DMARD discontinued because of lack of efficacy; recent treatment with any investigational drug, alkylating agents or parenteral steroids; and concurrent treatment with DMARDs other than MTX were not permitted. Changes in background RA treatment were not allowed for the first 12 weeks of the study, other than for safety reasons. Patients with clinically uncontrolled respiratory disease, active infection or high infection risk, and active or untreated latent tuberculosis were excluded. All patients provided written informed consent and were enrolled by the investigator or qualified designee. Study-stopping criteria are listed in the online supplementary material.

Randomisation and masking

Patients were randomised (interactive web response system) 1:1:1:1 to 150, 100 or 30 mg subcutaneous mavrilimumab or placebo eow in combination with stable dosages of MTX (7.5–25.0 mg/week) for 24 weeks, followed by transfer to a long-term, open-label extension (OLE) (NCT01712399) or a 12-week safety follow-up period. Study patients, investigators and sponsors were blinded to study treatment (see online supplementary material).

Procedures

During the 24-week treatment period, there were 14 scheduled visits (weeks 0, 1, 2 and eow until week 24). The safety follow-up period included visits at 4, 8 and 12 weeks after the last dose. Twelve weeks after treatment initiation, patients without adequate response (<20% improvement in both swollen and tender joint counts vs day 1) were eligible for early OLE entry. Corticosteroids (≤7.5 mg/day prednisolone or equivalent), analgesics and non-steroidal anti-inflammatory drugs were maintained at stable dosages for the study duration.

End points

Primary end points

Coprimary end points were change from baseline in DAS28–CRP score (week 12) and American College of Rheumatology (ACR) 20 (20% improvement in ACR criteria) response (week 24). Assessments performed are included in the online supplementary material.

Secondary end points

Secondary efficacy end points included: DAS28–CRP European League Against Rheumatism (EULAR) response rates, DAS28–CRP-defined remission (<2.6) and low disease activity (<3.2), ACR20/50/70 response rates at weeks 12 and 24, change from baseline or geometric means for ACR and DAS28 components over time and DAS28–ESR response. Assessments were performed at weeks 0, 1, 2, 4, 8, 12, 16, 20 and 24.

CRP and ESR geometric means were measured over time.

Exploratory end points

Exploratory end points, including disease activity and structural damage biomarkers, were examined at weeks 0, 1, 2, 4, 12 and 24. Multibiomarker disease activity (MBDA) Vectra DA score (Crescendo Biosciences, South San Francisco, California, USA)[25] was calculated to track the effects of mavrilimumab on inflammatory biomarkers at predefined time points.

An ELISA was used to measure serum concentrations of C1M,[26] a marker of tissue damage associated with structural progression.[27]

Safety assessments

Adverse events (AEs) and serious adverse events (SAEs) were summarised by severity and relationship to study drug by investigators. Laboratory evaluations (serum chemistry, haematology, urinalysis), vital signs, pulmonary function tests (PFTs), dyspnoea score and oxygen saturation were summarised by treatment group and time point. Serum was tested for antidrug antibodies (ADAs) and mavrilimumab concentrations throughout the study. Safety assessments were performed at every visit during the treatment period.

PFTs (forced vital capacity, forced expiratory volume in 1 and 6 s) were performed at screening, and at weeks 12 and 24. Dyspnoea score and oxygen saturation were assessed at each visit using the modified Borg scale and pulse oximetry, respectively. Adjudication of lung function abnormalities and pulmonary AEs was by an Independent Pulmonary Expert Committee.

Statistical analysis

The primary efficacy population was the modified intention-to-treat population (all randomised patients who received any study drug). The safety population included all patients who received study drug and had safety data available.

A sample size of 70 patients per treatment group provided 80% power to achieve statistical significance for DAS28–CRP and ACR20 at a two-sided significance level of 0.05. This assumed a 0.6-unit difference in change from baseline and a SD of 1.25 for DAS28–CRP, and a 25% difference in ACR20 response rate with a placebo response rate of 40%.

Change from baseline in DAS28–CRP was analysed using a mixed model for repeated measures (MMRM), with covariates for baseline DAS28–CRP, visit, treatment and visit-by-treatment interaction. Dosage–response assessment was performed using a test for linear trend on DAS28–CRP change from baseline at week 12. Two sensitivity analyses were performed for change

from baseline DAS28−CRP to allow for patients withdrawing from treatment (see online supplementary material). ACR20/50/70 response rates, DAS28-defined remission (<2.6) and response rates at each visit were analysed using logistic regression, with results presented as differences in response rates (95% CI; p value).[28] Individual ACR components were analysed using the same method as for DAS28−CRP analyses.

DAS28−CRP EULAR responses at each time point were analysed via a proportional odds model, with treatment as a factor. CRP and ESR were log-transformed prior to analysis. For discrete responder outcomes, patients who withdrew from treatment for any reason (including entering the OLE), started any new RA medication, or increased MTX dosage, were imputed as non-responders for all subsequent assessments. For continuous outcomes (DAS28−CRP and ACR components), missing data were handled by the MMRM analysis (including patients entering the OLE). For MBDA and C1M, results for each time point were analysed versus placebo using a non-parametric Mann-Whitney U test. AE and other safety data were summarised with descriptive statistics. An external independent safety data monitoring board oversaw the study.

RESULTS

Patients were recruited between September 2012 and June 2013, with evaluation until January 2014. Of 326 patients randomised, 305 (93.6%) completed the study. Patient disposition is presented in figure 1. Demographics and baseline clinical characteristics were similar between treatment groups, and indicated a cohort of patients with predominantly severe disease (DAS28−CRP >5.1) that would qualify for first-line biological therapy (see table 1 and online supplementary table S2).

Mavrilimumab significantly reduced DAS28−CRP scores from baseline compared with placebo at week 12, meeting the coprimary outcome (change from baseline (difference from placebo (95% CI)) 150 mg: −1.90 (−1.22 (−1.60 to −0.84)), 100 mg: −1.64 (−0.96 (−1.33 to −0.58)), 30 mg: −1.37 (−0.69 (−1.06 to −0.31)), placebo: −0.68; p<0.001, all dosages; figure 2A). Differences from placebo were detected at week 1, with treatment benefit increasing through week 12 (figure 2A). At week 24, significantly more patients receiving mavrilimumab 150 mg eow achieved an ACR20 response compared with placebo, with a dosage-dependent response (150 mg: 73.4%; 100 mg: 61.2%; 30 mg: 50.6%; placebo: 24.7% (p<0.001); figure 2B), indicating that the study also met its second coprimary outcome. There were significantly more ACR20 responders in the mavrilimumab 150 mg eow group than in the placebo group from the first assessment (week 1) and at every other assessment through to week 24 (figure 2B). Subgroup analyses of ACR20 by CRP concentration (normal or greater than the upper limit of normal), the presence of rheumatoid factor and/or anticitrullinated protein antibody (ACPA), and prior use of biologics and smoking status (see online supplementary table S3) suggest that clinical response is not dependent on baseline disease characteristics. Furthermore, mavrilimumab was demonstrated to be efficacious in patients who were rheumatoid factor negative and ACPA-negative at baseline (n=59; 18.1%).

DAS28−CRP/ESR EULAR good and moderate responses occurred more frequently with mavrilimumab 150, 100 and 30 mg eow than placebo at weeks 12 and 24 (figure 3). This was also true for ACR20 and ACR50 response rates (ACR50 response at week 24: 40.5%, 25.9%, 28.4% and 12.3%, respectively; p<0.05, all dosages; figure 3). Mavrilimumab 150 mg eow significantly improved ACR70 response rates compared with placebo at weeks 12 and 24 (week 12: 10.1% vs 1.2% (p=0.017); week 24: 13.9% vs 3.7% (p=0.026); figure 3). At week 24, there was a significantly greater ACRn response for patients receiving mavrilimumab compared with placebo (figure 3). Rates of DAS28−CRP remission (<2.6) were also significantly greater with mavrilimumab 150 mg compared with placebo at week 12 and all dosages of mavrilimumab compared with placebo at week 24 (p<0.05, all dosages; figure 3). There were significantly more patients with DAS28−CRP low disease activity scores (<3.2) in the mavrilimumab 150 mg eow group compared with placebo at weeks 12, 16, 20, 24 (31.6%, 40.5%, 43.0%, 41.8% vs 12.3%, 14.8%, 14.8%, 8.6%, respectively) and in all mavrilimumab groups compared with placebo at week 24 (p<0.001; figure 3). To confirm the robustness of the data, analyses of change from baseline in DAS28−ESR were also performed, and results were similar to those using DAS28−CRP (see online supplementary figure S1).

Results for components of composite outcomes were similar. Greater changes from baseline in ACR and DAS28 components, and patient-reported outcomes compared with placebo were observed at weeks 12 and 24 for patients receiving mavrilimumab 150 mg eow (see online supplementary table S4). As a greater number of patients in the placebo group than in the mavrilimumab group transferred to the OLE study between weeks 12 and 24 because of lack of response under 'rescue' criteria, it is important to interpret the week 24 data with caution.

A dosage-dependent, rapid (week 1) and sustained (week 24) reduction of both CRP and ESR concentrations was also observed, with CRP levels plateauing at approximately 3.3 mg/L (see figure 4A and online supplementary figure S2, respectively).

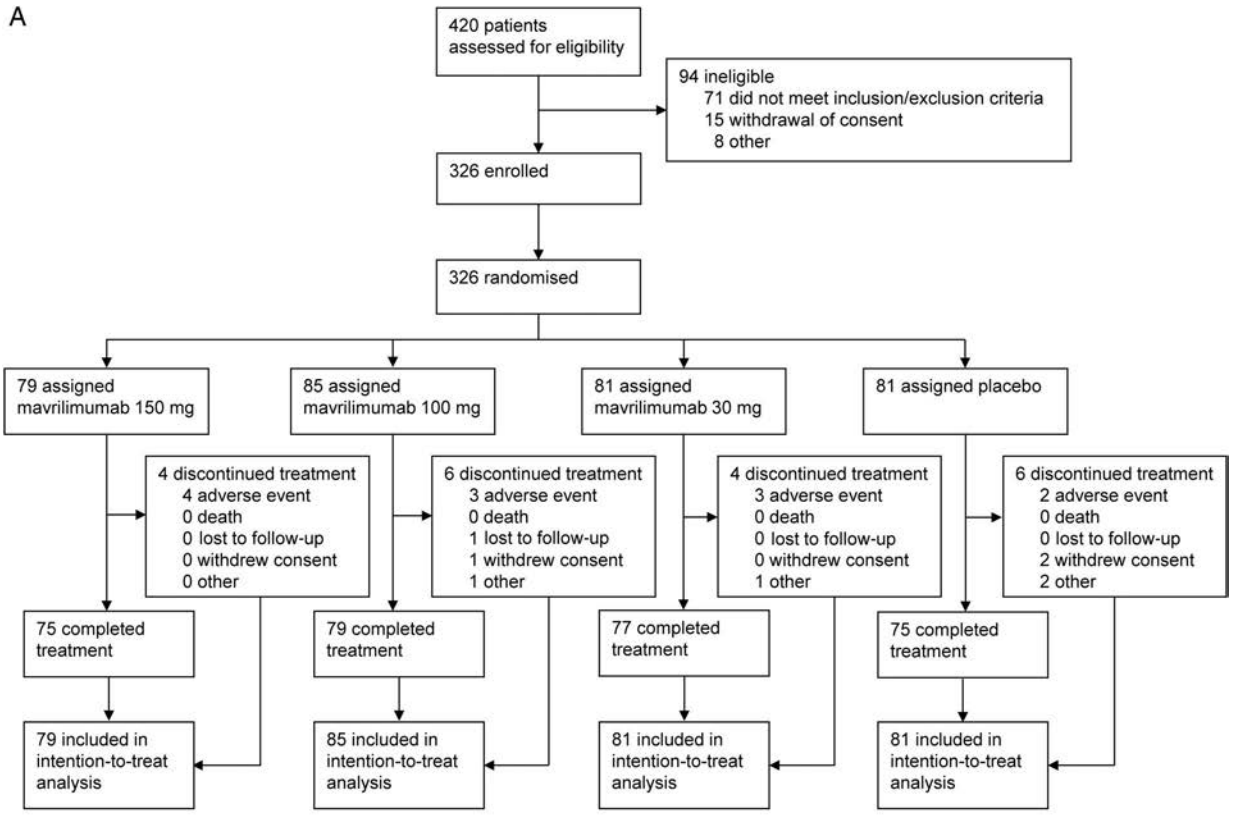
Of 326 patients, 120 reported at least one AE (150 mg: 43 (54.4%); 100 mg: 36 (42.4%); 30 mg: 41 (50.6%); placebo: 38 (46.9%)). The most common treatment-emergent AEs (TEAEs), and those leading to discontinuation or interruption of the study drug, are provided in table 2. SAEs were reported for two (2.5%), five (5.9%), four (4.9%) and one (1.2%) patients in the mavrilimumab 150, 100, 30 mg eow and placebo groups, respectively (table 2). Of these, only pneumonia (mavrilimumab 30 mg eow) and angioedema (mavrilimumab 150 mg eow) were considered to be related to treatment by the investigator.

Rates of pulmonary AEs for mavrilimumab 150, 100 or 30 mg eow were similar to the rate for placebo (6.3%, 3.5%, 6.2% vs 9.9%, respectively). There were no deaths or anaphylaxis. Two hypersensitivity AEs led to discontinuation (angioedema 6 days after first dose, mavrilimumab 150 mg eow; drug hypersensitivity 1 day after first dose, mavrilimumab 30 mg eow).

No clinically meaningful differences between mavrilimumab-treated and placebo-treated patients in haematology, including neutrophils, serum chemistry and urinalysis parameters, were observed. ADAs were detected in 0 (0.0%), 3 (3.5%), 13 (16.0%) and 2 (2.5%) patients in the mavrilimumab 150, 100, 30 mg eow and placebo groups, respectively (see online supplementary material). One injection-site reaction was observed (mavrilimumab 150 mg eow).

Pulmonary function values, dyspnoea scores and oxygen saturation were generally similar between mavrilimumab-treated and placebo-treated patients, with no evidence of a dosage-dependent decline in the mean values for patients receiving mavrilimumab (see online supplementary table S5). Any threshold changes in the percentage of PFT values were generally transient.

In biomarker analyses, treatment with mavrilimumab 150 and 100 mg eow induced early (week 1) and sustained (week 24) significant reductions in MBDA score versus placebo (p<0.01;



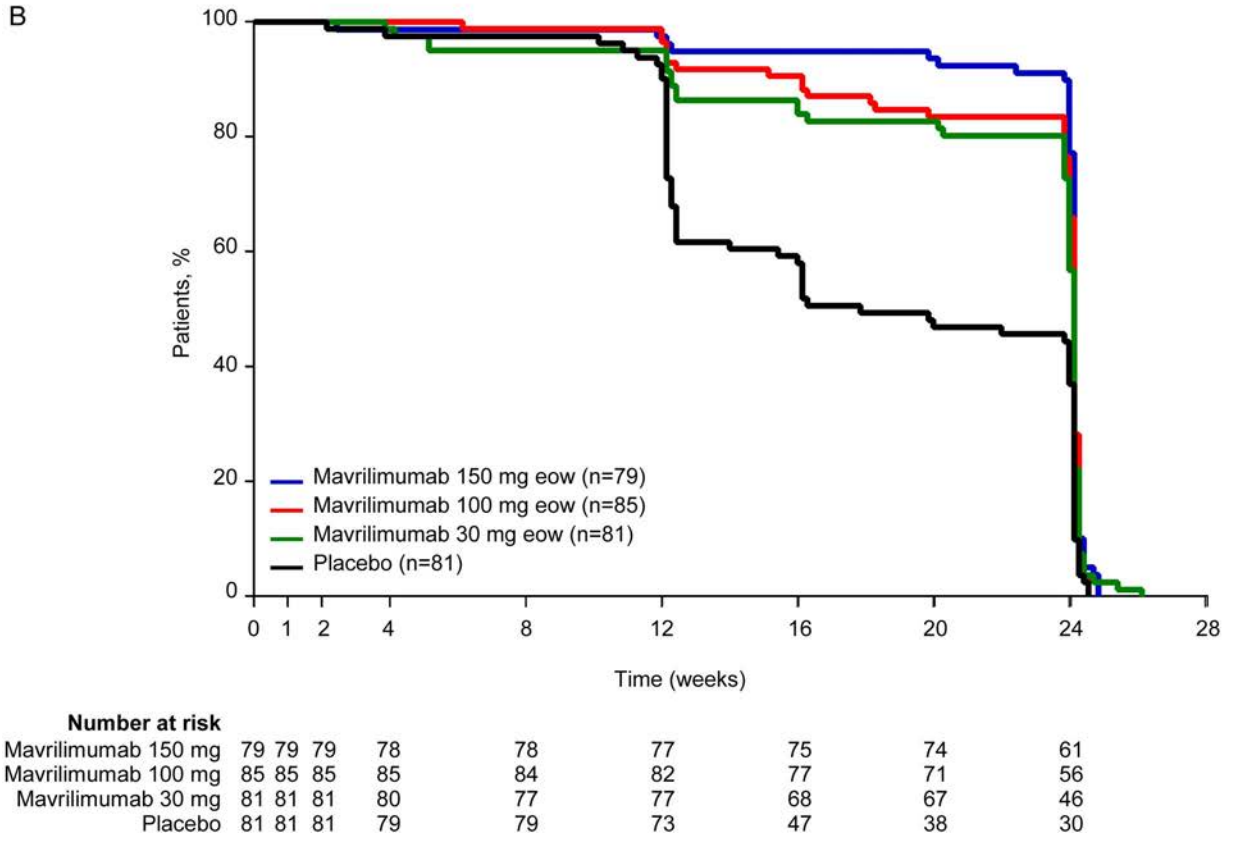


Figure 1 CONSORT diagram (A) and time to randomised study exit (B). Randomised study exit includes patients who withdrew from study treatment, patients who entered the OLE from week 12 as permitted in the protocol and those patients who entered the safety follow-up period at week 24. At week 12, 3 (3.8%), 8 (9.4%), 12 (14.8%) and 37 (45.7%) patients transferred to the OLE study because of lack of efficacy in the mavrilimumab 150, 100 and 30 mg groups and placebo group, respectively. eow, every other week; OLE, open-label extension.

Table 1 Patient demographics and baseline clinical characteristics

	Mavrilimumab			
	150 mg eow (n=79)	100 mg eow (n=85)	30 mg eow (n=81)	Placebo (n=81)
Demographics				
Age, mean (SD)	52.6 (10.3)	50.8 (11.9)	51.2 (11.6)	52.8 (10.6)
Female, n (%)	67 (84.8)	70 (82.4)	70 (86.4)	75 (92.6)
Race, n (%)				
White	74 (93.7)	81 (95.3)	76 (93.8)	76 (93.8)
Other	5 (6.3)	4 (4.7)	5 (6.2)	5 (6.2)
Weight, kg, mean (SD)	75.9 (17.6)	71.8 (16.2)	72.5 (15.2)	73.0 (15.2)
Body mass index, kg/m^2, mean (SD)	28.4 (6.2)	26.3 (5.3)	27.3 (5.1)	27.5 (5.1)
Baseline clinical characteristics				
Years since RA diagnosis, mean (SD)	8.5 (6.9)	7.2 (6.5)	7.8 (6.6)	7.6 (7.2)
Rheumatoid factor-positive, n (%)	60 (75.9)	68 (80.0)	67 (82.7)	65 (80.2)
ACPA-positive, n (%)	61 (77.2)	63 (74.1)	66 (81.5)	59 (72.8)
DAS28–CRP, mean (SD)	5.7 (0.8)	5.9 (0.9)	5.7 (0.9)	5.8 (0.8)
DAS28–ESR, mean (SD)	6.5 (0.9)	6.7 (0.9)	6.7 (1.0)	6.6 (0.9)
Swollen joint count, mean (SD)	15.7 (7.1)	16.8 (8.6)	17.8 (10.1)	14.4 (6.9)
Tender joint count, mean (SD)	26.7 (11.4)	27.0 (14.2)	27.5 (14.0)	26.3 (11.3)
HAQ DI, mean (SD)	1.58 (0.53)	1.58 (0.52)	1.52 (0.62)	1.63 (0.48)
CRP, mg/L, median (minimum–maximum)	5.6 (0.3–55.8)	9.0 (0.3–75.3)	5.2 (0.2–102.8)	6.3 (0.2–110.2)
Normal, n (%)	27 (34.2)	22 (25.9)	32 (39.5)	24 (29.6)
Greater than ULN, n (%)*	52 (65.8)	63 (74.1)	49 (60.5)	57 (70.4)
ESR, mm/hour, median (minimum–maximum)	38.0 (8–101)	40.0 (6–123)	40.0 (6–110)	42.0 (3–112)
MBDA score, mean (SD)	50.2 (14.0)	54.2 (16.7)	48.5 (17.3)	50.6 (17.9)
C1M, ng/mL, mean (SD)	83.7 (54.8)	107.1 (76.3)	88.6 (81.3)	98.1 (72.1)
Methotrexate use, n	79	84†	81	81
Dosage, mg/week, mean (SD)	14.5 (4.1)	15.1 (4.6)	14.6 (3.6)	15.0 (3.7)
<12.5 mg/week, n (%)	21 (26.6)	22 (26.2)	19 (23.5)	16 (19.8)
≥12.5 to <20 mg/week, n (%)	44 (55.7)	39 (46.4)	47 (58.0)	51 (63.0)
≥20 mg/week, n (%)	14 (17.7)	23 (27.4)	15 (18.5)	14 (17.3)
Corticosteroid use, n	46	51	50	43
Dosage, mg/day, mean (SD)	5.4 (1.7)	5.7 (1.3)	5.4 (2.4)	5.3 (1.7)
<5 mg/day	5 (10.9)	1 (2.0)	5 (10.0)	6 (14.0)
≥5 mg/day	41 (89.1)	50 (98.0)	45 (90.0)	37 (86.0)
Prior biological therapy, n (%)	10 (12.7)	13 (15.3)	12 (14.8)	12 (14.8)
Reason for discontinuation, n (%)				
Expense of medication	2 (2.5)	2 (2.4)	1 (1.2)	1 (1.2)
Medication only in clinical trial	7 (8.9)	9 (10.6)	8 (9.9)	8 (9.9)
Adverse event	0 (0.0)	1 (1.2)	3 (3.7)	1 (1.2)
Other	1 (1.3)	1 (1.2)	0 (0.0)	2 (2.5)

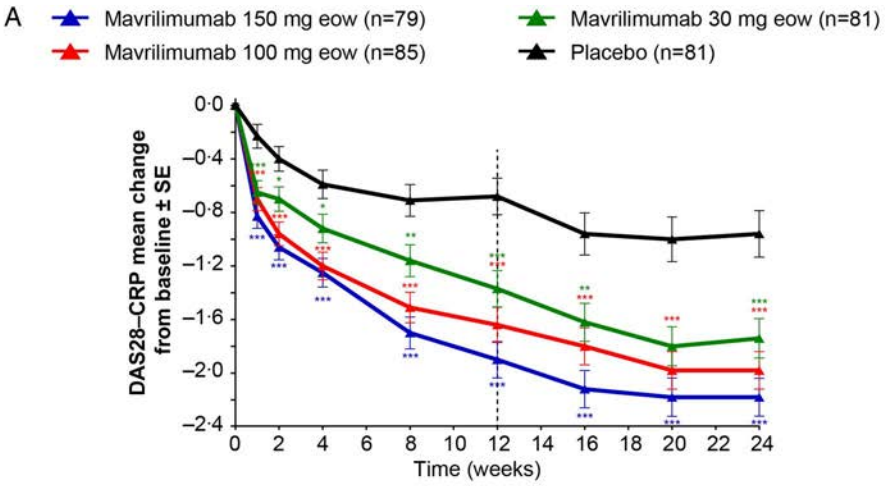
*The upper limit of normal for CRP (high sensitivity) was 3 mg/L.
†One patient did not receive methotrexate (not identified until after randomisation), and this was considered a protocol violation.
ACPA, anticitrullinated protein antibody; CRP, C reactive protein; DAS28, Disease Activity Score 28; eow, every other week; ESR, erythrocyte sedimentation rate; HAQ DI, Health Assessment Questionnaire Disability Index; MBDA, multibiomarker disease activity; RA, rheumatoid arthritis; SD, standard deviation; ULN, upper limit of normal.

figure 4B). Significant decreases from baseline in C1M concentrations were also observed for patients receiving mavrilimumab 150 and 100 mg eow compared with placebo from week 1 to week 24 ($p<0.01$; figure 4C).

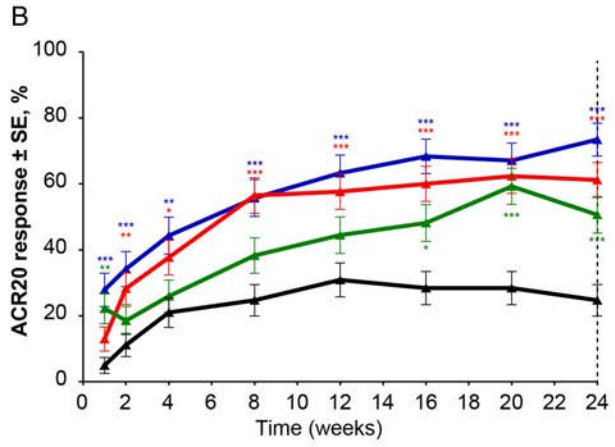
DISCUSSION

This phase IIb study met its coprimary outcomes, with mavrilimumab treatment resulting in dosage-related, significantly greater reductions from baseline in DAS28–CRP scores at week 12 and a significantly greater percentage of ACR20 responders at week 24, compared with placebo. The most effective dose was 150 mg eow. Mavrilimumab-treated patients also demonstrated significantly greater improvements than those receiving placebo across a range of secondary and patient-reported outcomes[29] (see online supplementary table S4). The data presented here are consistent with and build on those presented previously for mavrilimumab 100 mg by including a larger patient population, longer treatment duration and the higher (150 mg) mavrilimumab dosage.[19 20]

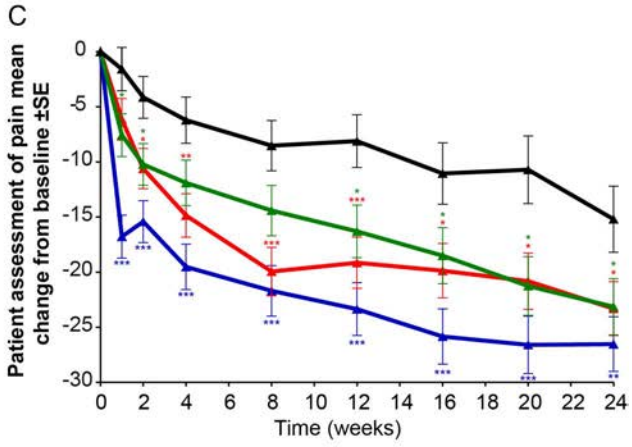
A rapid and sustained clinical response to mavrilimumab 150 and 100 mg eow was reflected in the reduction of CRP and ESR, concurrent decreases in MBDA score, a composite of soluble disease activity biomarkers and C1M concentration.[27] A clear dosage–response relationship was observed for mavrilimumab-treated patients in most efficacy outcomes analysed and for biomarker analyses, but not in AE rates or other



	Mavrilimumab			
	150 mg eow (n=79)	100 mg eow (n=85)	30 mg eow (n=81)	Placebo (n=81)
Baseline mean	5.67	5.91	5.73	5.78
Adjusted mean change from baseline at week 1	-0.83	-0.70	-0.65	-0.23
Adjusted mean change from baseline at week 12	-1.90	-1.64	-1.37	-0.68
Adjusted mean change from baseline at week 24	-2.18	-1.98	-1.74	-0.96



	Mavrilimumab			
	150 mg eow (n=79)	100 mg eow (n=85)	30 mg eow (n=81)	Placebo (n=81)
ACR response at week 1, n (%)	22 (27.8)	11 (12.9)	18 (22.2)	4 (4.9)
ACR response at week 12, n (%)	50 (63.3)	49 (57.6)	36 (44.4)	25 (30.9)
ACR response at week 24, n (%)	58 (73.4)	52 (61.2)	41 (50.6)	20 (24.7)



	Mavrilimumab			
	150 mg eow (n=79)	100 mg eow (n=85)	30 mg eow (n=81)	Placebo (n=81)
Baseline mean	62.35	63.58	62.65	62.16
Adjusted mean change from baseline at week 1	-16.78	-6.14	-7.57	-1.58
Adjusted mean change from baseline at week 12	-23.35	-19.15	-16.30	-8.11
Adjusted mean change from baseline at week 24	-26.53	-23.31	-23.14	-15.20

Figure 2 Changes from baseline in DAS28−CRP score (A), ACR20 response (B) and changes from baseline in patient assessment of pain (C) by visit. *p<0.05, **p<0.01, ***p<0.001 mavrilimumab versus placebo. ACR, American College of Rheumatology; DAS28−CRP, Disease Activity Score 28−C reactive protein; eow, every other week; SE, standard error.

safety parameters. However, as the study was powered specifically to assess the coprimary end points, the sample size and study duration were not sufficient to assess joint damage progression.

The number of patients who transferred to the OLE between weeks 12 and 24 because of lack of response was low in the mavrilimumab 150, 100 and 30 mg eow groups compared with placebo (3 (3.8%), 8 (9.4%), 12 (14.8%) and 37 (45.7%), respectively). This could be seen as an indication of the benefit of mavrilimumab treatment; however, it is a limitation of the study analysis, as the response of these patients at week 24, had they remained in the study, is unknown. To account for patients transferring to the OLE, a non-responder imputation for the ACR outcomes and a sensitivity analysis for DAS28−CRP were performed. The primary analysis method of MMRM resulted in a smaller difference from placebo than both the Last Observation Carried Forward (LOCF) and the Baseline Observation Carried Forward (BOCF) method (DAS28−CRP week 24 mavrilimumab 150 mg eow difference from placebo: MMRM=−1.21; LOCF=−1.46; BOCF=−1.37).

Mavrilimumab was generally well tolerated, with no substantial differences in AEs or SAEs between mavrilimumab-treated

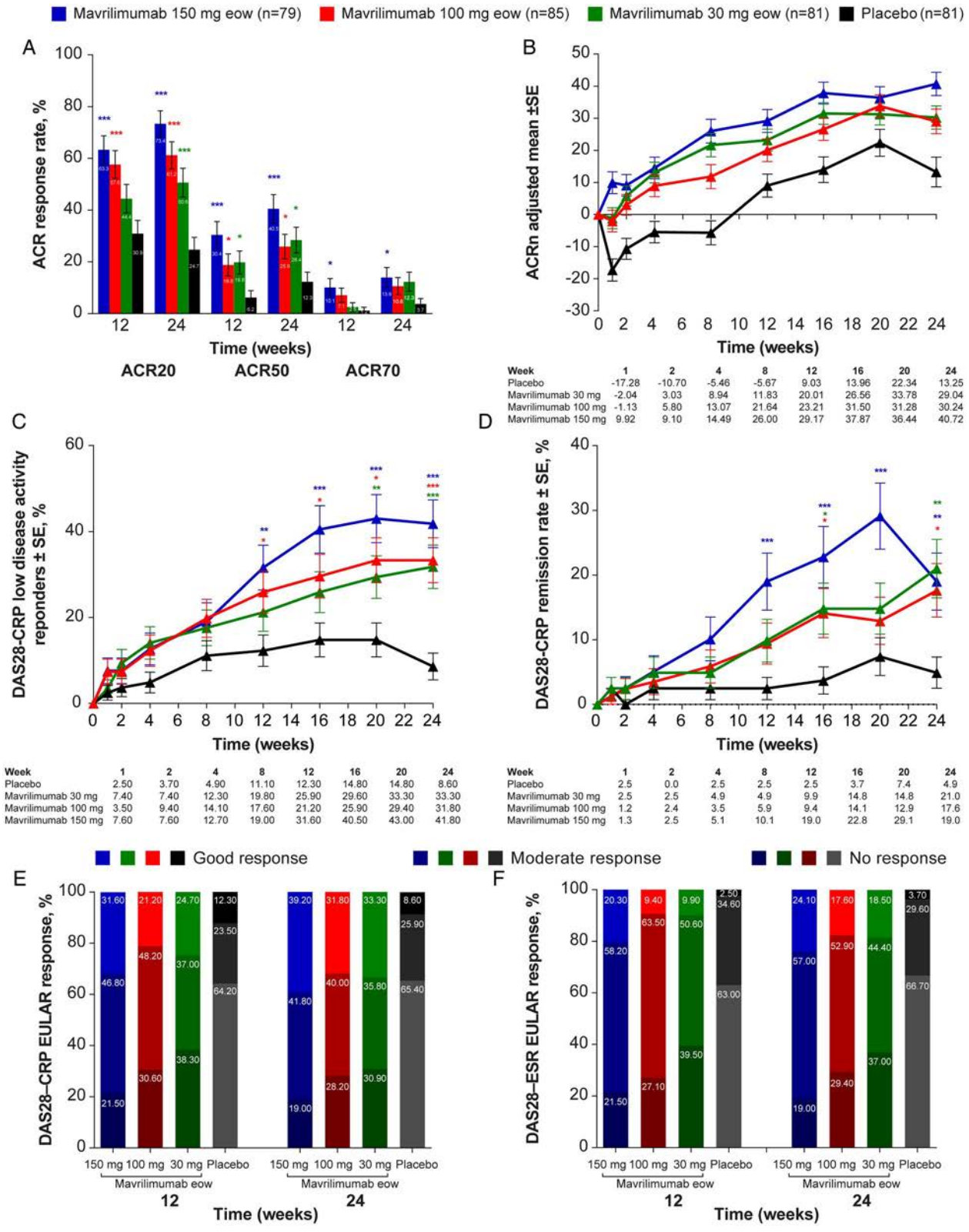
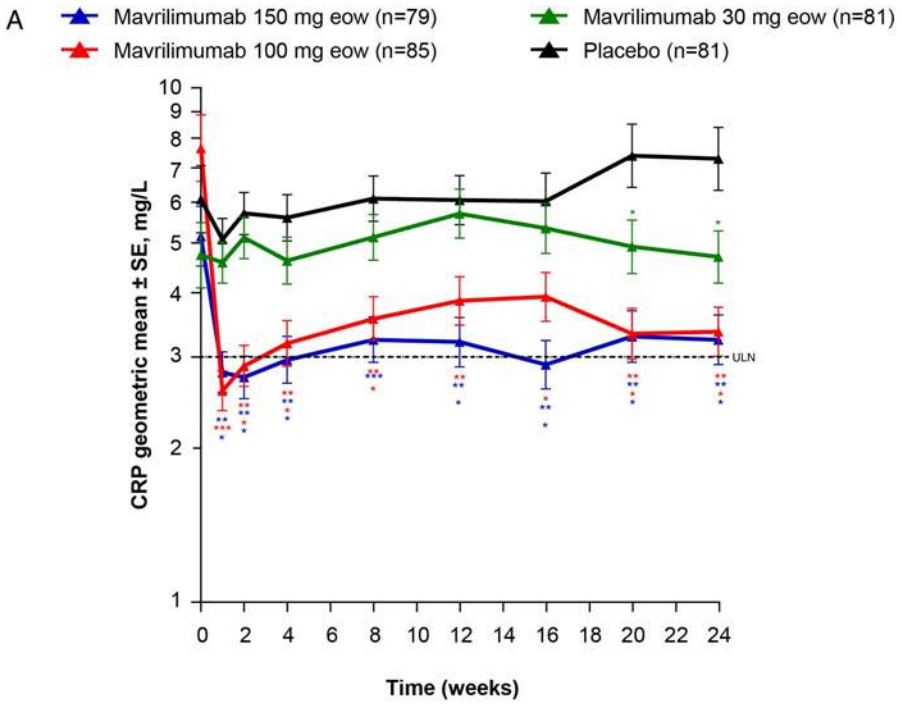
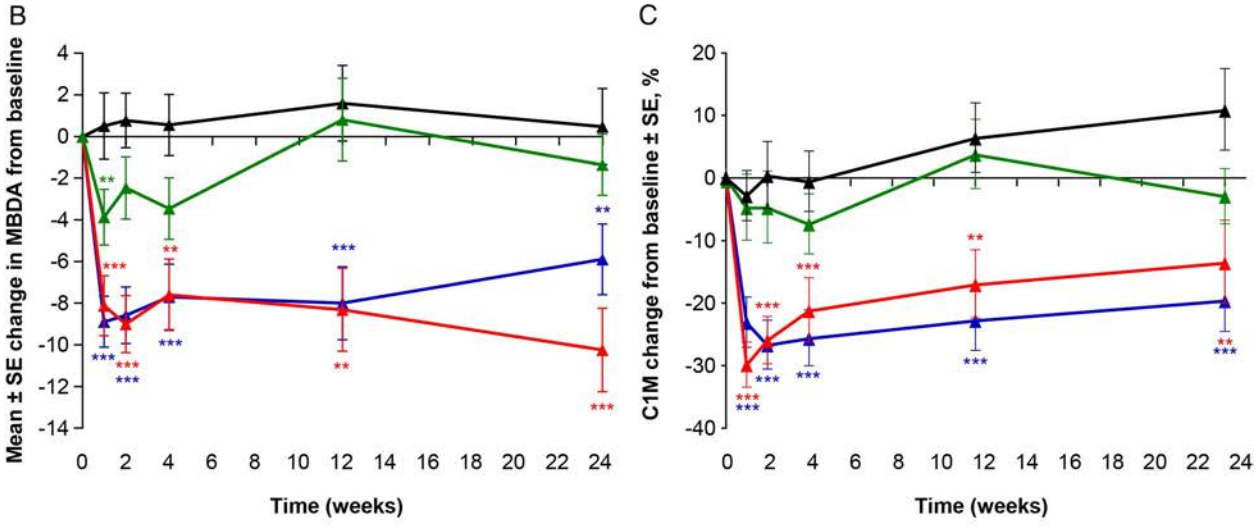


Figure 3 Analysis of secondary efficacy outcomes: ACR response rates (A), ACRn response over time (B), DAS28−CRP low disease activity responders (DAS28−CRP <3.2) (C), DAS28−CRP remission (DAS28−CRP <2.6) over time (D), DAS28−CRP European League Against Rheumatism (EULAR) response (E), DAS28−ESR EULAR response (F). ACR/EULAR response criteria are detailed in online supplementary table S6. DAS28−CRP remission defined as DAS28−CRP <2.6. DAS28−CRP low disease activity defined as DAS28−CRP <3.2. ACR, American College of Rheumatology; DAS28−CRP, Disease Activity Score 28−C reactive protein; ESR, erythrocyte sedimentation rate; eow, every other week; SE, standard error.

and placebo-treated patients (table 2). The percentage of patients experiencing TEAEs and TEAEs of special interest were similar in mavrilimumab versus placebo groups. The rate of serious infection was low (one serious pneumonia (mavrilimumab 30 mg) and one non-serious pneumonia (placebo)). Neutropenia was reported in three patients in the mavrilimumab 150 mg



Week	1	2	4	8	12	16	20	24
Placebo	5.080	5.710	5.600	6.100	6.060	6.030	7.390	7.290
Mavrilimumab 30 mg	4.580	5.120	4.620	5.130	5.700	5.340	4.920	4.700
Mavrilimumab 100 mg	2.580	2.880	3.190	3.560	3.860	3.930	3.330	3.360
Mavrilimumab 150 mg	2.800	2.740	2.960	3.240	3.210	2.900	3.290	3.240



Week	1	2	4	12	24
Placebo	0.508	0.775	0.559	1.600	0.479
Mavrilimumab 30 mg	-3.868	-2.466	-3.456	0.811	-1.350
Mavrilimumab 100 mg	-8.120	-9.000	-7.596	-8.314	-10.245
Mavrilimumab 150 mg	-8.885	-8.578	-7.596	-8.000	-5.890

Week	1	2	4	12	24
Placebo	-2.888	0.309	-0.626	6.312	10.793
Mavrilimumab 30 mg	-4.801	-4.805	-7.443	3.706	-2.984
Mavrilimumab 100 mg	-29.931	-26.003	-21.258	-17.115	-13.601
Mavrilimumab 150 mg	-23.150	-26.747	-25.672	-22.824	-19.669

Figure 4 Adjusted geometric mean ratio to baseline in CRP concentrations (A), change from baseline in MBDA score (B) and C1M (C). *p<0.05, **p<0.01, ***p<0.001 mavrilimumab versus placebo. For MBDA analyses (graph A), the number of patients for whom serum samples were analysed at each time point ranged from 57 to 64, 53 to 60, 53 to 61 and 40 to 59 for mavrilimumab 150, 100, 30 mg eow and placebo, respectively. For C1M analyses (graph B), the number of patients for whom serum samples were analysed at each time point ranged from 73 to 76, 74 to 84, 62 to 78 and 54 to 77 for mavrilimumab 150, 100, 30 mg eow and placebo, respectively. CRP, C reactive protein; eow, every other week; MBDA, multibiomarker disease activity; SE, standard error; ULN, upper limit of normal.

Table 2 Treatment-emergent adverse events occurring in ≥3% of patients in any group and all serious adverse events

Event, n (%)	Mavrilimumab 150 mg eow (n=79)	Mavrilimumab 100 mg eow (n=85)	Mavrilimumab 30 mg eow (n=81)	Placebo (n=81)
Treatment-emergent adverse events (≥3% patients in any group)				
Headache	6 (7.6)	4 (4.7)	5 (6.2)	2 (2.5)
Nasopharyngitis	6 (7.6)	3 (3.5)	4 (4.9)	6 (7.4)
Hypertension	3 (3.8)	4 (4.7)	4 (4.9)	2 (2.5)
Bronchitis	4 (5.1)	1 (1.2)	3 (3.7)	6 (7.4)
Hyperlipidaemia	3 (3.8)	0 (0.0)	2 (2.5)	0 (0.0)
Influenza	1 (1.3)	3 (3.5)	1 (1.2)	0 (0.0)
Rheumatoid arthritis	0 (0.0)	2 (2.4)	2 (2.5)	4 (4.9)
Neutropenia*	3 (3.8)	0 (0.0)	0 (0.0)	1 (1.2)
Treatment-emergent serious adverse events				
Atrial tachycardia	0 (0.0)	1 (1.2)	0 (0.0)	0 (0.0)
Supraventricular tachycardia	0 (0.0)	1 (1.2)	0 (0.0)	0 (0.0)
Dyspepsia	0 (0.0)	1 (1.2)	0 (0.0)	0 (0.0)
Cholelithiasis	0 (0.0)	1 (1.2)	0 (0.0)	0 (0.0)
Pneumonia†	0 (0.0)	0 (0.0)	1 (1.2)	0 (0.0)
Lower limb fracture	0 (0.0)	0 (0.0)	1 (1.2)	0 (0.0)
Tendon rupture	0 (0.0)	1 (1.2)	0 (0.0)	0 (0.0)
Osteoarthritis	0 (0.0)	0 (0.0)	1 (1.2)	0 (0.0)
Rheumatoid arthritis	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)
Adenocarcinoma of the cervix	0 (0.0)	1 (1.2)	0 (0.0)	0 (0.0)
Squamous cell carcinoma of the lung	1 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)
Cystocele	0 (0.0)	0 (0.0)	1 (1.2)	0 (0.0)
Angioedema	1 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)
Treatment-emergent adverse events resulting in permanent discontinuation of the study drug				
Patients reporting ≥1 event	5 (6.3)	3 (3.5)	3 (3.7)	2 (2.5)
Treatment-emergent adverse events resulting in interruption of the study drug				
Patients reporting ≥1 event	2 (2.5)	10 (11.8)	8 (9.9)	9 (11.1)
Treatment-emergent adverse events considered to be treatment-related				
	17 (21.5)	8 (9.4)	10 (12.3)	6 (7.4)
Treatment-emergent adverse events of special interest				
Patients reporting ≥1 event	11 (13.9)	5 (5.9)	7 (8.6)	10 (12.3)

*Grade 3 (placebo); grades 1, 2 and 3 (mavrilimumab 150 mg).
†One non-serious pneumonia was reported in the placebo group.
eow, every other week.

group (grades 1, 2 and 3) and one patient in the placebo group (grade 3). ADAs were detected more frequently in patients treated with lower mavrilimumab dosages and none at the 150 mg dosage, consistent with the previous observations that development of ADAs is inversely associated with dose.[30 31] The safety profile for mavrilimumab observed in this study was similar to that reported in previous mavrilimumab studies[17 19] and emerging data with other GM–CSF pathway inhibitors.[32]

No substantial increase in pulmonary events, or apparent dosage–response changes in pulmonary function, dyspnoea score or oxygen saturation, was noted for mavrilimumab-treated patients compared with those receiving placebo. Furthermore, mavrilimumab treatment was not associated with any confirmed or suspected case of PAP, as verified by an Independent Pulmonary Expert Committee. An open-label, phase II safety study (NCT01712399) aims to establish the long-term safety and efficacy profile of mavrilimumab 100 mg in patients with RA.

Despite the success of the currently available biologics in RA, a considerable percentage of patients do not achieve long-term responses to these therapies.[33] Consequently, new treatments employing different mechanisms of action from those currently available, such as GM–CSFR antagonism, are needed. Data from this study demonstrate that mavrilimumab, particularly at a dosage of 150 mg eow, provides a rapid, effective and well-tolerated potential treatment for patients with RA. Moreover, blockade of GM–CSF signalling could be applicable to patients for whom treatment with biologics targeting other pathways has failed or to those with other inflammatory/autoimmune diseases.[33] This proof-of-concept study confirms that inhibition of GM–CSF activity is a promising and novel therapeutic approach for patients with RA, including those who do not adequately respond to currently available therapies.

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Contributors GRB contributed to the study design, interpretation and generation of data, manuscript preparation and review; IBM contributed to study design, interpretation, manuscript preparation and review; JK contributed to study design, data analysis and interpretation, and manuscript preparation and review; PM contributed to data collection, review and interpretation; MK contributed to data collection and manuscript review; JV contributed to study design, data collection, analysis and interpretation, and manuscript preparation and review; AR-R contributed to study design, interpretation of data, manuscript preparation and review; EM contributed to protocol assessment, data analysis and interpretation, and manuscript preparation and review; MAS contributed to study design, data analysis and interpretation and manuscript preparation and review; AG contributed to study design and set-up, data cleaning, analysis and interpretation, and manuscript preparation and review; DS contributed to study design, data cleaning, analysis and interpretation, and manuscript preparation and review; XG contributed to data collection, analysis and interpretation, and manuscript preparation and review; WIW contributed to study design, data analysis and interpretation, and manuscript preparation and review; BW contributed to study design, data analysis and interpretation, and manuscript preparation and review; C-YW contributed to study design, data collection, and manuscript preparation and review; PCR contributed to study design, data collection, analysis and interpretation, and manuscript preparation and review; DC contributed to study design and conduct, data analysis and interpretation, and manuscript preparation and review; MEW contributed to study design and set-up, data analysis and interpretation, and manuscript preparation and review.

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Competing interests GRB is a consultant and has received lecture fees from Abbvie, Bristol-Myers Squibb, MSD, Pfizer, Roche, UCB; IM has received grants and personal fees from Abbvie, AstraZeneca, Bristol-Myers Squibb, Janssen, MedImmune, MSD, Pfizer, UCB; JK is a shareholder and employee of Corrona; has received grants from Abbvie, Amgen, Genentech, Lilly, Pfizer; and is a consultant for Abbvie, Amgen Genentech, Lilly, Pfizer, BMS and MedImmune; PM has received grants from MedImmune; JV is a consultant for Biotest and Samsung Bioepics and has participated in speaker bureaus for Abbvie, MSD, Pfizer, UCB, Roche; AR-R received honoraria for consultation and lectures from Abbvie, Amgen, Chugai, Roche, UCB, MSD, Pfizer, Lilly, Sanofi, Novartis, BMS; EM has received a grant from Organizicion medica de Investigacion; MAS was formerly a full-time employee of MedImmune, a wholly owned subsidiary of AstraZeneca; AG, DS, XG, WIW, BW, C-YW, PCR and DC are employees of MedImmune and hold AstraZeneca shares; MEW has received research grants from Bristol-Myers Squibb, Crescendo Bioscience and UCB, and is a consultant for Abbvie, Amgen, AstraZeneca, Bristol-Myers Squibb, Corrona, Crescendo Bioscience, Genentech/Roche, Janssen, Lycera, Lilly, MedImmune, Merck, Pfizer, Regeneron, Sanofi, UCB.

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EXTENDED REPORT

Infections and the risk of incident giant cell arteritis: a population-based, case-control study

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ABSTRACT

Objectives Alterations in the immune system and infections are suspected to increase susceptibility to giant cell arteritis (GCA). Recently herpes zoster has been directly implicated in the pathogenesis of GCA. We examined the association between prior infections, in particular herpes zoster, and incident GCA in a population-based cohort.

Methods A nested case-control study was performed using an electronic database from the UK. Cases with newly diagnosed GCA were identified using a validated algorithm and compared with age-matched, sex-matched and practice-matched controls. Conditional logistic regression was used to examine the relationship between any infection or herpes zoster infection on the development of GCA after adjusting for potential confounders; results were expressed as incidence rate ratios (IRRs).

Results There were 4559 cases of GCA and 22 795 controls. Any prior infection and herpes zoster were associated with incident GCA (IRR 1.26 (95% CI 1.16 to 1.36), p<0.01; and 1.17 (95% CI 1.04 to 1.32), p<0.01, respectively). A greater number of infections was associated with a higher risk of developing GCA (IRR for 1, 2–4 and ≥5 infections was 1.28, 1.60 and 2.18, respectively).

Conclusions Antecedent infections and, to a lesser extent, herpes zoster infections are modestly associated with incident GCA. These data provide population-level support for the hypothesis that long-standing alterations of the immune system are associated with susceptibility to GCA and suggest that herpes zoster is unlikely to play a major causal role in the pathogenesis of GCA.

INTRODUCTION

Giant cell arteritis (GCA) is a systemic vasculitis affecting medium and large arteries in adults over the age of 50 years. Potential causative mechanisms are still being elucidated but evidence suggests that GCA may be an antigen-driven disease although the exact trigger is still unknown.[1] The involvement of vascular dendritic cell activation, T lymphocytes, interferon-γ and macrophages in the pathogenesis of GCA may indicate that an infectious agent is the cause.[2]

Prior studies have investigated the potential association between microbes and GCA using temporal artery specimens but these studies had conflicting findings.[3–6] Recently, varicella zoster virus (VZV) has been directly implicated in the pathogenesis of GCA after VZV antigen was found in a large majority of temporal arteries of patients with GCA and use of antiviral medication has been suggested as adjunctive therapy in GCA;[7 8] however, independent confirmation of these findings are still needed. These studies raise new questions regarding the role of microbes in GCA and whether their involvement in the pathogenesis begins months and years prior to the onset of GCA. Alternatively, alterations in the immune system seen in patients with GCA, including ageing of the immune system (or immunosenescence), may also increase susceptibility to infections.[9 10]

The objective of this study is to determine the degree to which infections, including clinically evident herpes zoster, are associated with incident GCA using data from a large population-based cohort. Additionally, because prior infections may be temporally associated with the onset of GCA without playing a causal role in disease pathogenesis, the relationship between common infections and incident GCA was explored.

METHODS

Study design and data source

We conducted a nested matched case-control study with incidence density sampling using The Health Improvement Network (THIN), a population-based database from the UK.[11] This design is computationally more efficient than a cohort study and produces ORs that are unbiased estimates of incidence rate ratios (IRRs). The THIN database contains electronic medical records of over 11 million persons in the UK and is representative of the general UK population in terms of demographics and common illnesses.[12] THIN includes information on demographics, medical diagnoses and drug prescriptions. Medical diagnoses are recorded using Read codes, the standard classification system in the UK. The study was approved by the THIN Scientific Review Committee and considered exempt by the Institutional Review Board of the University of Pennsylvania.

Case definition

All patients receiving medical care from 1994 to 2015 from a THIN practitioner were eligible for inclusion. Using a previously validated algorithm, cases were defined as patients over the age of 50 years with at least one Read code for GCA, temporal arteritis or Horton disease, and a prescription for glucocorticoids.[13] Glucocorticoid use was defined as at least two prescriptions for oral glucocorticoids: one within 6 months of the diagnosis date and the second within 6 months of the first prescription. The index date was the date of the first diagnosis code. To avoid the inclusion of

prevalent cases, cases were included only if the initial Read code occurred at least 6 months after registration into the THIN database and after the practice implemented Vision software, a program which automatically codes and enters clinical data into the database. Patients with a prior diagnosis of polymyalgia rheumatica, based on Read codes, were excluded.

Control selection

The control group was selected based on incidence density sampling. For each case, five controls that were alive and free of GCA at the index date were selected and matched on age (within 5 years), sex and general practice.

Exposure and covariate assessment

The main exposures of interest were clinically evident herpes zoster infection or any infection prior to the index date based on a single Read code. Herpes zoster codes coupled with antiviral prescriptions written within 1 month were also examined to improve specificity for herpes zoster infection. Infections were identified using Read codes that were analogous to a list of international classification of disease V.9 codes for infections used previously.[14 15] Aside from herpes zoster infections, a prescription for antimicrobials was not required in order to include viral infections. We also examined the occurrence of any infection, number of infections and five common infections in the elderly (infections affecting the respiratory tract, urinary tract, gastrointestinal tract, conjunctiva, and skin and soft tissue).[16] The timing of infections prior to the index date were evaluated to determine if infections closer to the index date were more highly associated with GCA compared with more remote infections. Due to the possibility of misdiagnosis of symptoms of GCA as infections, infections that occurred within 6 months prior to the date of the diagnosis of GCA were not included in the primary analysis but were analysed in a sensitivity analysis (see online supplementary text).

Covariates included receipt of zoster vaccination, prior use of immunosuppressive therapies (glucocorticoids, azathioprine, ciclosporin, leflunomide, methotrexate, mycophenolate or sulfasalazine) earlier than 6 months from index date, any prior alcohol use, smoking history (ever—yes/no) and comorbidities at index date categorised using the Charlson Comorbidity Index.[17 18] The majority of prescriptions for oral immunosuppressive drugs are accurately captured in THIN.[19 20]

Statistical analysis

For comparison of the baseline characteristics, we used Student's t-test (or, if not normally distributed, Wilcoxon rank sum) and χ^2 test for continuous and categorical data, respectively. We used conditional logistic regression and, because the resulting ORs from the models accurately estimated the IRRs, the relationship between infection and the development of GCA was expressed with IRR and 95% CIs. Linear test for trend was performed to examine whether increasing categories of infection count was associated with a linear increase in GCA risk. All analyses were adjusted for prior glucocorticoid use, prior use of non-glucocorticoid immunosuppressive therapies, alcohol use, smoking history and the Charlson Comorbidity Index. To determine if receipt of the zoster vaccination was an effect modifier on the relationship between herpes zoster infection and GCA, a herpes zoster-by-vaccination interaction was included in the model along with herpes zoster infection and receipt of zoster vaccine as separate variables. Patients were also analysed according to the time period prior to index date in which the infection occurred to determine if recent infections were more highly associated with GCA compared with earlier infections; infections diagnosed within the 6 months prior to diagnosis were included in this analysis. To account for potential confounding due to differential use of glucocorticoids or other immunosuppressive medications among cases and controls, a sensitivity analysis excluding patients who previously received at least one prescription for an oral glucocorticoid or immunosuppressive medication was performed. Additional sensitivity analyses that were performed are listed in the online supplementary text. A significance level of 0.05 was used for all tests of hypothesis. All analyses were performed using Stata V.12.1 (Stata, College Station, Texas, USA).

RESULTS

Study population

There were 4559 cases of GCA and 22 795 matched controls. Patients with GCA were more likely to have received a prescription for oral glucocorticoids and other immunosuppressive

Table 1 Characteristics of study subjects

	Cases N=4559	Controls N=22 795	p Value
Age at index date, years	74 (9)	73 (10)	N/A*
Female sex, %	71%	71%	N/A*
Duration of follow-up before index date, years	6.4 (4.6)	6.5 (4.5)	0.35
Charlson Comorbidity Index	1 (0, 2)	0 (0, 2)	<0.01
Alcohol use, %	67%	66%	0.27
Tobacco use, %	51%	43%	<0.01
Prior use of glucocorticoid, %	28%	16%	<0.01
No. of prescriptions†, median (IQR)	3 (1, 10)	3 (1, 9)	<0.01
Time since last prescription (years)†, median (IQR)	1.4 (0.6, 4.2)	2.2 (0.8, 5.2)	<0.01
Prior use of immunosuppressive therapy, %	3%	2%	<0.01
No. of prescriptions†, median (IQR)	19 (7, 51)	22 (6, 56)	0.98
Time since last prescription (years)†, median (IQR)	0.8 (0.6, 3.7)	0.7 (0.5, 4.7)	0.14
Prior zoster vaccination, %	0.6%	0.4%	0.09
Time since last vaccination (months)†, median (IQR)	4 (2, 9)	5 (2, 9)	0.78

Data expressed as mean (SD) or percentage.
*Controls were age-matched and sex-matched to cases.
†Among patients who received a prescription.

Table 2 Unadjusted occurrence of infections among cases of GCA and controls

	Cases N=4559	Controls N=22 795	p Value
Any prior herpes zoster infection			
Percentage	9%	7%	<0.01
Time from zoster to GCA, years	6 (3, 10)	6 (3, 10)	0.99
Prior herpes zoster infection+ antiviral prescription			
Percentage	5%	4%	0.05
Time from zoster to GCA, years	5 (2, 8)	5 (2, 9)	0.93
Antiviral prescription among those who had zoster	53%	56%	0.34
Any prior infection	68%	61%	<0.01
Respiratory infection	58%	51%	<0.01
Urinary tract infection	19%	15%	<0.01
Gastrointestinal infection	6%	4%	<0.01
Skin or soft tissue infection	17%	14%	<0.01
Conjunctivitis	16%	12%	<0.01

Data expressed as median (IQR) or percentage.
GCA, giant cell arteritis.

therapy ≥6 months prior to the index date. There was no difference in the proportion who received the zoster vaccination between cases and controls (table 1). Evaluation of the individual diagnostic categories within the Charlson Comorbidity Index revealed that, prior to the index date, patients with GCA were more likely to have cerebrovascular disease (9% vs 7%, p<0.01), chronic pulmonary disease (22% vs 15%, p<0.01), mild liver disease (0.6% vs 0.4%, p=0.02), peptic ulcer disease (6% vs 4%, p<0.01), peripheral vascular disease (5% vs 3%, p<0.01) and renal disease (13% vs 11%, p<0.01), and less likely to have a pre-existing rheumatic disease (3% vs 5%, p<0.01) or dementia (1% vs 2%, p<0.01) compared with controls.

Herpes zoster and GCA

GCA cases were more likely to have had a prior herpes zoster infection (9% vs 7%, p<0.01; table 2). However, when the definition of a herpes zoster infection was restricted only to Read codes accompanied by prescription of an antiviral, the difference was attenuated and no longer significant.

After adjustment for potential confounders, a prior history of herpes zoster infection greater than 6 months before the index date was associated with an increased risk of developing GCA (adjusted IRR 1.17 (95% CI 1.04 to 1.32), p<0.01) (table 3). Similar results were found even after excluding patients who previously received oral glucocorticoids or immunosuppressive therapies (data not shown). The results of additional sensitivity analyses (see online supplementary text) led to similar results (see online supplementary table S1).

After stratifying by time period prior to the index date, there was no association between herpes zoster infection and GCA among the different time strata except for herpes zoster infections that occurred 5–10 years before the index date. Any infection was significantly associated with incident GCA in all time periods with the greatest association seen with infections which occurred in the first year before the index date (table 4).

There were 115 (0.4%) patients in the cohort who received the zoster vaccination. The herpes zoster-by-vaccination interaction term was statistically significant (p for interaction=0.04). That is, patients who received the zoster vaccine had a stronger association between herpes zoster infection and GCA (adjusted IRR 3.53 (95% CI 1.17 to 10.62)) compared with those who did not receive the vaccine (adjusted IRR 1.16 (95% CI 1.03 to 1.30)), even after adjusting for potential confounders.

Other infections and GCA

There was a higher occurrence of any infection in patients with GCA compared with controls (68% vs 61%, p<0.01) (table 2). Patients who had at least one prior infection were more likely to develop GCA (adjusted IRR 1.26 (95% CI 1.16 to 1.36), p<0.01) (table 3). A higher number of prior infections was also associated with a greater risk of GCA: 0 (reference), 1, 2–4 or ≥5 infections yielded IRRs of 1, 1.28, 1.60 and 2.18, respectively (test for trend: p<0.01).

Analysis of the five common types of infections showed a higher risk of GCA with each type of infection: respiratory tract (IRR 1.25 (95% CI 1.16 to 1.35), p<0.01), urinary tract (IRR 1.26 (95% CI 1.15 to 1.38), p<0.01), gastrointestinal tract (IRR 1.33 (95% CI 1.15 to 1.53), p<0.01), conjunctiva (IRR 1.25 (95% CI 1.14 to 1.37), p<0.01), and skin and soft tissue (IRR 1.10 (95% CI 1.00 to 1.21), p=0.04). A sensitivity analysis excluding patients who previously received glucocorticoids or other immunosuppressive therapies led to similar results (data not shown).

Infection was associated with incident GCA within all strata of time periods of infection prior to index date with the greatest association being within 6 months prior to the index date (table 4).

DISCUSSION

This study used a large population-based cohort to examine the association between infections and newly diagnosed GCA. We found that prior infections were associated with the

Table 3 The association of infections with incident giant cell arteritis

Exposure	Unadjusted IRR (95% CI)	p Value	Adjusted IRR (95% CI)*	p Value
Herpes zoster infection	1.24 (1.10 to 1.39)	<0.01	1.17 (1.04 to 1.32)	<0.01
Herpes zoster infection and antiviral therapy	1.16 (0.99 to 1.36)	0.05	1.09 (0.93 to 1.28)	0.27
Any infection	1.44 (1.34 to 1.56)	<0.01	1.26 (1.16 to 1.36)	<0.01
No. of infections				
0	1 (reference)	–	1 (reference)	–
1	1.38 (1.27 to 1.50)	<0.01	1.28 (1.18 to 1.40)	<0.01
2–4	1.88 (1.71 to 2.08)	<0.01	1.60 (1.44 to 1.77)	<0.01
5 or more	2.94 (2.58 to 3.36)	<0.01	2.18 (1.90 to 2.51)	<0.01
	Test for trend	<0.01	*Test for trend*	<0.01

*Adjusted for Charlson Comorbidity Index, alcohol use, smoking history, prior use of immunosuppressive therapies and prior use of oral glucocorticoids.
IRR, incident rate ratio.

Table 4 The association of herpes zoster and all infections with incident giant cell arteritis stratified by time period prior to index date

	Herpes zoster infection		Any infection	
	Adjusted IRR (95% CI)*	p Value	Adjusted IRR (95% CI)*	p Value
<1 year†	1.32 (0.95 to 1.82)	0.10	1.66 (1.54 to 1.79)	<0.01
1–2 years	1.11 (0.79 to 1.56)	0.55	1.32 (1.22 to 1.43)	<0.01
2–3 years	1.00 (0.70 to 1.43)	0.99	1.19 (1.09 to 1.29)	<0.01
3–4 years	0.92 (0.63 to 1.36)	0.69	1.19 (1.09 to 1.30)	<0.01
4–5 years	1.15 (0.77 to 1.70)	0.49	1.14 (1.05 to 1.25)	<0.01
5–10 years	1.36 (1.14 to 1.64)	<0.01	1.26 (1.17 to 1.35)	<0.01
>10 years	1.18 (0.99 to 1.42)	0.07	1.22 (1.13 to 1.32)	<0.01

*Adjusted for Charlson Comorbidity Index, alcohol use, smoking history, prior use of immunosuppressive therapies and prior use of oral glucocorticoids.
†Including 6 months prior to index date.
IRR, incident rate ratio.

development of GCA in a 'dose-dependent' fashion, but only a modest association was found between herpes zoster infection and GCA.

There is limited information on the relationship between common infections and incident GCA. We found that the risk of incident GCA was higher among patients who had prior infections and was similar among several common types of infections. Except for the 1st year prior to the index date, the association between infection and GCA was similar regardless of the time period in which the infection occurred, although these subgroup analyses were limited by smaller sample sizes. However the greatest association between infection and GCA was seen in the 1st year prior to the diagnosis. This finding may be due to misclassification bias if symptoms of GCA (such as fever) were misdiagnosed as infection. Alternatively, it is possible infections are directly involved in the pathogenesis of disease or that greater immune dysregulation occurs prior to the onset of disease. One prior study found an increased short-term effect of infections on the occurrence of GCA such that infections were three times more likely to occur in patients who developed GCA compared with matched controls.[21] Our study adds to this finding and shows that even years prior to onset of GCA, patients with GCA are more likely to have infections compared with controls. It is also notable that the occurrence of infection in GCA only modestly increases after diagnosis and initiation of glucocorticoids, also suggesting that medications alone cannot explain the increased association of infections in patients with GCA.[22]

Recently, the VZV antigen was found in the great majority of temporal artery biopsy specimens of patients with GCA[7] whereas prior studies also using histological specimens found no evidence of VZV in temporal artery biopsies.[5 6 23] Our study found a minimal-to-no association of clinically overt herpes zoster with GCA, thus not providing population-level support to recent observations on the association between VZV and GCA. The lack of a strong temporal association between herpes zoster infections and GCA further contradicts the possibility that herpes zoster has a causal role in the onset of GCA. However, it is possible that the histological findings can be explained by latent or subclinical VZV (eg, without classic skin manifestations) which our study was not able to assess. Similarly, reactivation of VZV at time of GCA onset may also explain the histological findings; however, when we included herpes zoster infections within 6 months of GCA diagnosis, results were similar suggesting that there was not a higher occurrence of clinically overt herpes zoster infections at the time of diagnosis.

There are several possible explanations for the association between infection and development of GCA. Infections have long been theorised to induce and perpetuate autoimmunity through alterations in the immune system.[24 25] Additionally, patients with GCA may simply be more predisposed to having infections possibly as a result of immune dysregulation. The association with GCA and older age suggests that immunosenescence, or the ageing of the immune system, is involved in susceptibility for GCA.[9 10] Whether infections are involved in the causal pathway or are simply a marker of immune dysfunction is still unclear. Lastly, the possibility of a vascular microbiome is emerging as studies now refute the idea that blood vessels are sterile.[26] Perturbances in the vascular microbiome from infections and/or antimicrobial therapy may increase susceptibility to GCA, although no studies have yet demonstrated this directly.[3 27 28]

Interestingly, the zoster vaccine was a significant effect modifier on the relationship between herpes zoster infection and GCA, such that herpes zoster infection conferred a much higher risk of GCA among patients who did versus patients who did not receive the zoster vaccine. However, this finding should be interpreted with caution since receipt of the zoster vaccine in this study was exceedingly rare (n=115 (0.4%)) given that the zoster vaccine became routinely available in the UK in September 2015 and only among individuals aged 70–78 years.[29] If a true interaction between herpes zoster infection and zoster vaccine exists, the possibility of an adjuvant effect to the immune system by re-inoculation with the antigen may explain this association. Alternatively, other risk factors may be present in patients with GCA prior to diagnosis which increase their likelihood of receiving vaccination. Additional studies with a larger number of patients who received the vaccine are needed to confirm this finding.

Our study has several strengths. Performing a nested case-control study within a population-based cohort enabled us to obtain a large sample of patients with a relatively rare disease. This study is one of the largest cohorts of patients with GCA. Use of the THIN database has several advantages over other administrative databases including availability of electronic medical record data (not just claims-based data) which provide greater depth of information as well as long periods of follow-up, and at times the lifelong health record of an individual patient, enabling us to examine long latency periods between exposure and outcome. The THIN data have been used extensively in epidemiological studies including studies of GCA.[13 22 30]

There are also several limitations of our study to consider. Misclassification may have occurred as Read codes for infections

were not validated and may not have accurately identified the exposures. Misclassification of the diagnosis of GCA may have occurred but would likely bias to the null, further strengthening our results. Glucocorticoids and other immunosuppressive therapies were more often prescribed to patients who later developed GCA suggesting that other potential comorbidities and/or immunosuppressive therapies not accounted for in the analysis may have confounded results. While we did not perform an in-depth exploration for the reasons for antecedent use of glucocorticoids, examination of the diagnostic categories comprising the Charlson Comorbidity Index revealed that a significantly greater proportion of patients with GCA had a chronic pulmonary disease and patients with GCA were less likely to have a pre-existing rheumatic disease (which included juvenile idiopathic arthritis, lupus, myositis, polymyalgia rheumatica, rheumatoid arthritis and scleroderma); the higher prevalence of pulmonary disease in patients with GCA may possibly explain the higher use of glucocorticoids prior to diagnosis. Furthermore, significant associations between infection and the risk of incident GCA were observed in multivariable models that adjusted for the use of immunosuppressive therapies and in analyses that excluded patients who had previously received glucocorticoids or other immunosuppressive medications. Lastly, patients with pre-existing polymyalgia rheumatica, which can often precede the diagnosis of GCA, were excluded which limited the ability to examine this important subgroup.[31]

In summary, this study found that antecedent infections are associated with incident GCA, although infections are probably a minor determinant of overall risk of GCA. The modest association seen between herpes zoster and GCA suggests that herpes zoster infections are unlikely to play a major causal role in the pathogenesis of GCA. These data provide population-level support for the hypothesis that long-standing alterations of the immune system are associated with susceptibility to GCA.

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Competing interests Intercontinental Marketing Services (IMS) Health Real World Evidence Solutions is an UK-based expert in anonymous patient data for the healthcare industry. IMS Health is a commercial organisation that supplies data and trains and supports researchers in the use of primary care patient data. Data are available for us in medical research in the academic setting as well as in industry for a fee which varies depending on the type of data requested. Aside from undergoing ethical review by The Health Improvement Network (THIN) Scientific Review Committee, independent academic groups who voluntarily act as an ethical review body, this protocol was not in any way discussed with IMS Health nor were any changes made by the company. We did not receive financial support or other forms of computational or analytical support from IMS/THIN. The data were collected by IMS and the general practitioners without knowledge of the study objectives and hypotheses.

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EXTENDED REPORT

Disease activity trajectories in early axial spondyloarthritis: results from the DESIR cohort

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ABSTRACT

Background Disease activity may change over time in axial spondyloarthritis (axSpA). The objectives were to identify patterns of disease activity evolution in patients with early axSpA.

Methods Patients from the prospective early axSpA cohort (DEvenir des Spondyloarthrites Indifférenciées Récentes (DESIR)) who fulfilled the Assessment in SpondyloArthritis Society (ASAS) criteria for axSpA at baseline and with at least three Ankylosing Spondylitis Disease Activity Score (ASDAS) values available over the 3 years of follow-up were analysed. *Statistical analyses:* trajectories were estimated by group-based trajectory modelling; predisposing baseline factors for such trajectories were identified by univariate and multivariable multinomial (logit) regression; work disability over time was compared between the trajectories by Cox hazard model.

Results In all, 370 patients were analysed: mean disease duration was 1.6 (±0.9) years. The five distinct trajectories of disease activity over the 3 years were (t1) 'persistent moderate disease activity' (n=134 (36.2%)); (t2) 'persistent inactive disease' (n=66 (17.8%); (t3) 'changing from very high disease activity to inactive disease' ((n=29 (7.8%)); (t4) 'persistent high disease activity' (n=126 (34.1%)) and (t5) 'persistent very high disease activity' (n=15 (4.1%)). After adjustment for other characteristics, t2 was associated with a white-collar job (OR=2.6 (95% CI 1.0 to 6.7)) and t3 with male gender (OR=7.1 (1.6 to 32.2)), higher education level (OR=9.4 (1.4 to 63.4)) and peripheral joint involvement (OR=6.2 (1.23 to 31.32)). Patients from (t4) and (t5) were more often declared work disabled over follow-up (HR=5.2 (1.5 to 18.0) and HR=8.0 (1.3 to 47.9), respectively).

Conclusions Trajectory modelling of disease activity was feasible in early axSpA: more than 30% patients (141/370) were in a trajectory with a persistent high disease activity. Persistent high disease activity trajectories were significantly associated with consequences on work.

Trial registration number NCT01648907.

Axial spondyloarthritis (axSpA) presentation can be phenotypically heterogeneous, for example, predominant axial involvement, predominant articular peripheral involvement with or without psoriasis, exclusive axial involvement with radiographic sacroiliitis, etc.[1] Due to this presentation diversity and also due to the different treatment modalities and other elements (eg, socioeconomic environment and access to healthcare, gender, etc.), disease activity can be heterogeneous, both at presentation and over time.[2 3] However, studies aiming to identify patterns of disease activity evolution over time in axSpA are sparse.[4] Furthermore, a disability is often associated with higher disease activity scores.[5] Therefore, a better identification and characterisation of homogeneous groups of patients based on disease activity would allow a better and tailored strategy for the follow-up of patients with axSpA.

In other disciplines, trajectory modelling has been applied to identify patterns of behaviour,[6–8] but only very few studies have applied these methodologies in the field of rheumatic diseases, to identify homogeneous groups of patients over follow-up.[9 10] To the best of our knowledge, those methods have not been applied in axSpA.

The Ankylosing Spondylitis Disease Activity Score (ASDAS) based on C-reactive protein (CRP) is a widely validated tool to measure disease activity in axSpA that integrates both patient-reported items and objective inflammatory markers.[11 12] Given its face validity and its psychometric properties,[13 14] we proposed to use ASDAS to define the trajectories of disease activity over time in an axSpA cohort.

Using the DESIR cohort[15] data, we aimed to identify (a) disease activity trajectories in patients with early axSpA over a 3-year follow-up period, (b) the baseline characteristics associated with such trajectories and (c) the outcomes associated with each trajectory in terms of treatment and disability.

PATIENTS AND METHODS

Study design

DESIR is a French prospective, multicentre, longitudinal observational cohort aiming to study patients with early inflammatory back pain (IBP) suggestive of SpA (clinicaltrials.gov NCT01648907).[15 16] This study fulfilled current good clinical practices and has obtained the approval of the appropriate ethical committee. Participants in the study gave their written informed consent.[17]

Patients

A total of 708 patients were included in DESIR: consecutive patients aged >18 and <50 years with IBP according to the Calin *et al*[18] or Berlin[19] criteria for more than 3 months but less than 3 years and symptoms suggestive of diagnosis for SpA score ≥5 (on a Numerical Rating Scale of 0–10, where 0=not suggestive and 10=very suggestive of SpA). None were taking tumour necrosis factor α inhibitors (TNFi) at baseline. For our

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analysis, only patients fulfilling the ASAS criteria for classification of axSpA[20 21] at baseline and for whom at least three ASDAS values were available during follow-up were included. The data set used was locked in December 2013.

Collected data

The collected data comprised demographics and clinical presentation of the disease at baseline and each 6 months for the first 2 years, and at year 3. Demographics included age and gender. Medico-economic data were also collected: highest degree of education; type of employment: blue collar (ie, physically demanding jobs, eg, farmer) versus white collar (ie, sedentary job, eg, secretary); employment state (ie, currently working, on sick leave or in permanent work disability) and days of sick leave over each period.

Disease activity for this analysis was evaluated by the ASDAS.[11] The ASDAS associates several activity criteria: total back pain, peripheral pain/swelling, duration of morning stiffness (questions 2, 3 and 6 of the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI),[22] respectively), patient global and CRP, combined in a single parameter. Since methodologies allowing the trajectories definitions require a single variable to define such trajectories,[23] this was used to define disease activity. Severity of the disease was assessed by the Bath Ankylosing Spondylitis Functional Index (BASFI).[24] Quality of life was evaluated according to the Short Form-36 (SF-36).[25] Non-steroidal anti-inflammatory drugs (NSAID) treatment was evaluated by the ASAS-NSAID score[26] during the 6 months preceding each study visit. Exposure to TNFi over follow-up was also collected.

Statistical analysis

a. Trajectories modelling: To identify distinctive trajectories, we used a semiparametric mixture model: group-based trajectory model (GBTM).[23 27] GBTMs model the relationship between a variable (here, ASDAS) and time: for each trajectory, the shape of the trajectory and the estimated proportion of the population belonging to each trajectory, and for each patient, the probability to belong to each different trajectory. Each participant is then assigned to the group for which her/his probability to belong to a trajectory is the highest. For this, we used the TRAJ procedure in the SAS software V.9.4: this procedure allows the estimation and comparison of models with several numbers of trajectories and shapes (constant, linear, quadratic or cubic). To select the best-fitting model, models with one to six trajectories and with several shapes were compared. The best-fitting model was selected according to the Bayesian information criterion. It was also required that means of individual posterior probability to belong to a trajectory were all superior to 0.7.

b. Baseline factors associated with each trajectory: Demographics, disease phenotype, disease severity, quality of life and NSAID treatment at baseline were compared in the different trajectories by univariate multinomial (logit) regression. Thereafter, multivariable multinomial regression was performed to identify the independent baseline factors associated with each trajectory, including in the model the baseline characteristics with a p value <0.10 after the univariate analyses. For these analyses, trajectory 1 ('persistent moderate disease activity') was used as the reference trajectory, because it was the trajectory with the largest number of patients and fitted better the data according to the Akaike Index Criterion. ASDAS was not included in the model because it was the parameter defining the trajectory. BASDAI was not included either, because of a concern of colinearity with ASDAS.

c. Outcomes associated with each trajectory: Exposure to TNFi and disability declaration were collected every 6 months, and evaluated over the 3 years of follow-up as a dichotomous

Table 1 Baseline disease characteristics of 370 early patients with axial spondyloarthritis

	All patients N=370	Trajectory 1 Moderate disease activity n=134	Trajectory 2 Inactive disease n=66	Trajectory 3 Changing disease activity n=29	Trajectory 4 High disease activity n=126	Trajectory 5 Very high disease activity n=15
Gender (male)	187 (51%)	70 (52%)	44 (62%)	22 (76%)	49 (39%)	5 (33%)
Age (years)	32 (7.4)	30.8 (7.3)	31.4 (7.2)	29.4 (7.8)	32.5 (7.3)	35.2 (6.3)
Education (university level)	242 (65%)	98 (73%)	55 (83%)	23 (79%)	60 (66%)	6 (40%)
White-collar job (vs blue-collar job)	125/310 (40%)	51/116 (44%)	**38/57 (67%)***	9/19 (47%)	**24/103 (23%)**	**3/15 (20%)**
Symptoms duration (years)	1.6 (0.9)	1.7 (1.0)	**1.3 (0.8)**	1.6 (0.8)	1.7 (0.9)	1.6 (0.9)
History of articular peripheral involvement	200 (54%)	62 (46%)	28 (42%)	**23 (79%)**	**74 (59%)**	**13 (87%)**
History of enthesitis involvement	167 (45%)	62 (46%)	**20 (30%)**	18 (62%)	60 (48%)	7 (47%)
Presence of HLA B27	309 (84%)	114 (85%)	59 (89%)	26 (90%)	100 (79%)	10 (67%)
Radiographic sacroiliitis†	92/314 (29%)	37/111 (33%)	10/56 (18%)	12/25 (48%)	31/108 (29%)	2/14 (14%)
MRI sacroiliitis†	185/364 (51%)	72/132 (55%)	28/66 (42%)	20/28 (71%)	58/124 (47%)	7/14 (50%)
ASDAS	2.6 (1.0)	2.2 (0.6)	1.5 (0.6)	4.0 (0.8)	3.2 (0.7)	4.1 (0.5)
BASDAI (0–100)	41.9 (20.5)	33.6 (15.4)	22.9 (15.9)	58.0 (15.3)	54.6 (15.5)	62.7 (15.9)
BASFI (0–100)	29 (22.2)	21.0 (16.9)	**10.7 (4.5)**	**42.5 (22.8)**	**39.7 (20.8)**	**52.4 (21.5)**
SF-36 physical component summary scale	41 (9.4)	42.6 (8.4)	**48.1 (7.7)**	**35.8 (7.4)**	**37.0 (8.7)**	**32.5 (6.2)**
SF-36 mental component summary scale	41 (11.4)	43.5 (10.0)	46.3 (10.5)	**36.6 (12.5)**	**37.2 (11.3)**	**34.8 (9.7)**
NSAID score‡	52 (42.1)	51.5 (42.6)	43.7 (44.1)	44.4 (34.3)	56.2 (42.6)	69.4 (31.3)

Results are presented as number (percentage) and mean (SD) for categorical variables and continuous variables, respectively.
*Results in bold are significantly different in univariate analysis when compared with trajectory 1.
†Radiographic sacroiliitis according to modified New York criteria was assessed by the local investigator. MRI sacroiliitis according to the local investigator.
‡NSAID score representing the intake during the 6 months preceding the study visit.[26] A score of 100 reflects a full NSAID dose intake during the study period.
ASDAS, Ankylosing Spondylitis Disease Activity Score; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; HLA, Human Leucocyte Antigen; NSAID, non-steroidal anti-inflammatory drugs; SF-36, Short Form-36.

state ('ever exposed' and 'ever disabled'). Both were compared in the different trajectories by Cox hazard models (proportional hazards assumption was confirmed by Schoenfeld residuals). The number of days of sick leave (if patients were declared in permanent work disability, they were considered as in sick leave all year) over follow-up were compared in the different trajectories by linear regression.

SAS V.9.4 was used for the TRAJ procedure; R software V.3.1.1 was used for the rest of the analyses.

RESULTS

Of the 708 patients included in the DESIR cohort at baseline, 439 (62.0%) fulfilled the ASAS criteria for axial SpA at baseline and 370 (52.3%) had at least three ASDAS values available during the 3 years of follow-up (table 1). Among the included patients, the percentage of patients with available data for ASDAS was 359 (82%), 319 (73%), 319 (73%), 295 (67%), 326 (74%) and 219 (66%) for the baseline, 6, 12, 18, 24 and 36 months, respectively.

Trajectories

The analyses yielded five distinctive trajectories of disease activity during the 3 years of follow-up (figure 1 and see online supplementary table S1).

Trajectory 1 (t1) (n=134 (36.2%)) included patients with 'persistent moderate disease activity'. Trajectory 2 (t2) (n=66 (17.8%)) included patients with 'persistent inactive disease'. Trajectory 3 (t3) (n=29 (7.8%)) included patients very high disease activity at baseline but reaching an inactive disease after 12 months and remaining in this state until the 36th month ('changing disease activity'). Trajectory 4 (t4) (n=126 (34.1%)) included patients who presented with 'persistent high disease activity' and trajectory 5 (t5) (n=15 (4.1%)) included patients with 'persistent very high disease activity'.

Baseline characteristics associated with each trajectory

Results of the multivariable analysis are presented in table 2: compared with patients in (t1), patients in (t2) ('persistent inactive disease') had more frequently a white-collar job (OR=2.6 (95% CI 1.0 to 6.7)), whereas patients from (t3) ('changing disease activity') were more frequently males (OR=7.1 (1.6 to 32.2)) with a higher degree of education (OR=9.4 (1.4 to 63.4) and more frequently a history of peripheral joint involvement (OR=6.2 (1.2 to 31.1)). Poorer quality of life (SF36 mental and physical components) at baseline was significantly associated with high disease activity trajectories in the univariable model, but was not retained in the multivariable model (table 1).

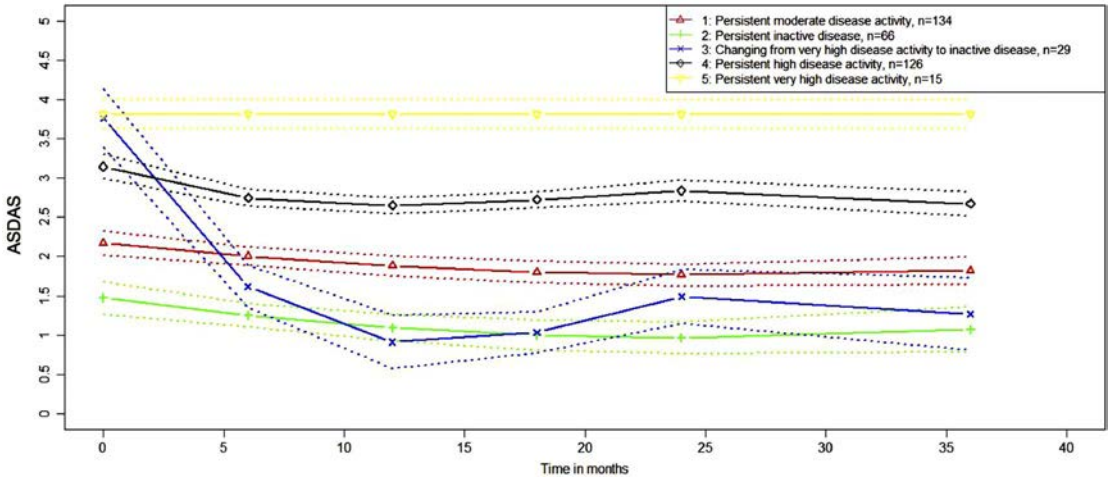

Figure 1 Trajectories of disease activity in early axial spondyloarthritis according to the group-based trajectory model technique. ASDAS, Ankylosing Spondylitis Disease Activity Score.

Table 2 Characteristics associated with trajectories in early axial spondyloarthritis (multinomial logit regression)*

	Trajectory 2† 'Inactive disease' n=66 OR (95% CI)	Trajectory 3 'Changing disease activity' n=29 OR (95% CI)	Trajectory 4 'High disease activity' n=126 OR (95% CI)	Trajectory 5 'Very high disease activity' n=15 OR (95% CI)
Gender (male)‡	1.4 (0.6 to 3.0)	**7.1 (1.6 to 32.2)**	0.8 (0.4 to 1.2)	0.4 (0.1 to 2.0)
Education (university)	0.9 (0.3 to 2.7)	**9.4 (1.4 to 63.4)**	0.5 (0.2 to 1.2)	0.4 (0.1 to 2.2)
White-collar job (vs blue-collar job)	**2.6 (1.0 to 6.7)**	1.1 (0.3 to 5.0)	0.7 (0.3 to 1.5)	0.2 (0.0 to 1.5)
Symptoms duration (years)	**0.5 (0.3 to 0.7)**	0.9 (0.4 to 1.8)	0.9 (0.6 to 1.3)	0.8 (0.4 to 1.8)
History of peripheral arthritis	0.9 (0.4 to 2.2)	**6.2 (1.3 to 31.3)**	1.2 (0.6 to 2.4)	10.2 (1.0 to 106.5)
BASFI	1.0 (0.9 to 1.0)	1.1 (0.9 to 1.0)	**1.0 (1.0 to 1.1)**	1.0 (1.0 to 1.1)

*Only variables independently associated with at least one trajectory are represented here.
†Trajectory 1 'Moderate disease activity' was the reference trajectory.
‡Statistically significant results are highlighted in bold.
BASFI, Bath Ankylosing Spondylitis Functional Index.

(T3) ('changing disease activity') and (t5) ('persistent very high disease activity') presented with almost identical ASDAS mean values at baseline (4.0 (±0.8) and 4.1 (±0.5) for (t3) and (t5), respectively). We compared these two subgroups in terms of baseline characteristics, and multivariable analysis only evidenced gender (male) (OR=17.59 (2.2 to 424.5)) and university education (OR=12.0 (1.5 to 279.2)) as baseline characteristics independently associated with (t3).

Outcomes associated with trajectories

TNFi intake

Twenty-five (18.7%), 4 (6.1%), 21 (72.4%), 42 (33.3%) and 8 (53.3%) patients received a TNFi during follow-up in (t1), (t2), (t3), (t4) and (t5), respectively (table 3). Compared with (t1), patients from (t3) ('changing disease activity') were the group of patients who received more frequently a TNFi over time (HR=4.5 (95% CI 3.5 to 5.9)). Interestingly, patients from trajectories (t4) ('persistent high disease activity') and (t5) ('persistent very high disease activity) also received more TNFi as compared with (t1) (HR=1.8 (1.4 to 2.2) and HR=2.63 (1.8 to 3.9), respectively).

Work disability

Patients from trajectories (t1), (t2), (t3), (t4) and (t5) presented a mean (±SD) number of days of sick leave over the 3 years of follow-up of 43 (±127), 15 (±41), 22 (±36), 75 (±116) and 300 (±312), respectively. Patients from (t5) were significantly more frequently on sick leave over follow-up (p<0.001) compared with patients from (t1). Over the 3 years, 1.5%, 3.0%, 0%, 7.9% and 13.3% patients from (t1), (t2), (t3), (t4) and (t5), respectively, were considered work disabled. Patients from (t4) and (t5), the trajectories with persistent high disease activity, were significantly more frequently declared work disabled over time (HR=5.2 (1.5 to 18.0) and HR=8.0 (1.3 to 47.9), for (t4) and (t5), respectively). Interestingly, despite the initial very high disease activity state, no patients from (t3) ('changing disease activity') were declared work disabled over follow-up (table 3).

DISCUSSION

In the era of personalised medicine and tailored treatment strategies, the identification of disease evolution is important to improve patients' management. Here, we have applied an original and validated methodology to determine longitudinal patterns of disease activity in an early axSpA cohort. This study identified five disease activity trajectories: two trajectories with stable moderate/low disease activity (t1 and t2), two trajectories with stable high/very high disease activity (t4 and t5) and a disease activity improving trajectory (t3); 141/370 patients (38%) belonged to trajectories of persistent disease activity.

These results highlight that even in a country with wide access to biologics,[28] axSpA remains a disease where more than a third of patients could remain in moderate to high disease activity over several years.

Nevertheless, some baseline characteristics were strongly associated with stable low and improving disease activity trajectories, that is, being a male, a higher degree of education and having a white-collar job. These results are consistent with what has been previously reported in rheumatoid arthritis (RA): in the COMOrbidities in Rheumatoid Arthritis (COMORA) cross-sectional study that included 3920 patients with RA worldwide, after adjustment, women (vs men) and low-educated (vs university) patients had higher disease activity.[29] In the field of SpA, feminine gender has also been found to be associated with

Table 3 Outcomes associated with distinct disease activity trajectories in early axial spondyloarthritis

	Trajectory 1 'Moderate disease activity' n=134 Reference Trajectory	Trajectory 2 'Inactive disease' n=66			Trajectory 3 'Changing disease activity' n=29			Trajectory 4 'High disease activity' n=126			Trajectory 5 'Very high disease activity' n=15		
	n (%)	n (%)	HR (95% CI)	p Value	n (%)	HR (95% CI)	p Value	n (%)	HR (95% CI)	p Value	n (%)	HR (95% CI)	p Value
TNFi treatment over 3 years	25 (18.7)	4 (6.1)	**0.5 (0.3 to 0.7)**	**<0.001**	21 (72.4)	**4.5 (3.5 to 5.9)**	**<0.001**	42 (33.3)	**1.8 (1.4 to 2.2)**	<0.001	8 (53.3)	**2.6 (1.8 to 3.9)**	**<0.001**
Disability over 3 years	2 (1.5)	2 (3.0)	1.3 (0.2 to 7.7)	NS	0 (0)	0 (NA to NA)	NS	10 (7.9)	**5.2 (1.5 to 18.0)**	**0.009**	2 (13.3)	**8.0 (1.3 to 47.9)**	**0.02**
	Mean (SD)	**Mean (SD)**	**Coefficient**	**p Value**	**Mean (SD)**	**Coefficient**	**p Value**	**Mean (SD)**	**Coefficient**	**p Value**	**Mean (SD)**	**Coefficient**	**p Value**
Days of sick leave over 3 years	43.0 (126.9)	15.3 (40.9)	−27.7	NS	22.3 (35.8)	−20.7	NS	74.7 (115.9)	−31.7	NS	**300.7 (312.1)**	**257.7**	**<0.001**

NS, not statistically significant; TNFi, tumour necrosis factor α inhibitors.

higher disease activity reported by the BASDAI despite lower acute phase reactants in several clinical trials,[30–35] and high-rank occupation has been found to be associated with lower disease activity in patients with SpA.[36] It is difficult to determine the causality of such links. Are educated men receiving better treatment (though of note, here, patients belonging to trajectories of persistent low disease activity received less frequently TNFi over follow-up), are they more adherent, do they have less severe disease or are they complaining less? Since ASDAS, the main criterion to define active disease here, is a mixed objective and subjective criterion, it is difficult to conclude on this point. In any case, physicians should be aware that when facing a patient with early axSpA, females with less formal education may be more at risk of persistent disease activity.

Another finding from this study was related to sick leave and work disability in early axSpA. First, the rate was rather high in this cohort (16/182 patients with available data on work disability, 10%). Second, it was strongly related to the disease activity trajectory, which validates both the methodology used here and the use of ASDAS as an outcome to assess disease activity. Such validations are important in the field of axSpA where assessments are often subjective and have not always been validated in terms of prediction of later outcomes.

Our study has several limitations and also some strengths. The main strength of our study is the innovative methodology allowing to evaluate disease activity patterns longitudinally. This validated methodology has been used in other disciplines, rarely in rheumatology and never in SpA. Furthermore, the large sample of patients presenting with early axSpA, according to the ASAS classification criteria, has allowed us to define distinctive trajectories of disease activity from an early time after onset of the disease. Nevertheless, most trajectories revealed a stable disease activity over follow-up and the main baseline characteristics associated with trajectories were demographic and socioeconomic. It is not impossible that the subjective patient-reported outcomes included in the ASDAS contributed more to the trajectories definitions rather than CRP. However, it is worth noting that the current guidelines recommend using both patient-reported outcomes and acute phase reactants (eg, CRP) for disease activity monitoring in SpA.[37]

Also, one may argue why ASDAS trajectories were not adjusted for TNFi use over time. TNFi use is associated with an important decrease of ASDAS.[38 39] Therefore, when modelling ASDAS trajectories over time, ASDAS trajectories inherently include TNFi use, and can thus be considered a reflection of the course of disease including its treatment.

Finally, we only assessed the outcome of the different trajectories in terms of disability and days of sick leave, and not in terms of structural damage (ie, radiographic sacroiliitis or syndesmophyte formation). However, structural progression is known to be very slow in axSpA[40] and particularly in this cohort,[41] that it did not seem appropriate to use such outcome for the first 3 years of follow-up.

Further studies evaluating longitudinally disease activity and the long-term outcomes of the different patterns of disease activity are needed to determine the validity of such trajectories in other patient groups.

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Competing interests None declared.

Ethics approval The DESIR cohort was approved by an Ethics Committee (*Comité de Protection des Personnes* Ile de France) and all patients gave their informed consent at the inclusion on the cohort.

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EXTENDED REPORT

Influence of geolocation and ethnicity on the phenotypic expression of primary Sjögren's syndrome at diagnosis in 8310 patients: a cross-sectional study from the Big Data Sjögren Project Consortium

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ABSTRACT

Objectives To analyse the influence of geolocation and ethnicity on the clinical presentation of primary Sjögren's syndrome (SjS) at diagnosis.

Methods The Big Data Sjögren Project Consortium is an international, multicentre registry designed in 2014. By January 2016, 20 centres from five continents were participating. Multivariable logistic regression analyses were performed.

Results We included 7748 women (93%) and 562 men (7%), with a mean age at diagnosis of primary SjS of 53 years. Ethnicity data were available for 7884 patients (95%): 6174 patients (78%) were white, 1066 patients (14%) were Asian, 393 patients (5%) were Hispanic, 104 patients (1%) were black/African-American and 147 patients (2%) were of other ethnicities. SjS was diagnosed a mean of 7 years earlier in black/African-American compared with white patients; the female-to-male ratio was highest in Asian patients (27:1) and lowest in black/African-American patients (7:1); the prevalence of sicca symptoms was lowest in Asian patients; a higher frequency of positive salivary biopsy was found in Hispanic and white patients. A north-south gradient was found with respect to a lower frequency of ocular involvement in northern countries for dry eyes and abnormal ocular tests in Europe (OR 0.46 and 0.44, respectively) and Asia (OR 0.18 and 0.49, respectively) compared with southern countries. Higher frequencies of antinuclear antibodies (ANAs) were reported in northern countries in America (OR=1.48) and Asia (OR=3.80) while, in Europe, northern countries had lowest frequencies of ANAs (OR=0.67) and Ro/La (OR=0.69).

Conclusions This study provides the first evidence of a strong influence of geolocation and ethnicity on the phenotype of primary SjS at diagnosis.

INTRODUCTION

Primary Sjögren's syndrome (SjS) is a systemic autoimmune disease that mainly targets the exocrine glands, leading to dryness of the main mucosal surfaces.[1] The histological hallmark is focal lymphocytic infiltration of the targeted organs and the key immunological markers include antinuclear antibodies (ANAs) (the most frequently detected), anti-Ro/SS-A (the most specific) and cryoglobulins and hypocomplementaemia (the main prognostic markers).[1] SjS overwhelmingly affects middle-aged women and its frequency varies widely according to study designs and the classification criteria used. More recent studies using the 2002 American-European classification criteria[2] have reported an incidence of 3–11 cases per 100 000 persons and a prevalence of between 0.01% and 0.72%.[3 4]

The influence of ethnicity on the phenotypic expression of systemic autoimmune diseases has been suggested by various studies, especially in systemic lupus erythematosus (SLE), which has been reported as being more frequent and having less favourable outcomes in non-white populations.[5 6]

With respect to the influence of geographical factors, a potential north-south gradient in the frequency of autoimmune diseases has been suggested.[7 8] In primary SjS, there is no information on the influence of ethnicity or geolocation on the phenotypic expression of the disease. Only one recent study, in the general population of Greater Paris,[4] has evaluated the influence of ethnicity on the frequency of primary SjS and this found a twofold higher prevalence in patients with non-European backgrounds compared with those with a European background.

The objective of this study was to determine the influence of geolocation and ethnicity on the clinical presentation of primary SjS at diagnosis in a large international cohort of patients.

METHODS

Patients

The Big Data Sjögren Project Consortium is an international, multicentre registry designed in 2014 to take a 'high-definition' picture of the main features of primary SjS at diagnosis by merging international SjS databases. International experts from the European League Against Rheumatism (EULAR)-SjS Task Force were invited to participate. Inclusion criteria were the fulfilment of the 2002 classification criteria;[2] in addition, a letter was sent to the corresponding authors of manuscripts published in the past 2 years in PubMed that included clinical data on at least 50 patients with primary SjS, inviting them to join the study. Exclusion criteria for considering SjS as a primary disease were chronic hepatitis C virus/HIV infections, previous lymphoproliferative processes and associated systemic autoimmune diseases. Diagnostic tests for SjS (ocular tests, oral tests and salivary gland biopsy) were carried out according to the recommendations of the European Community Study Group.[9] The study was approved by the Ethics Committee of the Coordinating Centre (Hospital Clinic, Barcelona, Spain, registry HCB/2015/0869).

Disease diagnosis was defined as the time when the attending physician confirmed fulfilment of the 2002 criteria. At this time, the main features of the disease were retrospectively collected and analysed (age, gender, ethnicity, country of residence, fulfilment of the 2002 criteria items, ANAs, rheumatoid factor (RF), C3 and C4 levels and cryoglobulins). By January 2016, the participant centres had included 8417 patients from 20 countries in five continents. Further confirmation was made by excluding cases in which fulfilment of the 2002 criteria could not be directly ensured according to the data provided (lack of information about items IV and VI—salivary biopsy and Ro/La autoantibodies).

Patients were classified according to the geolocation of the country of the diagnosing hospital. Patients were first classified by continent, with an additional north-south subclassification according to latitude in continents including patients from more than one country; the subclassification of the latitudes is not standard and was adapted to the geolocation of the countries included in the registry: latitude > or <50′N in Europe, equator > or < in America and latitude > or <30′N in Asia. Ethnicity was classified retrospectively (asking the patient or relatives, if necessary), according to the Office of Management and Budget Standards for Maintaining, Collecting, and Presenting Federal Data on Race and Ethnicity of the Food and Drug Administration,[10] using the following categories and definitions:

- Asian: A person having origins in any of the original peoples of the Far East, Southeast Asia or the Indian subcontinent, including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand and Vietnam.
- Black or African-American: A person having origins in any of the black racial groups of Africa.
- Hispanic or Latino: A person of Cuban, Mexican, Puerto Rican, South or Central American or other Spanish culture or origin, regardless of race. However, Spanish patients from Spain were included in the white definition.
- White: A person having origins in any of the original peoples of Europe, the Middle East or North Africa.
- Others: Native Hawaiian or Other Pacific Islander—defined as a person having origins in any of the original peoples of Hawaii, Guam, Samoa or other Pacific Islands; American Indian or Alaska Native—defined as a person having origins in any of the original peoples of North and South America (including Central America) and who maintains tribal affiliation or community attachment and patients with one or more racial designations.

Statistical analysis

Descriptive data are presented as mean and SD for continuous variables and numbers and percentages for categorical variables. The prevalence of a specific feature is stated as the number of cases with that feature/number of cases in which the feature was detailed. The χ^2 test was used to study categorical features at diagnosis according to geolocation (continent) and ethnic groups. One-way analysis of variance tests were used to compare the mean age at diagnosis. The following sub-analyses were made: (1) geolocation of countries by latitude (north versus south) in Europe (latitude > or <50′N), America (equator ><) and Asia (latitude > or <30′N); (2) Asian patients and country of residence (Asian versus non-Asian countries) and (3) Hispanic patients and country of residence (Latin American versus other countries). Clustered bar charts were constructed to compare ethnic clusters according to fulfilment of the 2002 criteria items with the baseline immunological profile.[11] Multivariable logistic regression analyses adjusted for ethnicity, age at diagnosis and gender were performed to study the association between geolocation with diagnostic tests for SjS and immunological markers at diagnosis. To handle missing data due to non-evaluated diagnostic tests for SjS or non-performed immunological markers, 'available case analysis' was assumed for the comparisons according to geolocation and ethnic groups. The missing data pattern shows that most variables had low percentages of missing data (see online supplementary figure S1). All significance tests were two-tailed and values of $p<0.05$ were considered significant. p Values were adjusted for multiple comparisons using the false discovery rate correction.[12] All analyses were conducted using the R (V.3.2.3) for Windows statistical software package (http://www.R-project.org/).

RESULTS

Baseline characterisation

Of the 8417 patients originally included in the database, 107 were excluded in the refinement process (lack of information about items IV and/or VI). The baseline characteristics of the final cohort (8310 patients) are summarised in table 1. The cohort included 7748 women (93%) and 562 men (7%) (female-to-male ratio, 14:1), with a mean age at diagnosis of primary SjS of 53.2 years (SD 14.2). The frequencies of fulfilment of the 2002 classification criteria items were 92% for dry eye (item I), 93% for dry mouth (item II), 86% for abnormal ocular tests (item III), 89% for positive minor salivary gland biopsy (item IV), 80% for abnormal oral diagnostic tests (item V) and 75% for positive anti-Ro/La antibodies (item VI). The frequency of immunological markers at diagnosis was as

Table 1 Baseline characteristics of 8310 patients with primary Sjögren's syndrome

Variable	Patients (%)
Gender (female)	7748 (93.2)
Age at diagnosis (n=8270) years	53.2±14.2
Dry eye	7660 (92.2)
Dry mouth	7700 (92.7)
Abnormal ocular tests	6228/7273 (85.6)
Schirmer's test	4903/6203 (79.0)
Rose bengal/other ocular dye score	2460/3302 (74.5)
Positive minor salivary gland biopsy	5305/5984 (88.7)
Abnormal oral diagnostic tests	4843/6063 (79.9)
Unstimulated whole salivary flow	3608/4938 (73.1)
Parotid sialography	702/873 (80.4)
Salivary scintigraphy	2160/2578 (83.8)
Positive anti-Ro/La antibodies	6177/8250 (74.9)
Anti-Ro antibodies	5950/8245 (72.2)
Anti-La antibodies	3599/8215 (43.8)
ANA-positive	6292/7746 (81.2)
RF-positive	3483/7154 (48.7)
C3 low	912/6554 (13.9)
C4 low	846/6540 (12.9)
Positive cryoglobulins	307/4118 (7.5)
Ethnicity	
White	6174/7884 (78.3)
Asian	1066/7884 (13.5)
Hispanic	393/7884 (5.0)
Black/African-American	104/7884 (1.3)
Others	147/7884 (1.9)
Geolocation	
Europe	6045 (72.7)
North (>50°N)	1393 (16.7)
South (<50°N)	4652 (56.0)
America	1134 (13.6)
North (>0°)	881 (10.6)
South (<0°)	253 (3.0)
Asia	940 (11.3)
North (>30°N)	300 (3.6)
South (<30°N)	640 (7.7)
Africa	45 (0.6)
Australia	146 (1.8)

ANA, antinuclear antibodies; RF, rheumatoid factor.

follows: positive ANA in 81% of patients, positive RF in 49%, low C3 levels in 14%, low C4 levels in 13% and positive serum cryoglobulins in 7.5% of patients.

Geolocation

The main results on geolocation (continent and subareas classified by latitude) are summarised in table 1. Patients came mainly from Europe (n=6045), America (n=1134) and Asia (n=940) (see online supplementary table S1). Table 2 compares the main geolocational features classified according to latitude in these three continents. Logistic regression analysis adjusted by ethnicity, age at diagnosis and gender showed that northern European patients (latitude > 50′N) had a lower frequency of ocular dryness (OR 0.46, 95% CI 0.37 to 0.57), abnormal ocular tests (OR 0.44, 95% CI 0.37 to 0.53), ANA (OR 0.67, 95% CI 0.58 to 0.78), low C3 levels (OR 0.76, 95% CI 0.62 to 0.93) and Ro/La autoantibodies (OR 0.69, 95% CI 0.60 to 0.79) and a higher frequency of abnormal oral tests (OR 2.12, 95% CI 1.71 to 2.64) and RF (OR 1.62, 95% CI 1.41 to 1.86) compared with southern European patients. North American patients had a lower frequency of positive salivary biopsy (OR 0.44, 95% CI 0.20 to 0.88) and a higher frequency of ANA (OR 1.48, 95% CI 1.05 to 2.09), RF (OR 2.04, 95% CI 1.49 to 2.82), Ro/La autoantibodies (OR 4.10, 95% CI 2.93 to 5.78) and low C4 levels (OR 6.94, 95% CI 3.17 to 18.32) compared with South American patients. Northern Asian patients had a lower frequency of dry mouth (OR 0.30, 95% CI 0.19 to 0.46), dry eyes (OR 0.18, 95% CI 0.12 to 0.28), abnormal ocular tests (OR 0.49, 95% CI 0.30 to 0.79) and positive salivary biopsy (OR 0.42, 95% CI 0.26 to 0.68) and a higher frequency of ANA (OR 3.80, 95% CI 2.54 to 5.73) and Ro/La autoantibodies (OR 2.78, 95% CI 1.89 to 4.09), compared with southern Asian patients.

Ethnicity

Ethnicity data were available for 7884 patients (95%) of the total cohort: 6174 (patients 78.3%) were classified as white, 1066 patients (13.5%) were classified as Asian, 393 patients (5%) were classified as Hispanic, 104 patients (1.3%) were classified as black/African-American patients and 147 patients (1.9%) were classified as of other ethnicities (table 1). Patients from European centres were overwhelmingly white compared with those from American centres (96% vs 52%, p<0.001); online supplementary table S2 summarises the main features of white patients compared with patients of other ethnicities. Table 3 shows the main features at presentation according to ethnicity: the highest percentage of men was in black/African-American patients and the lowest in Asian patients; the youngest age at diagnosis was in black/African-American patients and the oldest in white patients; the lowest frequency of sicca symptoms was in Asian patients and the highest in other ethnicities; the lowest frequency of abnormal diagnostic tests was in patients of other ethnicities and the highest in Hispanic patients and the highest frequency of Ro/La autoantibodies was in Asian patients and the lowest in patients of other ethnicities. Figure 1 includes clustered bar charts for the percentage of fulfilment of the six items of the 2002 classification criteria and figure 2 includes the percentage of abnormal results in the immunological profile according to ethnicity.

The potential effect of geolocation in patients classified in the same ethnic group was analysed in two sub-studies. Asian patients diagnosed in non-Asian countries had a higher frequency of dry mouth (p=0.007) and dry eye (p=0.003) compared with native patients (see online supplementary table S3). Hispanic patients living outside Latin American countries had a lower frequency of abnormal salivary biopsy (p=0.001) and positive RF (p=0.008) and a higher frequency of low C3 levels (p<0.001) compared with native patients (see online supplementary table S4).

DISCUSSION

The etiopathogenesis of primary SjS is unknown. The most frequently proposed hypothesis is based on the effect of multiple, mainly unknown, environmental factors affecting an individual with a specific genetic susceptibility. Geoepidemiological and ethnic studies may help elucidate the complex combination of genes and environment in systemic autoimmune diseases.[7] The most relevant studies have been carried out in SLE: US studies have reported a twofold to threefold higher incidence and

Table 2 Features at diagnosis according to geolocation

	Europe			America			Asia		
Variable	North (>50°N) (n=1393)	South (<50°N) (n=4652)	p Value†	North (>0°) (n=881)	South (<0°) (n=253)	p Value‡	North (>30°N) (n=640)	South (<30°N) (n=300)	p Value§
Gender (female)	1286 (92.3)	4311 (92.7)	0.741	821 (93.2)	247 (97.6)	0.022	623 (97.3)	287 (95.7)	0.366
Age at diagnosis, years	54±14.7	54±14	0.997	53.9±14.1	50±12.6	<0.001	50.2+ 14.1	43.7±10.4	<0.001
Dry eye	**1251 (89.8)**	**4414 (94.9)**	**<0.001**	856 (97.2)	247 (97.6)	0.951	**428 (66.9)**	**275 (91.7)**	**<0.001**
Dry mouth	1309 (94)	4322 (92.9)	0.250	867 (98.4)	243 (96)	0.057	**496 (77.5)**	**276 (92)**	**<0.001**
Abnormal ocular tests	**933/1175 (79.4)**	**3519/4024 (87.5)**	**<0.001**	664/780 (85.1)	199/243 (81.9)	0.333	**514/608 (84.5)**	**244/266 (91.7)**	**0.011**
Schirmer's test	884/1171 (75.5)	2483/2972 (83.5)	<0.001	499/774 (64.5)	190/243 (78.2)	<0.001	452/601 (75.2)	244/266 (91.7)	<0.001
Rose bengal/other ocular dye score	269/479 (56.2)	1436/1703 (84.3)	<0.001	431/660 (65.3)	77/97 (79.4)	0.018	237/350 (67.7)	NP	NA
Positive minor salivary gland biopsy	1092/1199 (91.1)	2864/3221 (88.9)	0.061	**559/655 (85.3)**	**176/185 (95.1)**	**0.002**	**282/353 (79.9)**	**244/270 (90.4)**	**0.001**
Abnormal oral diagnostic tests	**893/1007 (88.7)**	**2602/3365 (77.3)**	**<0.001**	566/760 (74.5)	205/243 (84.4)	0.005	494/596 (82.9)	3/3 (100)	1
Unstimulated whole salivary flow	861/995 (86.5)	1573/2382 (66)	<0.001	547/748 (73.1)	185/232 (79.7)	0.070	375/494 (75.9)	NP	NA
Parotid sialography	41/47 (87.2)	606/738 (82.1)	0.572	29/33 (87.9)	1/1 (100)	1	10/17 (58.8)	1/1 (100)	1
Salivary scintigraphy	210/224 (93.8)	1657/2019 (82.1)	<0.001	23/27 (85.2)	70/80 (87.5)	1	169/181 (93.4)	2/2 (100)	1
Positive anti-Ro/La antibodies	**955/1387 (68.9)**	**3470/4640 (74.8)**	**<0.001**	**679/880 (77.2)**	**138/246 (56.1)**	**<0.001**	**572/633 (90.4)**	**216/280 (77.1)**	**<0.001**
Anti-Ro antibodies	946/1386 (68.3)	3328/4637 (71.8)	0.019	631/879 (71.8)	136/246 (55.3)	<0.001	550/633 (86.9)	215/280 (76.8)	0.001
Anti-La antibodies	598/1379 (43.4)	1974/4625 (42.7)	0.741	406/880 (46.1)	60/246 (24.4)	<0.001	317/628 (50.5)	131/274 (47.8)	0.651
ANA-positive	**1020/1334 (76.5)**	**3528/4259 (82.8)**	**<0.001**	**672/848 (79.2)**	**179/248 (72.2)**	**0.039**	**555/601 (92.3)**	**216/284 (76.1)**	**<0.001**
RF-positive	**652/1094 (59.6)**	**1931/4086 (47.3)**	**<0.001**	**368/834 (44.1)**	**88/252 (34.9)**	**0.022**	295/595 (49.6)	135/248 (54.4)	0.366
C3 low	**148/806 (18.4)**	**546/3798 (14.4)**	**0.008**	31/728 (4.3)	8/252 (3.2)	0.668	132/601 (22)	35/212 (16.5)	0.201
C4 low	116/741 (15.7)	547/3850 (14.2)	0.416	**78/726 (10.7)**	**6/252 (2.4)**	**<0.001**	60/602 (10)	17/212 (8)	0.651
Positive cryoglobulins	36/237 (15.2)	262/3449 (7.6)	<0.001	3/42 (7.1)	0/108 (0)	0.048	2/124 (1.6)	0/94 (0)	0.724

Adjusted p values for 60 comparisons with false discovery rate correction corresponding to the comparison of European (†), American (‡) and Asian (§) countries.
In bold: statistically significant (p<0.05) variables associated with geolocation in the multivariable logistic regression analysis adjusted for ethnicity, age at diagnosis and gender.
ANA, antinuclear antibodies; NA, not available; NP, not performed; RF, rheumatoid factor.

Table 3 Features at diagnosis according to ethnicity groups

Variable	White (n=6174)	Asian (n=1066)	Hispanic (n=393)	Black/African-American (n=104)	Others (n=147)
Gender (female)	5720 (92.6)	1028 (96.4)	372 (94.7)	91 (87.5)	141 (95.9)
Age at diagnosis, years	54.2±14.2	48.3±13.3	47.8±12.9	47.2±12.4	52.5±13.7
Dry eye	5826 (94.4)	817 (76.6)	376 (95.7)	97 (93.3)	144 (98)
Dry mouth	5775 (93.5)	891 (83.6)	378 (96.2)	99 (95.2)	146 (99.3)
Abnormal ocular tests	4656/5354 (87)	818/953 (85.8)	334/373 (89.5)	65/78 (83.3)	115/146 (78.8)
Schirmer's test	3525/4351 (81)	746/940 (79.4)	301/345 (87.2)	33/52 (63.5)	59/146 (40.4)
Rose bengal/other ocular dye score	1949/2564 (76)	261/387 (67.4)	132/175 (75.4)	20/30 (66.7)	95/140 (67.9)
Positive minor salivary gland biopsy	3943/4406 (89.5)	592/709 (83.5)	258/272 (94.9)	66/81 (81.5)	103/138 (74.6)
Abnormal oral diagnostic tests	3664/4504 (81.3)	555/679 (81.7)	301/330 (91.2)	42/63 (66.7)	93/144 (64.6)
Unstimulated whole salivary flow	2625/3546 (74)	430/571 (75.3)	253/292 (86.6)	35/58 (60.3)	93/144 (64.6)
Parotid sialography†	655/813 (80.6)	14/22 (63.6)	31/35 (88.6)	1/2 (50)	NP
Salivary scintigraphy†	1880/2262 (83.1)	179/194 (92.3)	74/89 (83.1)	12/14 (85.7)	NP
Positive anti-Ro/La antibodies	4524/6154 (73.5)	896/1039 (86.2)	308/389 (79.2)	81/102 (79.4)	90/147 (61.2)
Anti-Ro antibodies	4347/6152 (70.7)	873/1039 (84)	299/387 (77.3)	79/101 (78.2)	80/147 (54.4)
Anti-La antibodies	2669/6130 (43.5)	510/1027 (49.7)	180/390 (46.2)	40/102 (39.2)	41/147 (27.9)
ANA-positive	4877/6062 (80.5)	876/1006 (87.1)	291/381 (76.4)	87/104 (83.7)	117/145 (80.7)
RF-positive	2695/5549 (48.6)	488/954 (51.2)	202/384 (52.6)	49/97 (50.5)	39/145 (26.9)
C3 low	684/5069 (13.5)	182/901 (20.2)	36/337 (10.7)	9/82 (11)	0/144 (0)
C4 low	685/5064 (13.5)	91/901 (10.1)	51/333 (15.3)	10/77 (13)	8/144 (5.6)
Positive cryoglobulins	296/3664 (8.1)	3/266 (1.1)	4/128 (3.1)	3/52 (5.8)	0/1 (0)

*All comparisons were statistically significant (adjusted p values for 20 comparisons with false discovery rate correction <0.05) except for parotid sialography with a p value equal to 0.086.
†p value was computed excluding others.
ANA, antinuclear antibodies; NP, not performed; RF, rheumatoid factor.

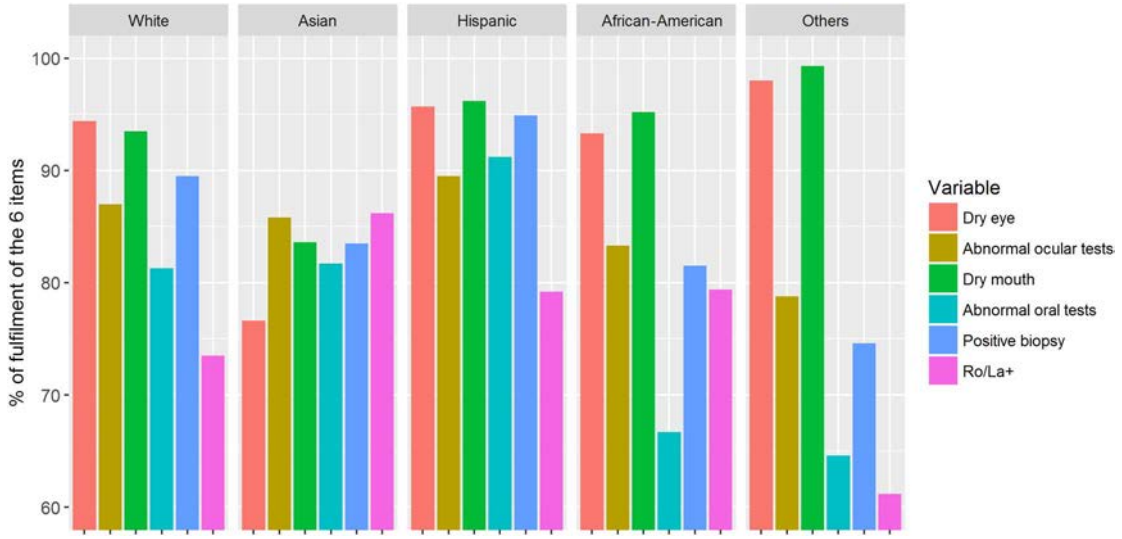

Figure 1 Clustered bar charts showing the percentage of fulfilment of the six items of the 2002 classification criteria according to ethnicity.

prevalence of disease rates in African-American patients, UK studies have reported up to eightfold higher rates in Afro-Caribbean and Asian patients and other studies have found higher prevalence in Native American Indians, Pacific People and Aborigines compared with European populations.[13] In systemic sclerosis, geoepidemiological studies have revealed a higher frequency in the USA and Australia than in Europe and Asia,[7] while in primary SjS, a twofold higher prevalence in patients with non-European backgrounds has recently been reported.[4]

Ethnicity also influences the phenotypic expression of autoimmune diseases, including the clinical course and outcomes. In SLE, the Lupus in Minorities: Nature versus Nurture (LUMINA) project found that African-American and Hispanic-American patients with SLE tend to develop the disease earlier and present with more severe disease.[14] In systemic sclerosis, a higher mortality rate has been reported in African-American populations compared with white populations,[7] while in systemic vasculitis, non-European patients with anti-neutrophil cytoplasmic antibodies vasculitis also showed more severe disease and higher damage scores.[15]

Until now, no studies have focused on the influence of geoepidemiology and ethnicity on the phenotypic expression of primary SjS. We evaluated these factors in the largest reported

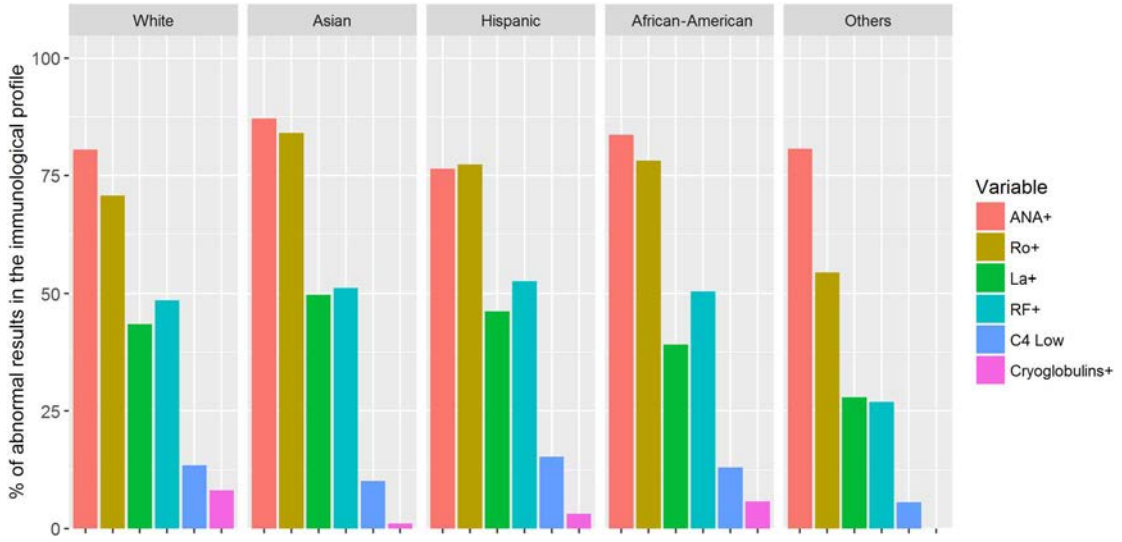

Figure 2 Clustered bar charts showing the percentage of abnormal results in the immunological profile according to ethnicity. ANA, antinuclear antibodies; RF, rheumatoid factor.

series of patients with primary SjS fulfilling the 2002 criteria from 20 countries across five continents. We found significant variations between ethnic groups. The disease was diagnosed a mean of 7 years earlier in black/African-American patients compared with white patients, a trend also reported by Maldini *et al*[4] in the Parisian multi-ethnic cohort. The female-to-male ratio also varied significantly, with the highest ratio (27:1) in Asian patients and the lowest (7:1) in black/African-American patients. The prevalence of sicca symptoms at diagnosis also varied significantly: the lowest frequencies were in Asian patients, a finding that has been related to cultural differences in previous studies.[16]

This is the first study to analyse the influence of ethnicity on the results of SjS diagnostic tests included in the current classification criteria. Hispanic patients had the highest rates of abnormal results and higher frequencies of subjective dryness symptoms. In contrast, patients of other ethnicities had the lowest rates of abnormal results but higher frequencies of sicca symptoms. This might suggest that ethnicity may influence the results of the objective diagnostic tests for dry eyes and mouth in patients with primary SjS, with Hispanic and white patients being more likely to have abnormal results compared with other ethnicities. With respect to the frequency of fulfilment of the histopathological criteria (salivary biopsy showing Chisholm-Mason grade 3 or grade 4), we found a pattern of ethnic association similar to that observed for objective tests for dryness, with a higher frequency of positive salivary biopsy in Hispanic and white patients compared with the other ethnicities; Maldini *et al*[4] reported a similar, although not significant, trend in the multi-ethnic Paris cohort.

The influence of ethnicity on the phenotypic expression of primary SjS at diagnosis could be driven by immunogenetic differences. Maldini *et al*[4] found a younger age at diagnosis and an increased frequency of polyclonal hypergammaglobulinemia and positive Ro/La antibodies in non-European patients, a reasonable association since patients with immunopositive SjS are often diagnosed earlier.[17] We have confirmed this association: patients from ethnic groups with the highest frequencies of positive anti-Ro antibodies (Asian, Hispanic and black/African-American) also had the youngest ages at diagnosis; a similar trend was observed for anti-La antibodies, except for black/African-American patients, who had the second-lowest frequency of all ethnic groups. Ro/La immunogenicity has traditionally been linked with genetic factors, mainly with specific human leucocyte antigen (HLA) class II alleles[18] and a joint contribution of HLA-DR and DQ alleles has been suggested as relevant for the development of antibodies against Ro/La autoantigens.[19] Most patients with SjS share a common allele (DQA1*0501) across racial and ethnic boundaries.[20] However, Kang *et al*[21] found significant differences in the frequency of some HLA-DR haplotypes (a higher frequency of DRB3 in Caucasians and DRB4 in Japanese and a lower frequency of DPB1 in Chinese patients). Future immunogenetic studies in primary SjS should evaluate the influence of ethnicity on the results, searching for possible immunogenetic differences.

A potential north-south autoimmune gradient, with rates seeming to increase according to distance from the Equator, has been suggested in the prevalence and incidence of several autoimmune diseases including type 1 diabetes mellitus, multiple sclerosis and inflammatory bowel disease.[7 22–25] Little geoepidemiological data were available for systemic autoimmune diseases and there are no data on primary SjS. The present study found some interesting results after comparing northern versus southern countries in the three continents for which data from more than one country are available (Europe, America and Asia). A north-south gradient was confirmed with respect to a lower frequency of ocular involvement and a higher frequency of cryoglobulinemic-related tests (cryoglobulins and hypocomplementemia) in northern compared with southern countries. The gradient was different in Europe with respect to the other components of primary SjS. For salivary gland involvement, the highest rates of abnormal results (including biopsy) in Europe were found in patients from northern countries, while in America and Asia the highest rates were reported in patients from southern countries. A similar gradient was observed with respect to autoantibodies (ANA, Ro, La): the highest frequencies in America and Asia were reported in northern countries, while in Europe, the highest frequencies were reported in southern countries. These results suggest, for the first time, that geolocation may influence the phenotypic expression of primary SjS at diagnosis, including significant geoepidemiological variations in the prevalence of dryness, the frequency of

abnormal diagnostic tests and the positivity of the main immunological markers.

We also analysed the influence of geoepidemiological migration on the phenotypic expression of primary SjS at diagnosis by comparing ethnic migrant with native populations. Interestingly, Asian patients diagnosed with primary SjS in non-Asian countries (overwhelmingly in Europe and the USA) had a higher frequency of sicca symptoms than Asian patients diagnosed in Asian countries. With respect to Hispanic patients diagnosed outside Latin America, differences were found in diagnostic tests, with a lower frequency of positive salivary biopsy, a lower frequency of positive RF and a higher frequency of low C3 values. No other studies have analysed this, although the study by Maldini *et al*[4] reported a differing clinical and immunological pattern of SjS expression in French patients with a non-European background.

The results, however, should be interpreted with caution and some limitations should be pointed out. Large studies may detect some differences which, although statistically significant, may not be relevant clinically, with further studies being necessary to confirm their clinical relevance. In addition, the predominant presence of European patients (due to the origin of the project in the EULAR-SjS Task Force Group) could limit the generalisation of the results, due to the small size of some ethnic subpopulations, such as black African-American patients. With respect to the study design, although studies comparing relative frequencies of clinical features should, ideally, be population-based, our study was designed according to a 'Data Sharing' approach, which is currently considered an alternative way of international scientific collaboration, especially in diseases with a low prevalence.[26] Since the participant centres are mainly tertiary university centres that are considered the referral centre in their corresponding cities (and in most cases, in their countries), the magnitude of the selection bias may vary between the 20 countries involved in the study and this could have an impact on the results (online supplementary figure S2 summarises the size of each cohort classified per city), as may differing medical practices across regions (availability of diagnostic tests included in the 2002 criteria); in fact, we found a negative correlation between the percentage of biopsied patients and the percentage of Ro/La-positive patients in each centre ($R=-0.55$) (see online supplementary figure S3). Other sources of heterogeneity may include the assays used by the different centres, although all are commercial tests and more than 80% used the same technique (ELISA) to test for Ro/La autoantibodies and ANA were overwhelmingly (>95%) tested for by indirect immunofluorescence and the missing data for some variables (see online supplementary figure S1).

In summary, this study provides the first evidence for a strong influence of geolocation and ethnicity on the phenotype of primary SjS at diagnosis. Genetic and environmental factors probably contribute to phenotypic variance in SjS and a recent study has attributed 54% of the predisposition to developing the disease to familial transmission (heritability plus shared environmental factors) and 46% to non-shared environmental factors.[27] Geoepidemiology and ethnicity should be considered as key variables that should be analysed in multi-ethnic studies of patients with primary SjS.

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OPEN ACCESS

EXTENDED REPORT

Incidence of hepatitis B virus reactivation in patients with resolved infection on immunosuppressive therapy for rheumatic disease: a multicentre, prospective, observational study in Japan

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ABSTRACT

Background Although the reactivation of hepatitis B virus (HBV) is recognised as a serious complication in patients with rheumatic disease (RD) receiving immunosuppressive drugs (ISDs), the incidence and risk factors for reactivation remain controversial.

Objectives To investigate the incidence and risk factors for HBV reactivation in patients with RD.

Methods We performed a multicentre, observational, prospective study over 2 years in patients with resolved HBV infection. Patients with RD treated with a dose of ≥5 mg/day prednisolone and/or synthetic or biological ISDs with negative HB virus surface antigen and positive anti-HB virus surface antibody (HBsAb) and/or anti-HB virus core antibody (HBcAb) were enrolled. Quantitative HBV DNA results and related data were regularly recorded.

Results Among 1042 patients, including 959 with rheumatoid arthritis, HBV DNA was detected in 35 (1.93/100 person-years), with >2.1 log copies/mL observed in 10 patients (0.55/100 person-years). None of the reactivated patients, including seven treated with a nucleic acid analogue, showed overt hepatitis. Low HBsAb titres and advanced age seemed to be risk factors for HBV reactivation; however, reactivation was observed in three patients with positive HBsAb and negative HBcAb test results. The risk of reactivation was lower with methotrexate but higher with prednisolone among the different types of ISDs. The intervals from the start of ISD to reactivation were relatively long (3–182 months; median, 66 months).

Conclusions The incidence of HBV reactivation with ISD use was 1.93/100 person-years in patients with RD with resolved HBV infection. No overt hepatitis was observed in the reactivated patients.

INTRODUCTION

It is estimated that approximately 350 million people are infected with the hepatitis B virus (HBV) worldwide and that one-third of the world's population is presently infected or has a history of past HBV infection. End-stage liver disease related to HBV is responsible for over 0.5–1 million deaths per year.[1]

HBV infection is responsible for 40.2%, and HBV reactivation due to immunosuppressive drugs (ISDs) has been observed in 6.8%, of fulminant hepatitis cases in Japan.[2] Because HBV reactivation could be caused by biological or non-biological disease-modifying antirheumatic drugs (DMARDs),[3] HBV infection is a serious problem for rheumatologists. HBV reactivation occurs in two forms: one involves the harmful proliferation of virus seen in HB virus surface antigen (HBsAg)-positive people, healthy carriers or patients with chronic HBV hepatitis and the other is seen in people with occult HBV infection who are HBsAg-negative and anti-HB virus core antibody (HBcAb)-positive and/or anti-HB virus surface antibody (HBsAb)-positive. Even though the latter is less frequently seen than the former, strict monitoring and preventive treatment are recommended by guidelines in the USA,[4] Europe,[1] Asia-Pacific[5] and Japan.[6–8]

Because the prevalence of resolved HBV infection in Japan (23.2%) is much higher than that in Western countries,[9] all patients with rheumatoid arthritis (RA) and other rheumatic diseases (RDs) in Japan who receive immunosuppressive DMARDs, including methotrexate (MTX), leflunomide (LEF), tacrolimus (TAC), mizoribine (MZB), corticosteroids and biological DMARDs, are recommended to be screened and managed according to the guideline developed by the Drafting Committee for Hepatitis Management Guidelines and the Japan Society of Hepatology.[7] Patients with negative HBsAg results should be screened for HBsAb and HBcAb. If the result for either of these antibodies is positive, the patient needs to be monitored for HBV DNA (using reverse transcription (RT)-PCR) every 1–3 months.

Because the guideline has been strictly followed in Japan, the costs for HBV DNA monitoring and preventive treatment with nucleic acid analogues (NAAs) have been increasing. However, there is insufficient clinical evidence to support the concepts of the guideline currently, and its effectiveness for preventing fatal hepatic damage is unknown.

Our objective was to elucidate the frequency and risk factors for HBV reactivation in patients with resolved HBV infection and RD.

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METHODS

This multicentre, observational, prospective study was conducted by a study group consisting of rheumatologists in Japanese Red Cross hospitals beginning in 2013 and spanning 2 years.

Subjects

Patients eligible for enrolment were those with RA or other RDs, over 18 years of age and attending a clinic for RDs in one of the 16 Japanese Red Cross hospitals in Japan. Patients being treated with corticosteroids (≥5 mg of prednisolone or its equivalent dose); immunosuppressive synthetic DMARDs, namely MTX, LEF, TAC, MZB or its equivalent and/or biological DMARDs, namely infliximab, etanercept, adalimumab, tocilizumab, abatacept, golimumab and certolizumab pegol were tested for HBsAg, HBsAb and HBcAb using chemiluminescent immunoassays. Patients with negative HBsAg (<0.05 IU/mL) and positive HBsAb (≥10.0 mIU/mL) and/or positive HBcAb (≥1.0 S/CO (sample/cut-off)) results were tested for HBV DNA with RT-PCR, and those with negative results were enrolled. Patients positive for HBsAb alone need HBV DNA monitoring except those with a history of HBV vaccination according to the Japanese Society of Hepatology guideline for the management of HBV infection,[7] as HBV reactivation is reported in such patients.[10 11] We excluded patients with positive HBsAb and negative HBcAb with a history of vaccination from this study.

Registration

All data of the enrolled patients were recorded anonymously and sent to the Japanese Red Cross Kyoto Daiichi Hospital Centre for Rheumatic Disease as password-protected digital information. The initial data collection was conducted from February 2013 to October 2014 and included the following information: basic patient characteristics, such as age, sex and disease duration; data related to hepatitis, such as HBsAg, HBsAb and HBcAb titres and aspartate transaminase and alanine transaminase levels within the last 3 months; immunological data, such as blood lymphocyte count and serum IgG levels; parameters related to disease activity, such as tender and swollen joints, Global Visual Analogue Scale score, Disease Activity Score 28, C reactive protein level and erythrocyte sedimentation rate and information about medications, such as dose of steroids and MTX and use or no use of a biologic or other ISDs. After the second year, serial results of quantitation of HBV DNA measured by RT-PCR, immunological data, parameters related to disease activity and medication information were recorded.

Primary and secondary end-points

We defined HBV reactivation as a positive conversion of HBV DNA measured using RT-PCR and included unquantifiable cases with positivity <2.1 log copies/mL. We consulted a hepatologist regarding the guidelines[6–8] for cases with positivity ≥2.1 log copies/mL and administered NAA if necessary without stopping ISDs. The primary end-point of this study was the frequency of HBV reactivation in HBsAg-negative and HBsAb-positive and/or HBcAb-positive patients with RD. We also examined risk factors for HBV reactivation and analysed the clinical and serological course after the reactivation as secondary end-points.

Statistical analysis

We analysed the primary end-point, which is the frequency of HBV reactivation in person/years. We used univariate Poisson regression analysis to evaluate risk factors for HBV reactivation and calculate risk ratios and its 95% CIs. We did not use multivariate analysis because the number of events was too small for analysis in a multivariate fashion.

Ethics

In this study, we evaluated only information that is collected in usual medical practice, and we substituted the agreement acquisition in the document with posting based on 'Ethical Guidelines for Epidemiological Research'.[12]

RESULTS

Characteristics of enrolled patients

Of 1330 patients, 1193 patients with RA and 137 other patients with RD, initially enrolled, 75 patients who were HBsAg-positive or who received NAA were excluded. We then excluded 213 other patients who dropped out for various reasons, including non-attendance, unrelated death or inadequate HBV DNA monitoring. Finally, we analysed 805 cases observed for 24 months and 237 patients observed for 12 months (figure 1). The characteristics of the enrolled patients at the initial registration are shown in table 1. The average dose of prednisolone in other patients with RD was more than twice the dose in patients with RA. In the RA group, the majority of patients were treated with MTX, and almost one-third used biologics, most (73.7%) of which were tumour necrosis factor (TNF) inhibitors. Other than MTX, TAC and MZB were used as ISDs in patients with RA.

The results regarding the presence of HBsAb and HBcAb are shown in table 2. The majority of patients were positive for both antibodies.

Incidence of HBV reactivation

HBV reactivation, as defined by positivity of HBV DNA, was found in 32 patients with RA and 3 with other RDs (in 1815 person-years) (table 3), and positivity ≥2.1 log copies/mL was seen in 8 patients with RA and 2 with other RDs (in 1831 person-years). Therefore, the frequency of HBV reactivation was calculated to be 1.93/100 person-years, and the frequency of quantitative positivity (≥2.1 log copies/mL) was 0.55/100 person-years. Seven of these patients were started on NAA

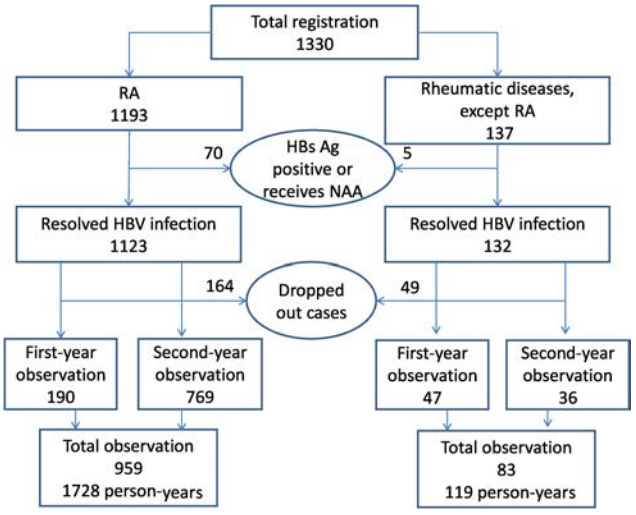

Figure 1 Flow diagram of patient selection. HBsAg, hepatitis B virus surface antigen; HBV, hepatitis B virus; NAA, nucleic acid analogue; RA, rheumatoid arthritis.

Table 1 Demographic features of enrolled patients at registration

	RA	Other RDs	Total
Patients (n)	959	83	1042
Age, years (median, IQR)	24–93 (69, 13)	40–92 (70, 16)	10–93 (69, 13.25)
Sex, female/male	257/702 (73.2)	27/57 (67.9)	284/759 (72.8)
Disease duration, months (median, IQR)	1–697 (98, 130)	3–350 (43, 76)	1–697 (93.5, 128)
Prednisolone			
Patients (n) (%)	373 (38.9)	81 (97.6)	454 (43.6)
Average dose, mg/day	4.02	9.03	5.10
≥5 mg, number (%)	186 (19.4)	71 (85.5)	257 (24.7)
Biologic DMARDs, number (%)	274 (28.8)		
Etanercept	103		
Infliximab	34		
Adalimumab	33		
Tocilizumab	48		
Abatacept	24		
Golimumab	29		
Others	3		
Methotrexate			
Patients (n) (%)	751 (79.1)	17 (18.9)	
Average dose, mg/week	7.52		
Other ISDs (%)	154 (16.2)	32 (35.6)	
Tacrolimus	122	10	
Mizoribine	28	8	
Leflunomide	6		
Azathiopurine		10	
Others		3	

Patients with other RDs, including 24 patients with polymyalgia rheumatica, 15 with systemic lupus erythematosus, 15 with vasculitis syndrome, 7 with myositis and 22 with others.
DMARDs, disease-modifying antirheumatic drugs; ISDs, immunosuppressive drugs; RA, rheumatoid arthritis; RD, rheumatic disease.

Table 2 HBV-related antibodies in enrolled patients

Group	HBcAb-negative, number (%)	HBcAb-positive, number (%)	Total
HBsAb-negative number (%)			
RA	0	177	177
Other RDs	0	13	13
Total*	0	190 (18.2)	190 (18.2)
HBsAb-positive number (%)			
RA	109	673	782
Other RDs	18	52	70
Total*	127 (12.2)	725 (69.6)	852 (81.8)
Total	127 (12.2)	915 (87.8)	1042

In the table, 'Total*' indicates the total number of patients in the upper two columns, RA and other RDs.
HBcAb, hepatitis B virus core antibody; HBsAb, hepatitis B virus surface antibody; HBV, hepatitis B virus; RA, rheumatoid arthritis; RD, rheumatic disease.

Table 3 Incidence of HBV reactivation in the first year and second year in patients with RA and other RDs

Group	Year of observation	Cases (n)	Sample size (person-years)	Incidence (/100 person-years)	Use of NAA
Reactivated cases					
RA	First	22	959	2.29	3
	Second	10	740	1.35	3
Other RDs	First	3	83	3.61	0
	Second	0	33	0	1
Total		35	1815	1.93	7
Cases with HBV DNA ≥2.1 log copies/mL					
RA	First	4	959	0.42	2
	Second	4	755	0.5	3
Other RDs	First	2	83	2.41	0
	Second	0	34	0	1
Total		10	1831	0.55	6

HBV, hepatitis B virus; NAA, nucleic acid analogue; RA, rheumatoid arthritis; RDs, rheumatic diseases.

medication, and none of the patients with HBV reactivation showed hepatic dysfunction during our observation. The incidence of reactivation in patients with negative HBsAb, 4.32/100 person-years, was higher than the patients with negative HBcAb or positive both antibodies, 1.36/100 person-years and 1.42/100 person-years, respectively (see online supplementary table S1).

Analysis of risk factors for HBV reactivation

According to the Poisson regression analysis for investigation of risk factors for HBV reactivation, the risk ratio of a low HBsAb titre below the median (71.4) was 2.8 (95% CI 1.3 to 6.8) and below the cut-off (titre <10.0) was 3.1 (95% CI 1.4 to 6.4). Advanced age over the median (69 years old) increased the risk to 3.3 (95% CI 1.5 to 8.4). Patients treated with MTX showed low risk ratios but those treated with prednisolone showed high risk ratios of 0.4 (95% CI 0.2 to 0.7) and 2.2 (95% CI 1.0 to 4.6), respectively (figure 2).

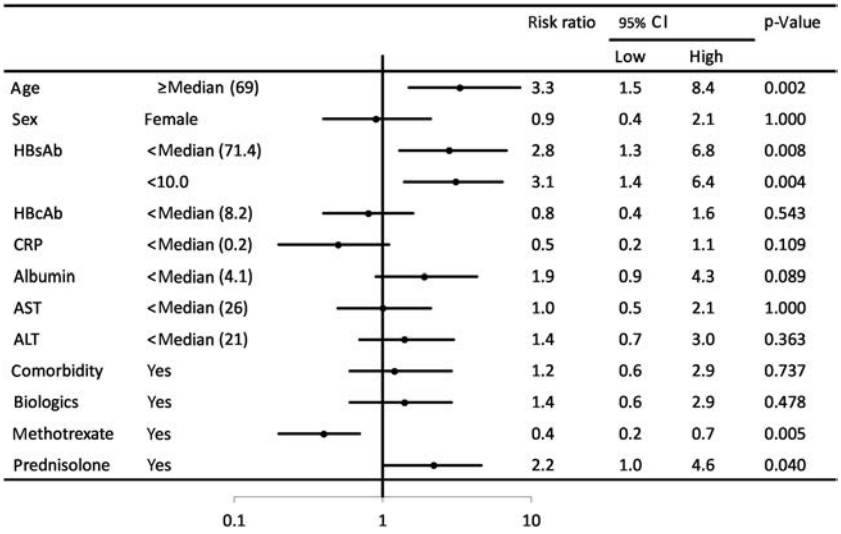

Figure 2 Risk ratios of clinical indicators for hepatitis B virus reactivation. The forest plot shows the risk ratios and 95% CIs of clinical parameters calculated by univariate Poisson regression analysis for HBV reactivation. ALT, alanine transaminase; AST, aspartate transaminase; CRP, C reactive protein; HBcAb, hepatitis B virus core antibody; HBsAb, hepatitis B virus surface antibody.

Clinical course of HBV reactivation

The interval between the beginning of ISDs and HBV reactivation ranged from 3 to 182 months (average, 66.2 months; median, 66 months; IQR, 60) in 35 reactivated cases (table 4). In 21 cases of HBV reactivation that we were able to observe 1 year later, NAA was started in seven cases, cases 2, 3, 4, 6, 7, 22 and 34, and has not yet been started in cases 5 and 33 with the increasing of HBV DNA in 1-year observation. HBV DNA spontaneously turned negative in six patients, cases 13–18, who were not administered NAA, and no deterioration was observed in the other six cases. HBV DNA negative conversion occurred immediately in all cases in which NAA was administered after the reactivation.

DISCUSSION

Some small-sized clinical studies of HBV reactivation in patients with RD with resolved infection were reported (see online supplementary table S2).[13–26] The incidence of reactivation in Japan (2–5.3%) is higher than that found in other south-eastern Asian countries (1.4–2.1%). In the reports from Europe, mainly in the cases for which TNF blockers were used, HBV reactivation was not found. In two systematic reviews, the incidence of HBV reactivation was reported as 5.4% and 1.7%, respectively. Differences in the prevalence of HBV infection and in the viral genotype or interval of HBV DNA monitoring could account for the differences in the incidence of reactivation among these countries.

In our study, the frequency of reactivation in patients with resolved HBV infection on immunosuppressive therapy for RD was 1.93/100 person-years, and >2.1 log copies/mL was observed in 0.55/100 person-years over 1–2 years of observation. Seven patients were treated with NAA and none developed overt hepatic damage. Although the frequency of HBV reactivation in patients with RD was lower in this study than in previous reports, it cannot be neglected as a complication of immunosuppressive therapy in RA and other RDs. On the other hand, the fact that overt hepatitis was not found in any of the reactivated cases shows that the prognosis of HBV reactivation is not always poor in RDs during a short-term follow-up.

Although a low HBsAb titre has been considered a candidate risk factor for HBV reactivation,[1 3] we had no direct evidence to support this idea. However, our results show that low HBsAb titres at baseline were significant risk factors for HBV reactivation. On the other hand, HBV reactivation was seen in eight cases with HBsAb titres higher than 100 mIU/mL and in three cases negative for HBcAb (see table 4 and online supplementary table S1). We should realise that although HBsAb is a neutralising antibody against HBsAg, it cannot completely prevent HBV reactivation in patients with RD. Although screening for resolved HBV infection only in those with positive HBcAb is recommended in some guidelines,[1 4] revision may be necessary considering the risk of reactivation in cases negative for HBcAb.

ISDs, that is, biologics, steroids, MTX and other synthetic DMARDs used for RD, can cause HBV reactivation. To evaluate the risk for reactivation for each drug is very important in order to stratify the patients to prevent HBV reactivation. According to a case–control study based on US Food and Drug Administration registration of patients with RA[27] the OR for HBV reactivation for steroids was 2.3, and the OR for TNF blockers was significantly lower than that for steroids or MTX. In our study, we showed the risk ratio of MTX was low and that of prednisolone was high among these groups of drugs. The discrepancy about the risk of MTX between these studies may be caused by the doses of MTX, which tend to be lower in Japan than in the USA or the combinations of drugs are variable in daily clinical practice. Since the results at this time are not enough to precisely evaluate the risk of each drug, we will continue this study to obtain more data for risk factor analysis.

HBV reactivation in patients with ISDs frequently evokes fulminant hepatitis, and its prognosis is very poor,[28] which is why careful follow-up and early preventive treatment are necessary for these patients. From the results of our study, the clinical course after reactivation in patients with RD was not very aggressive in either group of patients with and without NAA treatment, and a non-progressive course or spontaneous improvement was frequently seen, especially in cases with HBV DNA of <2.1 log copies/mL. These results support the effectiveness of preventive treatment with NAA in reactivated patients and the possibility that the cut-off value of HBV DNA for preventive therapy could be set at higher level.

HBV reactivation is supposed to have occurred in short term after the start of ISDs. Our study shows the interval between the start of ISD and reactivation ranged from 3 to 182 months (median, 66 months), which is longer than that reported in a previous study. Mochida *et al*[22] reported that the cumulative reactivation rate was 3.2% at 6 months, and the increase of the

Table 4 Characteristics of 35 HBV reactivated cases

No.	Diagnosis	Sex	Age (years)	Disease duration (months)	HBV DNA titre (log copies/mL)	NAA	Clinical course	HBsAb (mIU/ml)	HBcAb (S/CO)	Interval (months)	Medication
1	RA	F	81	16	2.6	–	–	222.6	0.5	10	MTX
2	RA	M	76	95	2.3	+	Worse	2.16	12	36	PSL
3	RA	M	86	303	7.1	+	Stable	25.5	10.3	30	ABC
4	RA	F	80	133	2.5	+	Stable	73.8	0.5	131	MTX
5	RA	F	60	85	2.3	–	Worse	124.7	1.16	5	TAC
6	RA	F	81	63	9.1	+	Worse	3.1	10.2	74	PSL
7	RA	F	70	6	3.5	+	Worse	28.6	9.01	138	PSL, MTX
8	RA	M	51	135	<2.1	–	Stable	0.5	8.54	86	TCZ, MTX
9	RA	F	71	220	<2.1	–	Stable	4.1	37.7	101	IFX, MTX
10	RA	F	80	64	<2.1	–	Stable	1000	95.6	52	MTX
11	RA	M	79	73	<2.1	–	Stable	11.8	19.1	73	MTX
12	RA	M	70	10	<2.1	–	Stable	113.5	19.3	14	PSL, TAC
13	RA	F	70	59	<2.1	–	Better	851	2.2	38	ADA, MTX
14	RA	F	73	409	<2.1	–	Better	13.5	7.12	27	ABC, MTX
15	RA	F	77	64	<2.1	–	Better	9.5	5	50	GLM, MTX
16	RA	F	76	141	<2.1	–	Better		12.63	157	MTX
17	RA	M	65	205	<2.1	–	Better	50.2	27.9	148	ETA, PSL
18	RA	F	72	201	<2.1	–	Better	147	0.5	3	GLM, PSL, MTX
19	RA	F	83	6	<2.1	–	–	45	10.15	14	ETA
20	RA	F	76	11	<2.1	–	–	10.8	1.23	19	MZB
21	RA	F	85	23	<2.1	–	–	47.1	6.04	32	PSL, MTX
22	RA	F	65	112	<2.1	+	Stable	5.7	8.96	90	MTX
23	RA	F	75	505	2.3	–	–	0.4	98.5	83	MZB
24	RA	F	72	39	<2.1	–	–	24.9	29.9	73	IFX, MTX
25	RA	F	67	193	<2.1	–	–	0	5.13	38	ABC, MTX
26	RA	M	75	35	<2.1	–	–	0	9.14	66	MTX
27	RA	F	81	434	<2.1	–	–	1.9	8.62	56	MZB
28	RA	M	76	65	<2.1	–	–	54.2	8.79	100	PSL
29	RA	F	84	157	<2.1	–	–	32.8	7.36	182	PSL
30	RA	F	82	614	<2.1	–	–	155.3	2.25	39	PSL
31	RA	F	66	171	<2.1	–	–	1000	5.71	87	MTX
32	RA	F	76	97	<2.1	–	–	1.4	88	12	GLM, PSL
33	PM	F	68	235	2.7	–	Stable	3.5	8.09	87	PSL
34	SLE	F	63	84	2.3	+	Worse	8.78	6.54	96	PSL
35	PMR	M	82	54	<2.1	–	Stable	0.4	12.1	70	PSL

Titres of HBsAb and HBcAb were collected at registration. 'Age', 'Disease duration', 'Medication' and 'Interval' between the beginning of immunosuppressive drugs and reactivation were at the point of reactivation. Administration of 'NAA' and 'Clinical course' was checked at the last observation. HBV DNA titre was the highest value during the observation period. In the column of 'clinical course', 'worse' means increase of HBV DNA and/or start of NAA, 'stable' and 'better' reveal 'unchanged' and 'decreased' HBV DNA levels without using NAA, respectively. The negative ranges of HBsAb and HBcAb are <10.0 mIU/mL and <1.0 S/CO, respectively.
ABC, abatacept; ADA, adalimumab; ETA, etanercept; F, female; GLM, golimumab; HBcAb, hepatitis B virus core antibody; HBsAb, hepatitis B virus surface antibody; HBV, hepatitis B virus; IFX, infliximab; M, male; MTX, methotrexate; MZB, mizoribine; NAA, nucleic acid analogue; PM, polymyositis; PMR, polymyalgia rheumatica; PSL, prednisolone; RA, rheumatoid arthritis; S/CO, sample/cut-off; SLE, systemic lupus erythematosus; TAC, tacrolimus; TCZ, tocilizumab.

rate at 48 months compared with that at 6 months was +1.5%. We must consider that, compared with cancer chemotherapy, treatment with ISDs in RDs usually results in patients being in a lower-grade and longer-term immunosuppressive state. The difference between intensity and duration of immunosuppression may be the basis of the differences in the pathophysiology of HBV reactivation.

As a limitation of this study, the risk and latency of immunosuppression caused by ISDs to HBV reactivation could not be accurately estimated for two reasons. First, we enrolled patients who were just starting ISDs and who were also already given ISDs. Second, the dosage and combination of ISDs were changed in many enrolled cases after the start of medication. Although it has been suggested that the clinical course of HBV reactivation in RD is different from that in cancer chemotherapy, we could not clarify the frequency or pathophysiology of de novo hepatitis due to viral replication in RD. As this may be a limitation of our study design (observational cohort study), we should consider a randomised control study to clarify the clinical question.

CONCLUSIONS

The incidence of HBV reactivation in patients with RD with resolved HBV infection was 1.93/100 person-years, and the incidence of quantitative HBV DNA positivity was 0.55/100 person-years. None evoked clinical hepatitis in reactivated patients.

Low titres or negative HBsAb and advanced age were risk factors in HBV reactivation in patients with RD, but in patients with high HBsAb titres and negative HBcAb, the possibility of

HBV reactivation could not be excluded. The risk ratio of MTX for HBV reactivation was lower than that of steroid and biologics.

The intervals from the start of ISD to HBV reactivation were variable, and the clinical course after reactivation was not always aggressive.

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EXTENDED REPORT

Improvement in 5-year mortality in incident rheumatoid arthritis compared with the general population—closing the mortality gap

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ABSTRACT

Objective Excess mortality in rheumatoid arthritis (RA) is expected to have improved over time, due to improved treatment. Our objective was to evaluate secular 5-year mortality trends in RA relative to general population controls in incident RA cohorts diagnosed in 1996–2000 vs 2001–2006.

Methods We conducted a population-based cohort study, using administrative health data, of all incident RA cases in British Columbia who first met RA criteria between January 1996 and December 2006, with general population controls matched 1:1 on gender, birth and index years. Cohorts were divided into earlier (RA onset 1996–2000) and later (2001–2006) cohorts. Physician visits and vital statistics data were obtained until December 2010. Follow-up was censored at 5 years to ensure equal follow-up in both cohorts. Mortality rates, mortality rate ratios and HRs for mortality (RA vs controls) using proportional hazard models adjusting for age, were calculated. Differences in mortality in RA versus controls between earlier and later incident cohorts were tested via interaction between RA status (case/control) and cohort (earlier/later).

Results 24 914 RA cases and controls experienced 2747 and 2332 deaths, respectively. Mortality risk in RA versus controls differed across incident cohorts for all-cause, cardiovascular diseases (CVD) and cancer mortality (interactions p<0.01). A significant increase in mortality in RA versus controls was observed in earlier, but not later, cohorts (all-cause mortality adjusted HR (95% CI): 1.40 (1.30 to 1.51) and 0.97 (0.89 to 1.05), respectively).

Conclusions In our population-based incident RA cohort, mortality compared with the general population improved over time. Increased mortality in the first 5 years was observed in people with RA onset before, but not after, 2000.

INTRODUCTION

Since the 1950s, studies have drawn attention to the premature mortality in rheumatoid arthritis (RA) compared with the general population.[1–7] Cardiovascular diseases (CVD) are the leading cause of excess mortality; infections and malignancies are other causes.[8–15] Mortality risk is generally lower in incident than prevalent cohorts,[2 4 5 8] as reflected by a lower standardized mortality ratio (SMR) (1.2 vs 1.9, respectively).[16] Studies evaluating mortality in the early years of RA report mixed results.[7] Some cohorts from early arthritis clinics, with early/aggressive disease-modifying antirheumatic drug (DMARD) treatment, report no excess mortality,[17–19] while others report increased mortality apparent from early years of disease and increasing with RA duration.[20–22] Others report excess mortality becoming apparent after 7–10 years.[23]

Increased mortality in RA is believed to be a consequence of inflammation, as markers of inflammation and disease severity have been associated with increased risk of death.[24–31] With more effective treatments and a paradigm shift in treating RA aimed at achieving remission, mortality would be expected to have improved over time.[32–37] Previous studies evaluating secular trends in RA mortality, including a meta-analysis,[4] have generally found no improvement relative to the general population, with some studies suggesting a widening mortality gap, due to improved mortality in the general population but not, or to a lesser extent, in RA.[4 16 23 38–43] However, these studies evaluated cohorts with RA onset up to the 1990s. In contrast, our study evaluates temporal trends in more contemporary incident cohorts with RA onset before versus after 2000, a period when RA treatment changed drastically[32 44–47] and awareness of cardiovascular risk in RA, and its link to inflammation, increased.[24 28 29 48–53]

The objective of our study was to evaluate secular trends in RA mortality, by assessing whether the mortality risk over the first 5 years of RA, compared with general population controls, differed between incident RA cases diagnosed in 1996–2000 and in 2001–2006.

METHODS

Study design

We conducted a longitudinal study of a population-based incident RA cohort with matched controls from the general population, using administrative health data from the entire province of British Columbia (BC), Canada.

Cohort definition

Incident RA cohort

All incident RA cases in BC who first met criteria for RA between January 1996 and March 2006 (using data from January 1990 onwards) were identified, using physician billing data from the Ministry of Health in a universal healthcare system, and were followed until December 2010. Using previously published criteria,[54] individuals were identified as RA cases if they had at least two physician visits at least 2 months apart within a 5-year

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period with an International Classification of Diseases, Ninth Revision (ICD9) code for RA (714.X).[55] To improve specificity, individuals were excluded if they had at least two subsequent visits with ICD9 codes for other forms of inflammatory arthritis (systemic lupus erythematosus, other connective tissue diseases, psoriatic arthritis, ankylosing spondylitis and other spondyloarthropathies). Cases were also excluded if a diagnosis of RA by a non-rheumatologist was never confirmed when the individual saw a rheumatologist; or if they had no subsequent RA diagnosis over more than 5 years of follow-up. These criteria have been validated in a subsample who participated in an RA survey. Using opinion of an independent rheumatologist reviewing medical records from their treating physicians as gold standard, we estimated the positive predictive value at 0.82.[56]

General population controls with no diagnoses of inflammatory arthritis were selected, using the same administrative databases as for the RA cohort, matching controls to RA cases in a 1:1 ratio on gender, birth year and index year.

The sample was divided into two cohorts: an earlier and later cohort (cases with RA onset in 1996–2000 vs 2001–2006, respectively, and their controls).

Data sources

Data were obtained from administrative databases of the Ministry of Health of British Columbia on all provincially funded healthcare services used since January 1990, including all physician visits, with one diagnostic code representing the reason for the visit, from the Medical Service Plan database[57] as well as hospital discharge data.[58] Vitals statistics data[59] were obtained, from January 1996 onwards, on deaths and primary cause of death derived from death certificates. All data were available until December 2010. Several population-based studies have been published using these data.[54 60–66]

Ethics

No personal identifying information was provided. All procedures were compliant with BC's Freedom of Information and Privacy Protection Act. The study received ethics approval from University of British Columbia.

Statistical analyses

Person-years of follow-up were calculated for incident RA cases and controls, from index date to end of follow-up (censored at 5 years to ensure equal follow-up in earlier and later cohorts), last healthcare utilisation or death. Index date was defined, for RA cases, as when they met incident RA inclusion criteria; and, for controls, as the date of a randomly selected healthcare encounter occurring in the same calendar year as the index year of their matched case. Mortality rates from all-cause, CVD, malignancy and infections were calculated for RA cases and controls, along with mortality rate ratios, with 95% CIs, representing the risk of mortality in RA relative to controls. We also used a parametric exponential proportional hazards (PH) model to estimate HRs representing the mortality risk in RA compared with controls, adjusted for age. Analyses adjusted for comorbidities which differed at baseline between RA and controls, and for the Romano modification of the Charlson comorbidity index (with RA excluded from comorbidities)[67–69] were also performed. To test if differences in excess risk of mortality (in RA relative to controls) changed over time, we tested the interaction between the indicators of RA (case vs control) and incident cohort (earlier vs later), in the exponential PH model. A statistically significant interaction indicates that the mortality risk in RA relative to the general population differs between the earlier and later cohorts.

Kaplan-Meier (KM) survival curves were estimated, describing survival from index date until death, stratified according to RA status, for all-cause and cause-specific mortality, censoring at 5 years of follow-up, last healthcare utilisation or death from other cause (for cause-specific analyses). Separate analyses were conducted for 1996–2000 and 2001–2006 cohorts. Sensitivity analyses estimated survival curves using all available follow-up time (ie, not censoring at 5 years). Analyses were performed using SAS V.9.4 (SAS Institute, Cary, North Carolina, USA).

RESULTS

The sample included 24 914 individuals with RA and 24 914 general population controls. Using BC population estimates, our cohorts yield age-standardised and sex-standardised incidence rates of 58 per 100 000 for the 1996–2000 cohort and 68 per 100 000 for the 2001–2006 cohort, consistent with reported RA incidence rates.[70 71] During the first 5 years, 2747 deaths were observed in the RA cohort and 2332 in controls.

Baseline characteristics of the RA cohorts and controls, measured at index date, are described in table 1. RA cases had more comorbidities than controls (higher rates of prior CVD, chronic obstructive pulmonary disease (COPD), hospitalised infections, hospitalisation rate), but no difference in overall comorbidity score (Romano score with RA excluded from comorbidities).[67–69] Of note, age at index date was slightly lower in the later than earlier RA cohort, although the difference is not clinically meaningful (mean (SD): 57.49 (12.8) and 58.35 (12.9) years, respectively). Furthermore, our analyses are age adjusted.

Mortality rates, and mortality risk in RA relative to general population controls, are shown in table 2. In the entire cohort, mortality rates of 24.43 and 20.72 per 1000 person-years were observed in RA and controls, respectively, yielding an all-cause mortality rate ratio of 1.18 (95% CI 1.12 to 1.25). Greater mortality in RA compared with controls was observed for mortality from CVD and infections.

When comparing mortality risk in RA relative to controls, across incident cohorts, we observed important differences between the 1996–2000 and 2001–2006 cohorts. A 39% increase in all-cause mortality was observed in the earlier RA cohort relative to the general population, whereas no increase was observed in the later cohort. Similarly, increased mortality was observed in earlier, but not later, RA cohorts relative to controls for deaths from CVD, cancer and infections. These time-differences in excess mortality were confirmed in age-adjusted exponential models, where significant interactions between RA (vs controls) and incident cohort (earlier vs later) were found for mortality from all-causes ($p<0.001$), CVD ($p<0.001$) and cancer ($p=0.002$), but not from infection ($p=0.097$).

To confirm our findings, we assessed age at death. Despite being matched on age to general population controls, RA cases in the earlier cohort died, on average, 1.3 years earlier than controls (mean (SD) age at death from all causes: 76.7 (12.9) vs 78.0 (11.0) years in controls), but not in the later cohort (77.3 (12.8) vs 77.8 (11.4) years in controls).

Similarly, KM curves revealed lower survival in RA cases than in general population controls for death from all-causes, CVD and cancer, in the earlier cohort (log rank test $p<0.01$), but not the later cohort ($p=0.695$, 0.583, 0.127, respectively) (figure 1). Of note, KM analyses require cautious interpretation as they are unadjusted and based on relatively few cause-specific mortality events. Too few deaths from infections were observed to allow

Table 1 Baseline characteristics of RA cases and controls in the earlier (incidence 1996–2000) and later (incidence 2001–2006) cohorts

Characteristic	Controls 1996–2000 cohort (n=10 798)	RA 1996–2000 cohort (n=10 798)	RA vs controls p Value	Controls 2001–2006 cohort (n=14 116)	RA 2001–2006 cohort (n=14 116)	RA vs controls p Value
Female, n (%)	7162 (66.33)	7162 (66.33)	1.000	9398 (66.58)	9398 (66.58)	1.000
Age at index date	58.34 (17.39)	58.35 (17.38)	0.983	57.49 (16.88)	57.49 (16.88)	0.978
RA duration at index date*, years, median (25Q;75Q)	N/A	0.63 (0.27;2.35)		N/A	0.36 (0.23;0.90)	
Romano Charlson comorbidity index†‡	0.36 (1.07)	0.38 (0.98)	0.200	0.36 (1.08)	0.37 (0.96)	0.215
Hospitalisation rate per year§¶	0.22 (0.34)	0.28 (0.40)	<0.001	0.19 (0.29)	0.23 (0.31)	<0.001
Cumulative no. of hospitalised days§¶	14.59 (78.08)	14.36 (51.12)	0.794	12.47 (62.77)	12.66 (29.22)	0.744
Diabetes‡, n (%)	605 (5.60)	586 (5.43)	0.571	956 (6.77)	907 (6.43)	0.240
COPD‡, n (%)	668 (6.19)	813 (7.53)	<0.001	718 (5.09)	940 (6.66)	<0.001
Renal failure‡, n (%)	72 (0.67)	78 (0.72)	0.623	144 (1.02)	145 (1.03)	0.953
Any CVD‡, n (%)	4115 (38.11)	4392 (40.67)	<0.001	5724 (40.55)	6115 (43.32)	<0.001
Prior CVA¶, n (%)	754 (6.98)	831 (7.70)	0.045	1053 (7.46)	991 (7.02)	0.154
Prior AMI¶, n (%)	536 (4.96)	548 (5.08)	0.708	698 (4.94)	721 (5.11)	0.531
Prior cancer¶, n (%)	1378 (12.76)	1391 (12.88)	0.791	2036 (14.42)	2078 (14.72)	0.479
Prior hospitalised infection¶, n (%)	839 (7.77)	1094 (10.13)	<0.001	1181 (8.37)	1531 (10.85)	<0.001

Unless otherwise indicated, values represent mean (SD); p values are from χ^2 for binary variables, or two-sample t-test for continuous variables.
*RA duration at index date was calculated as time from first RA visit to index date (ie, second RA visit at least 8 weeks later).
†Romano Charlson refers to the Romano adaptation of the Charlson comorbidity index developed for use with administrative health data, excluding RA as a comorbidity.[67–69]
‡Assessed over 1 year prior to index date.
§Hospitalisation rate was calculated as the number of hospitalisation events per year; cumulative number of hospitalised days was calculated as the sum of all days spent in hospital during all hospitalisations.
¶Assessed over all available data preindex date, that is, from 1990 onwards.
25Q;75Q, 25th and 75th percentile; AMI, acute myocardial infarct; COPD, chronic obstructive pulmonary disease; CVA, cerebrovascular accident; CVD, cardiovascular disease.

Table 2 Mortality risk in RA compared with general population controls

	Mortality rate RA (per 1000 PY)	Mortality rate controls (per 1000 PY)	Mortality rate ratio (95% CI) RA vs controls	aHR* (95% CI) RA vs controls	aHR† (95% CI) RA vs controls	aHR‡ (95% CI) RA vs controls
Entire cohort						
All-cause mortality	24.43	20.72	1.18 (1.12, 1.25)	1.17 (1.11, 1.24)	1.14 (1.08, 1.20)	1.15 (1.09, 1.22)
Mortality from CVD	8.49	7.04	1.21 (1.10, 1.33)	1.20 (1.09, 1.31)	1.16 (1.05, 1.27)	1.19 (1.08, 1.32)
Mortality from cancer	6.48	6.20	1.05 (0.94, 1.16)	1.04 (0.94, 1.15)	1.04 (0.94, 1.16)	1.02 (0.92, 1.14)
Mortality from infection	1.45	0.91	1.58 (1.23, 2.05)	1.57 (1.23, 2.01)	1.51 (1.18, 1.94)	1.50 (1.15, 1.95)
All-cause mortality						
Incident cohort 1996–2000	32.68	23.45	1.39 (1.29, 1.51)	1.40 (1.30, 1.51)	1.34 (1.24, 1.45)	1.38 (1.27, 1.49)
Incident cohort 2001–2006	18.29	18.59	0.98 (0.91, 1.07)	0.97 (0.89, 1.05)	0.95 (0.88, 1.03)	0.95 (0.88, 1.04)
Mortality from CVD						
Incident cohort 1996–2000	12.30	8.50	1.45 (1.27, 1.64)	1.45 (1.28, 1.65)	1.39 (1.23, 1.58)	1.45 (1.27, 1.65)
Incident cohort 2001–2006	5.66	5.90	0.96 (0.83, 1.11)	0.94 (0.81, 1.08)	0.93 (0.80, 1.07)	0.94 (0.81, 1.09)
Mortality from cancer						
Incident cohort 1996–2000	8.61	7.08	1.22 (1.05, 1.41)	1.22 (1.06, 1.41)	1.22 (1.06, 1.41)	1.20 (1.03, 1.40)
Incident cohort 2001–2006	4.90	5.52	0.89 (0.76, 1.04)	0.88 (0.75, 1.02)	0.89 (0.76, 1.03)	0.86 (0.74, 1.01)
Mortality from infections				(0.097)	(0.142)	(0.104)
Incident cohort 1996–2000	1.88	0.97	1.93 (1.34, 2.79)	1.94 (1.37, 2.75)	1.82 (1.28, 2.59)	1.86 (1.28, 2.70)
Incident cohort 2001–2006	1.13	0.87	1.30 (0.91, 1.88)	1.27 (0.90, 1.81)	1.26 (0.89, 1.78)	1.20 (0.83, 1.75)

*aHR adjusted for age at index date.
†aHR adjusted for age at index date, plus baseline CVD, COPD, infection, hospitalisations per year and Romano modification of Charlson comorbidity score excluding RA from list of comorbidities.
‡aHR adjusted for age at index date; results from sensitivity analysis excluding all cases/controls with <6 years of enrolment in the Medical Service Plan at index date.
aHR, adjusted HR; COPD, chronic obstructive pulmonary disease; CVD, cardiovascular diseases; PY, person-years; RA, rheumatoid arthritis.

adequate interpretation of results. KM results were similar when survival was uncensored (log rank test p≤0.01 for mortality from all-causes, CVD and cancer in earlier cohort; and p=0.210, 0.510 and 0.473, respectively, for the later cohort with up to 8 years of follow-up) (see online supplementary figure S1).

Robustness of our results was tested in sensitivity analyses. Because individuals moving to BC could appear to be incident cases, we excluded all cases/controls with <6 years of Medical Services Plan enrolment at index date. This yielded similar results (table 2). Because the lead-in time to differentiate

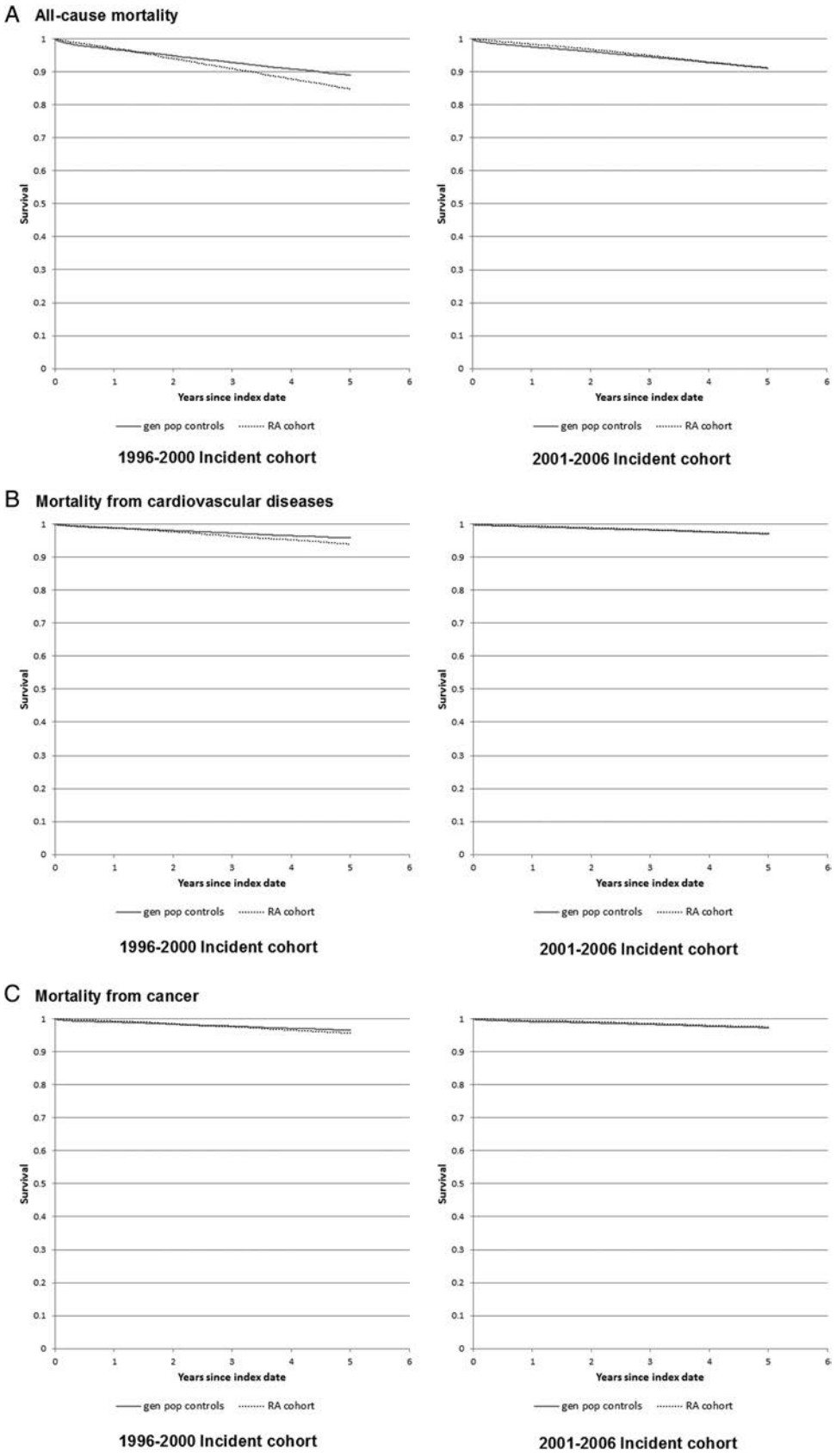

Figure 1 Survival from all-causes, cardiovascular diseases, cancer and infection, in rheumatoid arthritis (RA) and general population (gen pop) controls. (A) All-cause mortality; log rank test comparing Kaplan-Meier (KM) survival in RA versus controls for 1996–2000 cohort: p<0.001; and for the 2001–2006 cohort: p=0.695. (B) Mortality from cardiovascular diseases; log rank test comparing KM survival in RA versus controls for 1996–2000 cohort: p<0.001; and for the 2001–2006 cohort: p=0.583. (C) Mortality from cancer; log rank test comparing KM survival in RA versus controls for 1996–2000 cohort: p=0.007; and for the 2001–2006 cohort: p=0.127.

incident from prevalent RA was longer for the 2001–2006 cohort (11 vs 6 years), we limited the 2001–2006 cohort's lead-in time to 6 years. Results did not differ (all-cause mortality rate ratio: 0.96 (0.89 to 1.04) vs 0.98 (0.91 to 1.07)). To ensure baseline differences in RA duration across the two cohorts did not confound the interaction,[72] we adjusted the exponential PH models for RA duration at index date. To avoid near-collinearity between RA/control status and RA duration (which, by definition, equals 0 for all controls), RA duration in RA cases was centred to a mean of 0.[73] Results were similar (all-cause mortality adjusted HR 1.48 (1.37 to 1.60) for earlier; 0.92 (0.85 to 1.00) for later cohorts) and the interaction remained significant ($p<0.001$).

DISCUSSION

We conducted a population-based study of a large incident RA cohort with general population controls, using administrative health data in a universal healthcare system, to evaluate RA mortality trends over time. Our study reveals that the risk of death in RA compared with the general population has improved over time. Statistically significant improvement was observed for all-cause mortality, as well as deaths from CVD, and cancer, but not from infections. In our cohort, over 5 years of follow-up, an increased risk of mortality in RA compared with the general population was observed in people with RA onset on or before 2000, but not in people with RA onset after 2000. Although improvement in mortality risk was clearly observed in our study, one should be cautious about interpreting our results as indicating that mortality differences between RA and the general population no longer exist, as 5 years of follow-up is relatively short, and it is possible that differences between RA and the general population would be observed with longer follow-up, especially since previous studies have suggested that the greatest increase in mortality risk may occur after 7–10 years of disease.[23] Of note, in sensitivity analyses, KM survival curves estimated using all available follow-up (up to 8 years for the later cohort) yielded similar results.

Strengths of our study include the population-based nature of our cohort with complete capture of all RA cases in BC, ensuring the sample is representative of the entire spectrum of RA disease and of patients treated in everyday clinical practice; the inclusion of incident cases with complete follow-up from RA onset to death or study end and the large sample size providing adequate power to look at rare events such as mortality.

Limitations of our study are those inherent to observational studies and studies using administrative data. They include uncertainty around RA diagnosis identified using administrative data and possible effect of unmeasured confounding. Measuring the timing of RA onset is imprecise with administrative data. For these to influence the observed difference in mortality risk between the earlier and later cohorts, they would have to differentially affect both cohorts. Temporal differences in billing code practices during the prediagnosis phase could influence timing of the index date. Temporal differences in accuracy of billing data could influence the number of 'true/false' RA cases included. However, over the period examined, one would expect improved accuracy as a result, for example, of the introduction of electronic medical records. This would constitute a conservative bias as less non-RA cases would be included in the later cohort. We observed a small increase in RA incidence rate over 1996–2006. A similar trend was reported in Olmsted County, USA over the same period.[74] Nonetheless, inclusion of a greater number of non-RA cases in the later cohort could bias results towards a reduced mortality difference with the general population.

Survival could appear improved in the later incident cohort if RA was diagnosed earlier, or if people presented to care earlier, as a result of recent efforts to raise awareness about the importance of early RA diagnosis and treatment. Of note, median RA duration at index date was slightly shorter (3 months) in the 2001–2006 than the 1996–2000 cohort; and mean age at index date was only slightly lower in the later cohort (57.5 vs 58.4 years in the earlier cohort). Furthermore, a younger age at death in RA relative to controls was observed in the earlier, but not the later, cohort. However, caution is warranted when comparing age at death, as a number of factors can influence it.

It is also possible that incident cohorts are contaminated with prevalent cases. Given our minimum lead-in period of 6 years, this would require gaps between consecutive RA visits longer than 6 years. In our cohort, this occurred very infrequently (only 0.44% of periods between consecutive RA visits were >6 years; 94.3% were <1 year; 97.3% <2 years). Because of potential differential effect on the two cohorts (with less prevalent cases in the 2001–2006 cohort due to longer lead-in time), we performed sensitivity analyses limiting the lead-in time of the 2001–2006 cohort to 6 years, which yielded similar results. Furthermore, we explored how much misclassification of prevalent cases as incident cases in the earlier cohort would be necessary to eliminate the difference in excess mortality observed. Analyses (described in online supplementary file) revealed that highly unlikely assumptions were required, indicating that misclassification cannot reasonably account for the observed results. Prevalent RA cases moving to BC during the study period could also be included. However, this would likely not differentially affect the two cohorts, and sensitivity analyses excluding individuals registered <6 years prior to index date yielded similar results.

Two other recent studies, until now published only in abstract form, also support the concept that the mortality risk in RA compared with the general population has recently improved.[75 76] A population-based study of Olmsted County found a significant reduction in cardiovascular mortality among patients with RA onset in 2000–2007 vs 1990–1999 (HR: 0.43, 95% CI 0.19 to 0.94),[75] with no difference with the general population for the 2000–2007 patients. However, their results are based on a small sample (315 patients with RA in 2000–2007 cohort), and only 8 deaths observed in RA and 9 in controls. Our analyses provide more robust confirmation of these findings in an independent, much larger sample, with >2000 deaths in RA and controls. Interestingly, previous studies from the same group described a gradual widening of the mortality gap, relative to the general population, for patients with RA with more recent onset over 1955–2000.[38 39] This suggests that improvement in mortality may be limited to patients with RA onset after 2000, as observed in our study. The second study used an electronic medical record database in the UK to compare all-cause mortality in two incident RA cohorts (RA onset 1999–2005 followed until end of 2005; and RA onset 2006–2012 followed until end of 2012) compared with general population controls.[76] Consistent with our findings, they found a significant reduction in the HR for all-cause mortality, relative to controls, for the later RA cohort ($p=0.027$ for interaction). However, even in the later cohort, RA mortality remained increased compared with the general population (HR 1.21, 95% CI 1.05 to 1.39).

Our findings have important implications for people living with arthritis, clinicians and health policy makers. It provides

reassuring evidence suggesting that time trends in the disease itself and/or its management are having a beneficial impact on an outcome of utmost importance to people living with arthritis. Exploring why mortality has improved over time is beyond the scope of this study. We speculate that it may be due to improved RA treatment, with more effective control of inflammation, from availability of more effective DMARDs and from the paradigm shift in RA management towards early, aggressive treatment with the aim of eradicating inflammation. Alternatively, improved survival could be due to improved prevention, detection or management of life-threatening comorbidities, such as CVD, as a result of increased awareness of its role as a leading cause of premature death. It is also possible that the natural history of RA has evolved over time. Exploration of these reasons warrants further study. Furthermore, future long-term studies should compare mortality in RA versus general population over >10 years of follow-up, since RA onset.

In conclusion, in our population-based incident RA cohort, the 5-year mortality risk compared with the general population has improved for patients with RA onset in the 21st century. Statistically significant relative risk reductions were observed for all-cause mortality, as well as deaths from CVD, and cancer, but not from infections. In our cohort, during the first 5 years after RA diagnosis, the mortality gap between RA and the general population observed in people with RA onset on or before 2000 was not observed for people with RA onset after 2000. Longer follow-up is needed before concluding that mortality differences between RA and the general population no longer exist.

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EXTENDED REPORT

Effect of rituximab on malignancy risk in patients with ANCA-associated vasculitis

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ABSTRACT

Objectives Patients with antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) treated with cyclophosphamide have an increased malignancy risk compared with the general population. We investigated whether treatment with rituximab instead of cyclophosphamide has decreased the malignancy risk in patients with AAV.

Methods The study included patients with AAV treated at a tertiary vasculitis referral centre between 2000 and 2014. The malignancy incidence in these patients was compared with the incidence in the general population by calculating standardised incidence ratios (SIRs), adjusted for sex, age and calendar year. Malignancy incidence was compared between rituximab-treated and cyclophosphamide-treated patients.

Results Of the 323 included patients, 33 developed a total of 45 malignancies during a mean follow-up of 5.6 years. This represented a 1.89-fold increased (95% CI 1.38 to 2.53) malignancy risk, and a non-significantly increased risk if non-melanoma skin cancer was excluded (SIR, 1.09; 95% CI 0.67 to 1.69). The risk of non-melanoma skin cancer was 4.58-fold increased (95% CI 2.96 to 6.76). Cyclophosphamide-treated patients had an increased malignancy risk compared with the general population (SIR, 3.10; 95% CI 2.06 to 4.48). In contrast, rituximab-treated patients had a malignancy risk similar to the general population (SIR, 0.67; 95% CI 0.08 to 2.43). The malignancy risk in cyclophosphamide-treated patients was 4.61-fold higher (95% CI 1.16 to 39.98) than in rituximab-treated patients.

Conclusions The malignancy risk in patients with AAV was lower in rituximab-treated patients than in cyclophosphamide-treated patients. Notably, rituximab treatment was not associated with an increased malignancy risk compared with the general population. Rituximab could therefore be a safe alternative to cyclophosphamide in the treatment of AAV.

INTRODUCTION

Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is a systemic autoimmune disease that affects small-sized to medium-sized blood vessels in multiple organs. AAV comprises granulomatosis with polyangiitis (formerly Wegener's granulomatosis), microscopic polyangiitis and eosinophilic granulomatosis with polyangiitis (formerly Churg-Strauss syndrome).[1] Autoantibodies against proteinase 3 (PR3-ANCA) and myeloperoxidase (MPO-ANCA) assist in the diagnosis of AAV, but patients can also be negative for ANCA.[2] Although the introduction of cyclophosphamide therapy for AAV has improved patient survival considerably,[3 4] the carcinogenic effects of cyclophosphamide put patients at increased risk of developing malignancies. Several studies have reported increased malignancy risks in patients with AAV who were treated with cyclophosphamide compared with the general population, especially for non-melanoma skin cancer, bladder cancer, malignant lymphoma and leukaemia.[5–12] Moreover, two studies found a dose–response association between cyclophosphamide and malignancy risk.[8 13] These results are restricted to patients with granulomatosis with polyangiitis and microscopic polyangiitis, and the malignancy risk in patients with eosinophilic granulomatosis with polyangiitis has not been investigated in detail before.

International efforts have been devoted to find less cytotoxic regimens for the treatment of AAV. In particular, the cumulative cyclophosphamide doses have been lowered,[14 15] and rituximab has emerged as a promising substitute for cyclophosphamide.[16 17] The initial findings from randomised controlled trials showed similar treatment efficacy in patients treated with either cyclophosphamide or rituximab.[18–20] However, concerns were raised about a possible higher malignancy rate in patients treated with rituximab.[21 22] Notably, the trials focused on treatment efficacy; thus, their results regarding malignancy incidence should be interpreted in light of their small sample sizes and the short follow-up of a maximum of 24 months.

This study investigated the long-term malignancy risk in 323 patients with AAV. This is, to our knowledge, the first study to compare the long-term malignancy risks between patients treated with rituximab and patients treated with cyclophosphamide.

METHODS

Study population

The study included patients with AAV (granulomatosis with polyangiitis, microscopic polyangiitis or eosinophilic granulomatosis with polyangiitis) who were treated at the Vasculitis and Lupus Clinic at Addenbrooke's Hospital, Cambridge, UK, between 2000 and 2014. The diagnosis was established according to the European Medicines Agency algorithm.[23] Follow-up began on the date of diagnosis and ended on the date of death, the date the patient was lost to follow-up or on 1 July 2015, whichever occurred first. Follow-up surveillance was performed at Addenbrooke's Hospital. This

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study was conducted in accordance with the ethical principles stated in the Declaration of Helsinki.

Clinical data

The following data were obtained from the medical records of the patients: demographic characteristics, diagnosis, date of diagnosis, ANCA serotype, organ involvement, therapy regimen, renal transplantation and the occurrence of malignancies. Patients with incomplete or missing medical records were excluded from further analyses. The cumulative doses of cyclophosphamide and rituximab during follow-up were determined. For subgroup analysis, patients were categorised according to their cyclophosphamide and/or rituximab exposure into the following categories: patients treated only with cyclophosphamide, patients treated only with rituximab, patients treated with both cyclophosphamide and rituximab, or patients who were not treated with either cyclophosphamide or rituximab. In all categories, the treatment may also have included other immunosuppressive agents, such as glucocorticoids, azathioprine, mycophenolate mofetil, methotrexate and/or tumour necrosis factor (TNF)-α inhibitors.

Standardised incidence ratio calculations

Standardised incidence ratios (SIRs) were calculated to compare the malignancy incidence between the study cohort and the general UK population, expressing the malignancy risk relative to the general population and matching for sex, age and calendar year. The SIR is the observed number of malignancies divided by the expected number of malignancies. The observed number of malignancies was the total number of primary invasive malignancies. The expected number of malignancies was the number of person-years at risk in our cohort multiplied by the malignancy incidence rates in the general UK population as obtained from the Office for National Statistics and matched for sex, 5-year age group and 1-year calendar time period.[24] Since the malignancy incidence rates were available until 2013, the malignancy incidence rate in 2013 was extrapolated to 2014 and 2015. The SIR was calculated for malignancies at all sites, for all malignancies except non-melanoma skin cancers and for each malignancy site as reported in the study population. SIRs were stratified by sex, age category at diagnosis (younger than the median age of 59 years vs 59 years or older), clinical diagnosis, ANCA serotype, renal transplantation and follow-up duration. Moreover, SIRs were compared in different treatment categories and according to the cumulative doses of cyclophosphamide and rituximab.

Statistical analyses

Student's t-test, the χ^2 test, Fisher's exact test and the one-way analysis of variance (ANOVA) test were used to compare the characteristics of different subgroups (SPSS statistical software, V.23). SIR values were compared between subgroups by calculating relative risks (RRs). Exact Poisson regression analysis was used to calculate 95% CIs for the SIR and RR values assuming a Poisson distribution of the observed number of cases (SAS software, V.9.3; SAS Institute).[25–27] p Values less than 0.05 were considered significant in all analyses.

RESULTS

Patient characteristics

The characteristics of the 323 patients with AAV included in this study are shown in table 1. The mean (SD) age at diagnosis was 56.4 (16.1) years, and the mean follow-up was 5.6 (3.2) years (1802 person-years). A total of 160 (49%) patients were diagnosed with microscopic polyangiitis; 109 patients (34%) were diagnosed with granulomatosis with polyangiitis; and 54 patients (17%) were diagnosed with eosinophilic granulomatosis with polyangiitis. Finally, 12 patients (4%) underwent renal transplantation, and 39 patients (12%) died during follow-up.

Malignancy occurrence

Of the 323 patients, 33 developed a total of 45 malignancies during follow-up. The sex, age and calendar year-adjusted malignancy risk was 1.89-fold higher in the patients with AAV than in the general population (95% CI 1.38 to 2.53) (table 2). There were 13 different malignancy types, with non-melanoma skin cancer occurring most frequently (10 basal cell carcinomas and 15 squamous cell carcinomas). The SIR for non-melanoma skin cancer was significantly increased (SIR, 4.58; 95% CI 2.96 to 6.76), while the risk for all malignancies excluding non-melanoma skin cancer was comparable to that of the general population (SIR, 1.09; 95% CI 0.67 to 1.69) (table 2).

Malignancy occurrence in the subgroups

The SIR for overall malignancy risk was stratified by gender, age, clinical diagnosis, ANCA serotype, renal transplantation and follow-up duration (see online supplementary table S1). Patients with eosinophilic granulomatosis with polyangiitis had the highest malignancy risk (SIR, 2.75; 95% CI 1.19 to 5.40), followed by those with granulomatosis with polyangiitis (SIR, 2.20; 95% CI 1.20 to 3.68) and those with microscopic polyangiitis (SIR, 1.59; 95% CI 1.01 to 2.38). Transplanted patients had a higher malignancy risk (SIR, 4.31; 95% CI 1.17 to 11.04) than patients who did not undergo renal transplantation (SIR, 1.79; 95% CI 1.29 to 2.43). The treatment duration and cumulative doses of cyclophosphamide and rituximab in subgroups are shown in online supplementary table S2.

Effects of cyclophosphamide and rituximab on malignancy risk

Patients treated only with cyclophosphamide had a 3.10-fold higher (95% CI 2.06 to 4.48) malignancy risk than the general population (table 3), and a 1.14-fold higher (95% CI 0.49 to 2.25) malignancy risk if non-melanoma skin cancer was excluded. Patients treated only with rituximab had no increased malignancy risk compared with the general population (SIR, 0.67; 95% CI 0.08 to 2.43), which was similar if non-melanoma skin cancer was excluded (SIR, 0.88; 95% CI 0.11 to 3.19). The malignancy risk in patients treated only with cyclophosphamide was 4.61-fold higher (95% CI 1.16 to 39.98) than in patients treated only with rituximab and was 3.05-fold higher (95% CI 1.40 to 7.35) than in patients treated with both cyclophosphamide and rituximab (table 4). The mean cumulative cyclophosphamide dose was lower in patients treated only with cyclophosphamide than in patients treated with both cyclophosphamide and rituximab (7.3 g vs 11.1 g; p=0.002). The duration of follow-up was longer for patients who received rituximab than for patients who did not receive rituximab (p<0.001). In terms of mean organ involvement, the disease extent did not differ between the treatment groups (p=0.07) (table 3). Patients treated with cyclophosphamide received azathioprine maintenance therapy more frequently than those treated with rituximab (81% vs 42%; p<0.001). The SIR of malignancy for patients receiving a combination of cyclophosphamide and azathioprine was 3.20 (95% CI 2.05 to 4.76; p<0.001), whereas patients receiving a combination of rituximab and azathioprine expressed a comparable malignancy risk to that of the general population (SIR, 1.52; 95% CI 0.18 to 5.50; p=0.38).

Table 1 Characteristics of the patients with ANCA-associated vasculitis who were included in this study*

	All patients (n=323)	No malignancy occurrence (n=290)	Malignancy occurrence (n=33)	p Value†
Age (years) at diagnosis, mean (SD)	56.4 (16.1)	55.9 (16.3)	61.3 (12.7)	0.03
Follow-up (years), mean (SD)	5.6 (3.2)	5.5 (3.2)	6.3 (3.2)	0.20
Male, n (%)	149 (46)	135 (47)	14 (42)	0.65
Clinical diagnosis, n (%)				0.64
Microscopic polyangiitis	160 (49)	146 (50)	14 (42)	
Granulomatosis with polyangiitis	109 (34)	97 (33)	12 (36)	
Eosinophilic granulomatosis with polyangiitis	54 (17)	47 (16)	7 (21)	
ANCA serotype, n (%)‡				0.89
MPO-ANCA	110 (34)	99 (34)	11 (33)	
PR3-ANCA	152 (47)	136 (47)	16 (49)	
Organ involvement, mean (SD)	2.3 (1.5)	2.3 (1.5)	2.2 (1.2)	0.85
Deaths, n (%)	39 (12)	30 (10)	9 (27)	0.01
Relapsing disease, n (%)	86 (28)	79 (28)	7 (22)	0.54
Renal transplantation, n (%)	12 (4)	11 (4)	1 (3)	1.00
Treatment, n (%)				
Glucocorticoids	318 (99)	286 (99)	32 (97)	0.33
Cyclophosphamide	233 (72)	207 (72)	26 (79)	0.38
Rituximab	155 (48)	144 (50)	11 (33)	0.07
Cyclophosphamide and rituximab	114 (35)	105 (36)	9 (27)	0.31
Azathioprine	218 (68)	196 (68)	22 (67)	0.89
Mycophenolate mofetil	154 (48)	141 (50)	13 (39)	0.31
Methotrexate	39 (12)	35 (12)	4 (12)	1.00
TNF-α inhibitors	19 (6)	15 (5)	4 (12)§	0.12

*Values are reported as means (SD) or as numbers (%).
†p Values were calculated using Student's t-test, χ^2 test or Fisher's exact test.
‡ANCA serotype data were not available for 61 patients.
§Four of the 19 patients (21%) who received TNF-α inhibitors developed, in total, two basal cell carcinomas, one breast carcinoma and one prostate carcinoma. All four patients were also treated with cyclophosphamide, and one was treated with rituximab. Malignancy risk was similar in patients treated with and without a TNF-α inhibitor.
ANCA, antineutrophil cytoplasmic antibody; MPO-ANCA, myeloperoxidase-ANCA; PR3-ANCA, proteinase 3 ANCA; TNF, tumour necrosis factor.

Table 2 SIR for malignancies overall and per observed malignancy site*

Malignancy or malignancy site	Observed malignancies (n)	Expected malignancies (n)	SIR (95% CI)†	p Value†
All sites	45	23.80	1.89 (1.38 to 2.53)	<0.001
Non-melanoma skin cancer	25	5.46	4.58 (2.96 to 6.76)	<0.001
All malignancies excluding non-melanoma skin cancer	20	18.33	1.09 (0.67 to 1.69)	0.76
Lung	4	2.61	1.53 (0.42 to 3.92)	0.53
Breast	3	2.82	1.06 (0.22 to 3.11)	1.00
Colon or rectum	3	1.98	1.52 (0.31 to 4.44)	0.63
Prostate	2	2.74	0.73 (0.09 to 2.64)	0.97
Bladder	1	0.65	1.53 (0.04 to 8.57)	0.96
Pancreas	1	0.52	1.94 (0.05 to 10.81)	0.81
Testis	1	0.04	24.66 (0.62 to 137.41)	0.08
Ovary	1	0.39	2.54 (0.06 to 14.14)	0.65
Melanoma	1	0.66	1.52 (0.04 to 8.49)	0.96
Tongue	1	0.07	13.70 (0.35 to 76.34)	0.14
Central nervous system	1	0.25	3.94 (0.10 to 21.95)	0.45
Kidney	1	0.49	2.03 (0.05 to 11.32)	0.78

*SIR is the ratio of the observed to expected malignancies adjusted for sex, age (per 5-year age group) and calendar time period (per 1-year calendar time period).
†Calculated by exact Poisson regression analysis.
SIR, standardised incidence ratio.

Effects of cumulative cyclophosphamide and rituximab doses on malignancy risk

The mean (SD) cumulative cyclophosphamide and rituximab doses were 9.1 (9.0) g and 5.9 (3.4) g, respectively. The highest cyclophosphamide dose was 108 g, given intermittently for 7.6 years, during a follow-up period of 8.1 years, in which the patient experienced no relapses. The highest rituximab dose was 18 g, given intermittently over 6.1 years, during a follow-up period of 9.1 years, in which one relapse occurred. A positive dose–response relationship was found between

Table 3 SIR stratified according to treatment category*

Treatment†	Patients (n)	SIR (95% CI)‡	SIR p Value‡	Cyclophosphamide cumulative dose (g), mean (SD)§	Follow-up (years), mean (SD)¶	Organ involvement, mean**
Only cyclophosphamide	119	3.10 (2.06 to 4.48)	<0.001	7.26 (4.94)	4.92 (3.10)	2.11 (1.49)
Only rituximab	41	0.67 (0.08 to 2.43)	0.86	0.00	6.34 (3.56)	2.35 (1.09)
Both	114	1.01 (0.46 to 1.93)	1.00	11.05 (11.63)	6.60 (2.84)	2.56 (1.63)
None	48	2.10 (0.77 to 4.56)	0.14	0.00	4.20 (2.94)	1.96 (1.44)

*Values are reported as means (SD) unless otherwise indicated. The SIR is the ratio of the observed to expected malignancies adjusted for sex, age (per 5-year age group) and calendar time period (per 1-year calendar time period).
†The 'only cyclophosphamide' group was treated with cyclophosphamide but not with rituximab. The 'only rituximab' group was treated with rituximab but not with cyclophosphamide. 'Both' indicates a group that received cyclophosphamide and rituximab. 'None' indicates a patient group that neither received cyclophosphamide nor rituximab, but instead had various heterogeneous treatments including glucocorticoids, azathioprine, mycophenolate mofetil and methotrexate. Other immunosuppressive drugs were also administered in all of the groups.
‡Calculated by exact Poisson regression analysis.
§The mean cumulative cyclophosphamide dose differed between the 'only cyclophosphamide' and 'both' groups (Student's t-test, p=0.002).
¶The mean follow-up duration differed between groups (ANOVA, p<0.001). The mean follow-up duration also differed when the 'only rituximab' and 'both group' were compared with the 'only cyclophosphamide' and 'none' group (Student's t-test, p<0.001).
**The mean organ involvement did not differ between groups (ANOVA, p=0.07).
ANOVA, analysis of variance; SIR, standardised incidence ratio.

Table 4 Relative risks (RR) according to treatment category

Treatment*	RR (95% CI)†	p Value†
Only cyclophosphamide vs only rituximab	4.61 (1.16 to 39.98)	0.03
Only cyclophosphamide vs both	3.05 (1.40 to 7.35)	0.003
Only cyclophosphamide vs none	1.48 (0.60 to 4.36)	0.52

*The 'only cyclophosphamide' group was treated with cyclophosphamide but not with rituximab. The 'only rituximab' group was treated with rituximab but not with cyclophosphamide. 'Both' indicates a group that received cyclophosphamide and rituximab. 'None' indicates a group that did not receive cyclophosphamide or rituximab. Other immunosuppressive drugs were also administered in all of the groups.
†RR represents the risk of malignancy compared with the reference group. Calculated by exact Poisson regression analysis.

cyclophosphamide therapy and the overall malignancy risk (table 5), and between cyclophosphamide therapy and the risk of non-melanoma skin cancer (see online supplementary table S3). The opposite relationship was found for patients treated with rituximab: the higher the cumulative rituximab dose, the lower the overall malignancy risk (table 5), and the lower the risk of non-melanoma skin cancer (see online supplementary table S3). Patients who did not receive rituximab had a 2.86-fold higher (95% CI 1.98 to 3.99) malignancy risk than the general population. No increased risk was observed when patients had a cumulative rituximab dose below 6.0 g (SIR, 1.41; 95% CI 0.57 to 2.90). A total of 83 patients received more than 6.0 g rituximab, and these patients had a non-significantly lower malignancy risk than the general population (SIR, 0.45; 95% CI 0.09 to 1.32) and a 6.32-fold lower (95% CI 1.99 to 32.15) malignancy risk than patients who did not receive rituximab (table 5). The cumulative cyclophosphamide and rituximab doses individually received by the patients who developed a malignancy during follow-up are shown in online supplementary table S4.

DISCUSSION

This study compared the malignancy risks in patients with AAV treated with rituximab versus cyclophosphamide. Strikingly, patients treated with cyclophosphamide had a 4.61-fold higher risk than those treated with rituximab. In patients treated with cyclophosphamide, the malignancy risk was 3.10-fold higher than in the general population; in contrast, patients treated with rituximab did not show an increased risk compared with the general population. Patients treated with both rituximab and cyclophosphamide (N=114) had a lower malignancy risk than those treated with only cyclophosphamide, even though the mean cyclophosphamide dose was lower in the latter group. In addition, there was a non-significant trend towards an inverse dose–response relationship between the cumulative rituximab dose and malignancy risk: the more rituximab a patient received, the lower the malignancy risk, with the risk actually falling below the risk in the general population if more than a cumulative dose of 6.0 g was given. The relative risk for

Table 5 SIR stratified according to cumulative cyclophosphamide and rituximab doses*

Cumulative dose (g)	Patients (n)	N observed malignancies	SIR (95% CI)†	SIR p Value†	RR (95% CI)†	RR p Value†
Cyclophosphamide						
0	89	8	1.37 (0.59 to 2.70)	0.47	1 (reference)	
0.1–20	207	31	1.91 (1.30 to 2.71)	0.001	1.39 (0.63 to 3.50)	0.52
20–108	16	5	5.06 (1.64 to 11.82)	0.007	3.69 (0.95 to 12.78)	0.06
Rituximab						
0	167	34	2.86 (1.98 to 3.99)	<0.001	1 (reference)	
0.1–6	70	7	1.41 (0.57 to 2.90)	0.47	0.49 (0.18 to 1.13)	0.11
6–18	83	3	0.45 (0.09 to 1.32)	0.10	0.16 (0.03 to 0.50)	<0.001

*SIR is the ratio of the observed to expected malignancies adjusted for sex, age (per 5-year age group) and calendar time period (per 1-year calendar time period). SIR represents the malignancy risk compared with the general population, and the RR represents the malignancy risk compared with the reference group.
†Calculated by exact Poisson regression analysis.
RR, relative risk; SIR, standardised incidence ratio.

developing a malignancy was more than six times lower in patients who had received a cumulative dose of rituximab of more than 6.0 g than in patients who had not received rituximab at all.

Interestingly, our findings—although the number of patients was relatively low—may point towards the possibility that rituximab has a protective role in the development of malignancies. This hypothesis is underlined by data showing a trend of an inverse dose–response relationship, and by the difference in malignancy development of the combined treatment group (ie, patients receiving both cyclophosphamide and rituximab). Depletion of B cells due to rituximab may increase antitumour immunity, as was demonstrated in mouse models in which B-cell-deficient mice are resistant to the development of certain malignancies.[28 29] The enhanced antitumour immune response in these mice is probably caused by decreased IL-10 production by B cells, leading to enhancement of the antitumour effects of cytotoxic T cells.[28] There is emerging evidence that regulatory B cells are the main mediators of this mechanism.[30] In humans, the hypothesis that rituximab enhances the antitumour immune response is supported by the trend towards a lower risk of developing a second primary malignancy in patients with non-Hodgkin's lymphoma treated with rituximab-containing chemotherapy compared with patients treated with chemotherapy that does not include rituximab.[31 32] However, clarification of the effects of B-cell depletion on antitumour immunity in humans requires further investigation.

The increased risks of bladder and haematological malignancies that have been previously reported for patients treated with cyclophosphamide did not materialise in this study, possibly reflecting the ongoing efforts to reduce cumulative cyclophosphamide doses.[11] In accordance with two recent studies, only the risk of non-melanoma skin cancer was increased in the current study.[9 11] To prevent the development of these lesions, all patients were given written information concerning the risks of non-melanoma skin cancer. Moreover, they were advised to avoid ultraviolet radiation, to use sunscreens and to promptly report skin lesions. Of the patients who developed non-melanoma skin cancer despite these preventative measures, the majority had received azathioprine as maintenance therapy before the occurrence of this malignancy. Therefore, the previously reported association between non-melanoma skin cancer and azathioprine exposure is confirmed in our study.[33–37] However, in our study, only the combination of cyclophosphamide and azathioprine treatment was associated with an increased malignancy risk. In contrast, patients treated with rituximab and azathioprine had a malignancy risk similar to the general population. Lowering cyclophosphamide and azathioprine exposure will most likely decrease the malignancy risk. For patients with AAV who receive azathioprine, especially those who received cyclophosphamide as induction therapy, regular skin cancer screening should be started to control and prevent the development of non-melanoma skin cancers. Moreover, patients should be advised as to how to protect themselves against ultraviolet radiation.[38]

Previous studies that investigated the malignancy risk in patients with AAV were restricted to patients with granulomatosis with polyangiitis and microscopic polyangiitis, and did not include patients with eosinophilic granulomatosis with polyangiitis. Eosinophilic granulomatosis with polyangiitis has a lower incidence than granulomatosis with polyangiitis and microscopic polyangiitis, and it is treated similarly.[39] The 54 patients with eosinophilic granulomatosis with polyangiitis who were included in this study had a 2.75-fold increased malignancy risk compared with the general population. We therefore recommend that clinicians monitor patients with eosinophilic granulomatosis with polyangiitis for malignancies as carefully as patients with granulomatosis with polyangiitis and microscopic polyangiitis.

One limitation of this study is its retrospective design. However, it excluded patients with unclear or missing data. A second limitation is the relative short follow-up, with a mean of 5.6 years. Longer follow-up studies are now required to validate our findings. A third limitation of this study is the relatively small number of patients, particularly in the subgroup analyses. This could explain the non-significance of the inverse dose–response relationship between rituximab and malignancy risk. This relationship merits further investigation in larger studies. Finally, the study involved just one medical centre, so the findings may not be generalisable to other settings. One strength of this study is the large study population, in which, for the first time, the malignancy risk was evaluated in patients treated with rituximab during long-term follow-up. This is also the first study to analyse the malignancy risk in patients with eosinophilic granulomatosis with polyangiitis. Another strength of our study is the calculation of cumulative cyclophosphamide and rituximab doses. Finally, the calculation of sex, age and calendar-year period-matched SIRs ensured reliable comparisons between our cohort and the general population.

In conclusion, we demonstrated that patients with AAV who are treated with rituximab have a decreased burden of malignancy, which surpasses expectations from clinical trials data.[18 19] Moreover, our results suggest that rituximab may protect against the occurrence of malignancies, a possibility that should be explored in further detail using larger cohort populations. Patients with AAV treated with rituximab had a strikingly lower malignancy risk than those treated with cyclophosphamide and no increased malignancy risk compared with the general population. Therefore, the rituximab dose currently used in clinical practice could be a safe alternative to cyclophosphamide in the treatment of AAV.

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Contributors EEvD, DRJ, IMB and CR conceived and designed the study. RR, AK and CR acquired the data. EEvD, RW and CR performed statistical analyses. EEvD wrote the first draft of the manuscript. All authors critically revised the manuscript and approved the final version. EEvD is the guarantor of the study.

Competing interests None declared.

Ethics approval Following the Governance Arrangements for research ethics committees, this study did not require review by a research ethics committee, since it used non-identifiable information that was previously collected for normal care.

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OPEN ACCESS

EXTENDED REPORT

Effect of secukinumab on clinical and radiographic outcomes in ankylosing spondylitis: 2-year results from the randomised phase III MEASURE 1 study

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ABSTRACT

Objective To evaluate the effect of secukinumab, an interleukin-17A inhibitor, on clinical signs and symptoms and radiographic changes through 2 years in patients with ankylosing spondylitis (AS).

Methods In the phase III MEASURE 1 study, patients were randomised to receive intravenous secukinumab 10 mg/kg (at baseline, week 2 and week 4) followed by subcutaneous secukinumab 150 mg (intravenous 150 mg; n=125) or 75 mg (intravenous 75 mg; n=124) every four weeks, or matched placebo (n=122). Placebo-treated patients were re-randomised to subcutaneous secukinumab 150 or 75 mg from week 16. Clinical efficacy assessments included Assessment of SpondyloArthritis international Society 20 (ASAS20) response rates through week 104. Radiographic changes at week 104 were assessed using the modified Stoke Ankylosing Spondylitis Spine Score (mSASSS).

Results 97 (77.6%) and 103 (83.1%) patients in the intravenous 150 mg and intravenous 75 mg groups, respectively, completed week 104. In the full analysis set (intent-to-treat), ASAS20 response rates at week 104 were 73.7% and 68.0% in the intravenous 150 mg and intravenous 75 mg groups, respectively. Among patients with evaluable X-rays who were originally randomised to secukinumab (n=168), mean change in mSASSS from baseline to week 104 was 0.30±2.53. Serious adverse events were reported in 12.2% and 13.4% of patients in the 150 mg and 75 mg groups, respectively.

Conclusions Secukinumab improved AS signs and symptoms through 2 years of therapy, with no unexpected safety findings. Data from this study suggest a low mean progression of spinal radiographic changes, which will need to be confirmed in longer-term controlled studies.

Trial registration number NCT01358175.

INTRODUCTION

Ankylosing spondylitis (AS), a chronic inflammatory disease primarily affecting the axial skeleton, can be associated with progressive irreversible structural damage, resulting in functional deterioration.[1–3] Long-term treatment goals are to maximise quality of life through control of signs and symptoms, prevention of structural damage and preservation of physical function.[4]

Tumour necrosis factor (TNF) inhibitors are the recommended pharmacotherapy for patients with high disease activity despite treatment with non-steroidal anti-inflammatory drugs (NSAIDs),[4 5] based on their proven efficacy in improving signs, symptoms and physical function.[6–10] However, up to 40% of patients do not respond to or cannot tolerate TNF inhibitors,[11] and loss of efficacy can occur over time.[12] Moreover, their effect on spinal osteoproliferative changes, a major component of structural damage in AS, is unclear.[13–16]

The pro-inflammatory cytokine interleukin-17A (IL-17A) is implicated in various pathophysiological features of spondyloarthritis, including inflammation and pathogenic bone remodelling.[17–29] In placebo-controlled phase III studies, secukinumab, a fully human anti-IL-17A monoclonal antibody, significantly improved the signs and symptoms of psoriasis,[30] psoriatic arthritis (PsA),[31] and AS.[32] Here, we present an update on the efficacy and safety of secukinumab in patients with AS during the non-controlled continuation phase of MEASURE 1 (NCT01358175) through 2 years. Results of exploratory radiographic endpoints at 2 years are also presented.

METHODS

Study design and patients

Study design and patient eligibility criteria have been described previously.[32] Briefly, patients were ≥18 years of age, with AS fulfilling the modified New York Criteria, and active disease as defined by a Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) score of ≥4[33] and a spinal pain score of ≥4 cm on a visual analogue scale (0–10 cm), despite treatment with maximal tolerated doses of NSAIDs. Patients who previously received one anti-TNF agent could enrol if they had an inadequate response or had stopped treatment for safety or tolerability reasons (anti-TNF-IR). Patients could continue on stable doses of sulfasalazine, methotrexate, prednisone and NSAIDs. Key exclusion criteria are detailed in the online supplementary materials.

Between 9 November 2011 and 21 January 2013, eligible patients were randomly assigned (1:1:1) to one of two secukinumab arms or placebo (randomisation procedures are described in the online supplementary materials). Patients randomised to secukinumab received a 10 mg/kg intravenous infusion at baseline and weeks 2 and 4,

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followed by subcutaneous injections of 150 mg (secukinumab intravenous 150 mg) or 75 mg (intravenous 75 mg) every four weeks from week 8; patients in the placebo group were treated using the same intravenous-to-subcutaneous schedule. Placebo-treated patients were randomly reassigned (1:1) to receive secukinumab 150 or 75 mg subcutaneous every four weeks from week 16 (non-responders) or week 24 (responders), with response based on Assessment of SpondyloArthritis international Society 20 (ASAS20) response criteria.[3]

Procedures and end points

Disease activity and efficacy assessments[3 34 35] were conducted at baseline and throughout the study, with key assessments at weeks 16 and 52 (as previously reported),[32] and week 104; further details are provided in the online supplementary materials.

Lateral view radiographs of the cervical and lumbar spine were obtained at baseline and week 104. Images were digitised centrally and patient identifiers and temporal indicators removed to ensure blinding. Radiographs were scored using the modified Stoke Ankylosing Spondylitis Spine Score (mSASSS) method (total score range: 0–72; see online supplementary materials for further details).[36 37] Scoring was conducted by two central, independent readers, blinded with regard to treatment group and temporal sequence, who both read each film; statistical analyses used the mean score from the two readers. The top 5% of cases with the highest between-reader differences for change in radiographic score were identified for adjudication, with the two readers performing a third consensus read. Data from X-ray completers (patients with X-rays at both baseline and week 104) are presented as mean change from baseline to week 104.

Safety analyses assessed adverse events (AEs), serious AEs and routine laboratory values. Biochemical investigations were classified according to the Common Terminology Criteria for Adverse Events (V.4.03).[38] Blood samples were collected at baseline through week 104 for assessment of secukinumab immunogenicity using a MesoScale Discovery bridging immunoassay.[39]

Statistical analysis

Clinical efficacy analyses at week 104 were carried out on data from patients originally randomised to secukinumab. Statistical analyses for binary variables up to week 104 used multiple imputation to account for missing data, while mixed-effect model repeated measures, with missing data assumed missing at random, were used for continuous variables (except high-sensitivity C-reactive protein (hsCRP) was reported as observed). Data are also reported 'as observed' among patients with evaluable data. Radiographic analyses were assessed in X-ray completers who were initially randomised to secukinumab treatment, and in X-ray completers who switched from placebo to secukinumab at week 16 or 24 (placebo switchers). In each population, data were pooled between treatment groups.

RESULTS

Patients

Of 371 patients originally randomised to treatment, 97/125 (77.6%) patients in the secukinumab intravenous 150 mg group, 103/124 (83.1%) in the intravenous 75 mg group and 90/122 (73.8%) in the placebo group (no placebo given beyond week 24) completed week 104. AEs (6.4% of patients), lack of efficacy (4.4%) and patient/guardian decision (5.8%) were the main reasons for discontinuation among secukinumab-treated patients (see online supplementary figure S1).

Demographic and baseline characteristics have been reported previously.[32] Mean time since diagnosis in the three randomised groups was 6.5–8.3 years, mean total BASDAI scores were 6.1–6.5 and median hsCRP levels were 7.4–9.2 mg/L. Approximately 73% of patients were anti-TNF-naive.

Concomitant medications through 104 weeks were very similar to 52 weeks and baseline, and included sulfasalazine (35.0%), methotrexate (15.8%), systemic glucocorticoids (19.7%) and NSAIDs (93.6%).

Clinical efficacy

Among patients who continued on secukinumab treatment, ASAS20 and ASAS40 response rates similar to those achieved at weeks 16 and 52[32] were observed at week 104 (figure 1A, B). ASAS20/40 response rates at week 104 (with multiple imputation of missing values) were 73.7/55.7%, respectively, in the secukinumab intravenous 150 mg group and 68.0/48.5% in the intravenous 75 mg group (table 1). Response rates using observed data are reported in table 1. Improvements consistent with those reported previously at weeks 16 and 52[32] were also observed in total BASDAI score (figure 1C) and all other secondary end points at week 104 (table 1).

In prespecified subgroup analyses, ASAS20 and ASAS40 response rates in both anti-TNF-naive and anti-TNF-IR patients were higher with secukinumab than placebo at week 16 (see online supplementary figure S2). Observed data showed that 59/69 (85.5%) and 47/65 (72.3%) anti-TNF-naive patients in the secukinumab intravenous 150 mg and intravenous 75 mg groups, respectively, achieved an ASAS20 response at week 104; an ASAS40 response was achieved by 48/69 (69.6%) and 34/65 (52.3%) patients, respectively. In anti-TNF-IR patients, 10/18 (55.6%) and 15/21 (71.4%) patients achieved an ASAS20 response in the intravenous 150 mg and intravenous 75 mg groups, respectively, while 8/18 (44.4%) and 12/21 (57.1%) patients achieved an ASAS40 response.

Radiographic findings

Of the 97 patients in the secukinumab intravenous 150 mg group and 103 patients in the intravenous 75 mg group who completed week 104, 86 (88.7%) and 82 (79.6%), respectively, had evaluable X-rays at baseline and week 104 (X-ray completers) meeting predefined statistical analysis windows. Baseline characteristics of the X-ray completer cohort were similar between dose groups (table 2) and to those previously reported in the overall population.[32] Overall, 104 (61.9%) X-ray completers had syndesmophytes at baseline and 105 (62.5%) had hsCRP >5 mg/L. Baseline characteristics of the 89 placebo-switcher X-ray completers (see online supplementary table S1) were similar to those originally randomised to secukinumab.

Figure 2 shows the cumulative probability plot for change from baseline in mSASSS at week 104 among X-ray completers randomised to secukinumab at baseline. The change in mSASSS (mean±SD) over 104 weeks was 0.30±2.53 in the pooled secukinumab group, 0.30±1.94 in the intravenous 150 mg group and 0.31±3.04 in the intravenous 75 mg group (table 3). Changes among placebo switchers were 0.54±2.45 (pooled), 0.44±2.09 (150 mg) and 0.64±2.79 (75 mg) (see online supplementary table S2 and figure S3). The smallest detectable change (SDC) was 1.838 and 2.814 at an 80% and 95% level of agreement, respectively. As defined by SDC at the 80% level of agreement, a large proportion of patients (>80%) had no spinal radiographic progression. The Bland-Altman plot in online supplementary figure S4 shows the level of agreement between the two readers; variability was observed, particularly when an mSASSS change of >0 was recorded. The intraclass

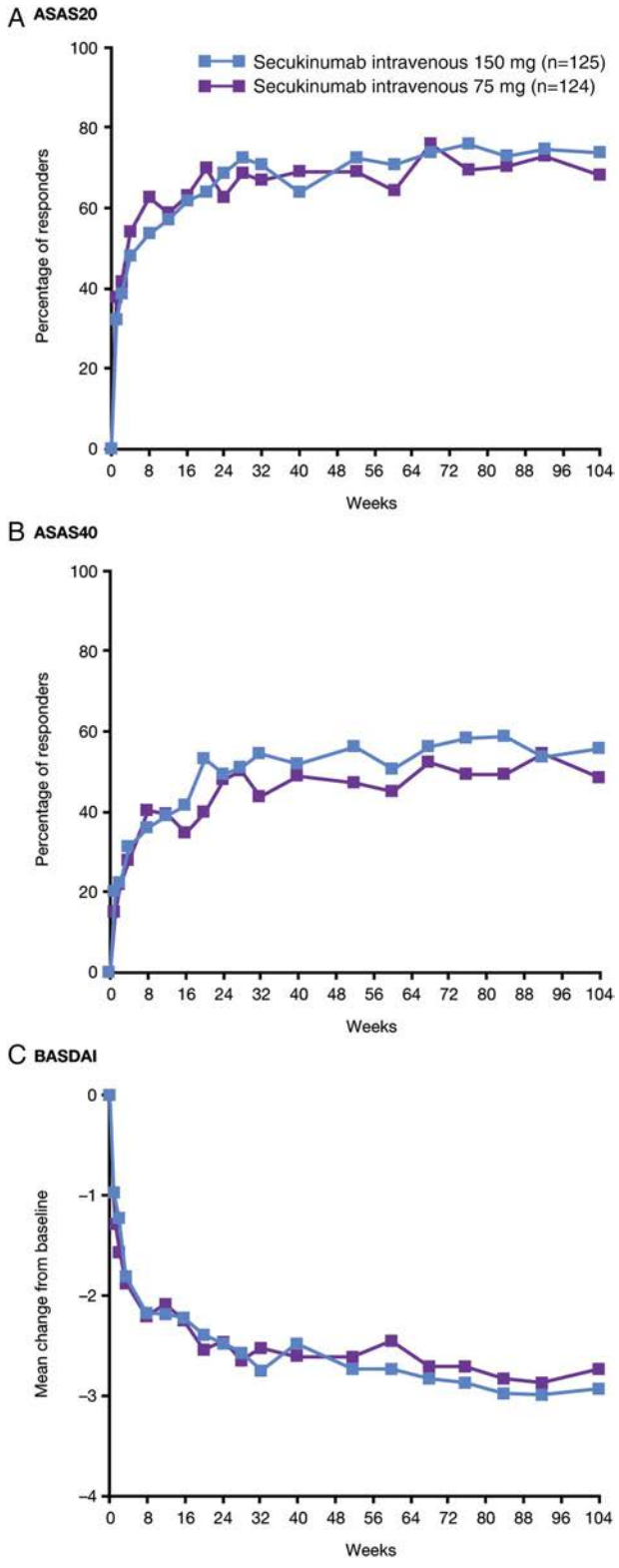

Figure 1 Summary of selected clinical efficacy end points through week 104 for patients randomised to secukinumab at baseline. Assessment of SpondyloArthritis international Society response criteria (ASAS20) (A) and ASAS40 (B) response rates, and mean change in total Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) score (C) over time to week 104 is shown. Multiple imputation applied to handle missing data for ASAS20 and ASAS40, mixed-effects model repeated measures used for BASDAI. Patients received a 10 mg/kg loading dose of secukinumab at baseline and weeks 2 and 4, before receiving indicated dose of secukinumab subcutaneously every four weeks from week 8.

correlation coefficient (ICC) score for agreement between readers was 0.570 for change in mSASSS.

Baseline mSASSS was higher among patients with known predictors of radiographic progression compared with those without (table 3). Mean mSASSS change at week 104 among X-ray completers randomised to secukinumab was higher in males than females, in those with baseline syndesmophytes versus those without, in patients with elevated versus normal baseline hsCRP levels and in smokers versus non-smokers (table 3). Among the X-ray completers who had no syndesmophytes at baseline and who were randomised to secukinumab, 61/64 (95.3%), remained free from syndesmophytes at week 104. Of the 104 X-ray completers with syndesmophytes at baseline, approximately 70% did not develop additional syndesmophytes through week 104 (see online supplementary table S3).

Safety

The most common AEs with secukinumab were nasopharyngitis, diarrhoea, headache, upper respiratory tract infections and pharyngitis (table 4). Discontinuations due to AEs were infrequent. The incidence of serious AEs was low, and similar between the two secukinumab groups (table 4; see online supplementary table S4).

One death was reported among secukinumab-treated patients throughout the study; on day 706, a patient in the intravenous 75 mg group with a history of arterial hypertension and smoking died due to acute respiratory failure secondary to cardiac failure and pulmonary fibrosis. No suicides or suicidality-related AEs were reported among secukinumab-treated patients.

Candida infections were reported in four secukinumab-treated patients (0.7 cases per 100 patient-years of secukinumab exposure); two oral candidiasis (one in each dose group), one cutaneous *Candida* infection (150 mg group) and one genital candidiasis (75 mg group). All four events were considered mild and non-serious, resolved spontaneously or with standard antifungal therapy, and did not result in study discontinuation. Herpes viral infections — mainly oral herpes and herpes zoster infections — were reported by 8.3% of patients in the 150 mg group and 2.2% in the 75 mg group. All cases were non-serious. One led to treatment discontinuation (herpes zoster infection in the 150 mg group).

Grade 3 neutropenia occurred at a single visit in each of three patients receiving secukinumab 75 mg and one receiving secukinumab 150 mg. Grade 4 neutropenia was reported in one patient (75 mg group) at a single visit. None of these events led to treatment interruption or discontinuation. One grade 3 case was associated with a non-serious upper respiratory tract infection.

Crohn's disease was reported as a non-serious AE in four patients in the 75 mg group (of whom two had a history of Crohn's disease and one a history of a polyp and colon adenoma) and one patient in the 150 mg group, equivalent to 0.8 cases per 100 patient-years of secukinumab exposure. Two patients (one in each group; one with a history of Crohn's disease) discontinued because of an AE of Crohn's disease. A history of uveitis was reported in 62 (16.7%) patients at baseline. An AE of uveitis was reported in 12 patients (seven with history of uveitis) on secukinumab, equivalent to 2.0 cases per 100 patient-years of secukinumab exposure. One was a serious AE (150 mg group); this resolved and did not require discontinuation of study treatment. Nineteen (5.1%) patients had a history of psoriasis. Five patients reported an AE of psoriasis during the study (two on 75 mg, three on 150 mg),

Table 1 Summary of efficacy data at week 104 among patients randomised to secukinumab at baseline

	Imputed		Observed	
Efficacy end point	Secukinumab intravenous 150 mg (n=125)	Secukinumab intravenous 75 mg (n=124)	Secukinumab intravenous 150 mg	Secukinumab intravenous 75 mg
ASAS20 response	73.7%	68.0%	69/87 (79.3%)	62/86 (72.1%)
ASAS40 response	55.7%	48.5%	56/87 (64.4%)	46/86 (53.5%)
hsCRP, median change from baseline (min, max) (mg/L)	N/A	N/A	–4.20 (–143.6, 50.0) (n=88)	–2.7 (–97.5, 30.2) (n=87)
ASAS5/6 response	57.9%	52.2%	56/87 (64.4%)	49/86 (57.0%)
BASDAI, mean change from baseline	–2.93 (0.18)	–2.75 (0.18)	–3.41 (2.12) (n=87)	–3.04 (1.81) (n=86)
SF-36 PCS score, mean change from baseline	6.88 (0.68)	6.36 (0.69)	8.06 (8.08) (n=87)	7.41 (6.83) (n=85)
ASQoL score, mean change from baseline	–4.38 (0.45)	–4.34 (0.45)	–4.82 (4.83) (n=86)	–4.58 (4.44) (n=85)
ASAS partial remission	25.6%	19.3%	28/87 (32.2%)	20/86 (23.3%)

Binary variables are reported using multiple imputation (percentage of responders) to account for missing data and as observed data (n/m (%), where n=number of patients with response and m=number of patients with evaluable data). For continuous variables, mean change from baseline is reported as least-square mean change (SE) where mixed-effects model repeated measures analysis was performed and as observed data (SD). Patients received a 10 mg/kg loading dose of secukinumab at baseline and weeks 2 and 4, before receiving indicated dose of secukinumab subcutaneously every four weeks from week 8.
ASAS, Assessment of SpondyloArthritis international Society response criteria; ASQoL, Ankylosing Spondylitis Quality of Life; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; hsCRP, high-sensitivity C-reactive protein; N/A, not available; SF-36 PCS, Short Form-36 physical component summary.

Table 2 Baseline characteristics of the X-ray completer cohort randomised to secukinumab at baseline

Characteristic	Secukinumab intravenous 150 mg (n=86)	Secukinumab intravenous 75 mg (n=82)
Age, mean (SD) years	39.6 (12.0)	42.4 (13.1)
Male gender, n (%)	63 (73.3)	60 (73.2)
Weight, mean (SD) kg	75.2 (16.4)	78.4 (19.3)
Time since AS diagnosis, mean (SD) years	6.6 (7.0)	7.8 (8.9)
HLA-B27 positive, n (%)	64 (74.4)	72 (87.8)
Current smoker, n (%)	25 (29.1)	17 (20.7)
Anti-TNF-naive, n (%)	60 (69.8)	62 (75.6)
Medication use at baseline, n (%)		
Methotrexate	14 (16.3)	12 (14.6)
Sulfasalazine	27 (31.4)	32 (39.0)
Glucocorticoids	13 (15.1)	10 (12.2)
hsCRP, median (min, max), mg/L	7.8 (0.2, 147.7)	9.7 (0.4, 100.0)
Elevated hsCRP >5 mg/L, n (%)	55 (64.0)	50 (61.0)
Total BASDAI, mean (SD)	6.2 (1.6)	6.0 (1.5)
BASFI, mean (SD)	5.6 (2.2)	5.4 (2.0)
BASMI (linear), mean (SD)	3.9 (1.8)	4.2 (1.7)
mSASSS, mean (SD)	9.6 (16.6)	10.8 (16.7)
Syndesmophyte present, n (%)	51 (59.3)	53 (64.6)
Total back pain, mean (SD)	63.9 (17.2)	63.3 (18.6)
Patient's global assessment of disease activity, mean (SD)	64.6 (17.7)	60.5 (18.6)

Data are n (%), mean (SD), or median (minimum–maximum).
AS, ankylosing spondylitis; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; BASMI, Bath Ankylosing Spondylitis Metrology Index; HLA, human leucocyte antigen; hsCRP, high-sensitivity C-reactive protein; mSASSS, modified Stoke Ankylosing Spondylitis Spine Score; TNF, tumour necrosis factor.

three of which were described as worsening or exacerbation of psoriasis. No psoriasis AEs were considered serious or required treatment interruption or discontinuation.

Four secukinumab-treated patients (two in each dose group) had cardiovascular events adjudicated as meeting major adverse cardiac event (MACE) criteria: three myocardial infarctions and one ischaemic stroke (see online supplementary table S5). None of these events led to treatment discontinuation. The incidence of MACE was 0.6 per 100 patient-years of secukinumab exposure.

Four cases of malignant/unspecified tumours were reported (0.6 per 100 patient-years of secukinumab exposure), all before week 52, and have been described previously.[32]

Treatment-emergent anti-secukinumab antibodies were detected through week 104 in two patients in the secukinumab 150 mg group; no neutralising antibodies were detected and neither patient experienced a loss of ASAS20 response.

DISCUSSION

This analysis demonstrates that secukinumab improves the clinical signs and symptoms of AS through 2 years of continued therapy. Secukinumab was effective in both anti-TNF-naive and anti-TNF-IR patients, although absolute response rates were generally higher in anti-TNF-naive patients. In contrast to the anti-TNF-naive subgroup, ASAS20 and ASAS40 response rates were highest with secukinumab intravenous 75 mg in anti-TNF-IR patients. However, these results should be viewed in the context of the relatively small number of anti-TNF-IR patients, particularly at week 104, and the heterogeneity of this subpopulation, which comprised patients who failed anti-TNF treatment for any one of several reasons, including lack of primary or secondary efficacy, or intolerance. Overall, given the lack of therapeutic alternatives, these findings indicate that secukinumab may address an unmet clinical need in

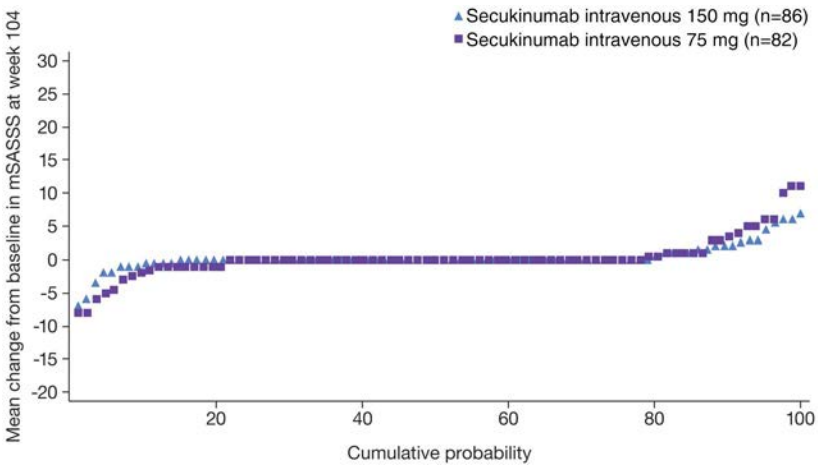

Figure 2 Cumulative probability plot for change from baseline in the modified Stoke Ankylosing Spondylitis Spine Score (mSASSS) at week 104 in X-ray completers randomised at baseline to secukinumab (observed data). X-ray completers are those patients with X-rays at both baseline and at week 104. Patients received a 10 mg/kg loading dose of secukinumab at baseline and weeks 2 and 4, before receiving indicated dose of secukinumab subcutaneously every four weeks from week 8.

anti-TNF-IR patients, as well as providing a high level of efficacy as first-line therapy in anti-TNF-naive patients.

Prevention of structural damage is a long-term treatment goal in AS. The effect of anti-TNFs on radiographic progression in AS is unclear. mSASSS changes of 0.8–0.9 over 2 years have been reported with TNF inhibitors in AS clinical trials compared with 0.9–1.3 in respective matched biologic-naive historical control cohorts from the Outcomes in AS International Study (OASIS).[14–16] Authors concluded that the results do not provide evidence that anti-TNF therapy inhibits radiographic progression over 2 years. More recent studies have suggested that longer-term (approximately 8 years) treatment with an anti-TNF or earlier treatment initiation may be required to demonstrate an effect on radiographic progression.[40 41] In the present study, the mean change in mSASSS through 2 years of secukinumab therapy was 0.30 (SD 2.53) overall, and 0.38–0.52 among patients with known predictors of radiographic progression at baseline, such as syndesmophytes or elevated CRP.[42] Comparisons between these findings and those reported in the aforementioned studies and historical cohorts cannot be made because of differences in study designs and populations. Data from OASIS are from a period when treatment options were limited, and early TNF inhibitor trials are likely to have had more severe disease than those enrolled in MEASURE 1 and other recent placebo-controlled trials. Indeed, patients with evidence of radiographic progression are now more likely to be prescribed an approved TNF inhibitor than enter a clinical trial in which they might be randomised to placebo, and the anti-TNF trials included patients with longer disease duration (~10–11 years vs ~7 years in X-ray completers)[8 14–16] and more severe radiographic disease at baseline (mSASSS ~16–20[13–16] vs ~10) than in our study. Agreement between readers in mSASSS change was modest, with an ICC within the expected range of those reported from other studies.[13–15 43] The Bland-Altman plot indicates that there is inter-reader variability, particularly where changes in mSASSS have occurred. The low overall rate of progression seen with secukinumab requires further exploration in long-term controlled studies before definite conclusions can be reached on whether anti-IL-17A therapy is effective in inhibiting mSASSS progression in patients with AS. Comparing radiographic changes associated with secukinumab with the historical cohorts, matched in terms of baseline disease, is also a possible area of future research.

Secukinumab showed an acceptable safety profile over 622.5 patient-years of exposure, with no new safety signals or unexpected safety findings compared with the first 52 weeks[32] or with the safety profile reported in PsA and psoriasis.[30 31] The incidence of AEs was higher with 150 mg than with 75 mg, driven primarily by non-serious infections. Serious infections were infrequent in both secukinumab groups. *Candida* infections, a known risk with IL-17 inhibitors given the role of IL-17 in mucosal defence,[44] were infrequent, mild and clinically manageable with antifungals. No dose dependence was observed for other safety risks. All four patients who reported MACE events on secukinumab treatment had pre-existing cardiovascular risk factors. The incidence of MACE in our study (0.6 per 100 patient-years) is consistent with reported data in AS.[45]

Spondyloarthritis is often associated with extra-articular manifestations such as uveitis, psoriasis and inflammatory bowel disease.[2] Patients with previous or stable presentation of these conditions could enrol in MEASURE 1. The incidence of extra-articular manifestations reported as AEs in our study was low and consistent with expected rates in AS. IL-17A has been reported to have either a pathogenic or a protective role in Crohn's disease.[46 47] The incidence of Crohn's disease in the present study (0.8 per 100 patient-years of secukinumab exposure) compares with reported rates of 0.2–1.3 per 100 patient-years for TNF inhibitors in the AS population.[48]

Limitations of this analysis include the lack of comparator group beyond week 16, limiting interpretation of long-term findings to a comparison between secukinumab doses and to longitudinal evaluation. Despite the use of accepted statistical methods to account for missing data during the continuation period of the study, there remains a possible bias from the fact that patients who stay on study are those who do well on study treatment. Nevertheless, retention rates were high throughout the study and only 4.4% of patients withdrew because of lack of efficacy during the 2-year period. The slow nature of radiographic disease progression in AS means long follow-up periods are required to show measureable changes. This prohibits the inclusion of a placebo comparator and can introduce confounding effects of concomitant treatment with other drugs that influence radiographic progression.[49] Although blinded to treatment groups and sequence, readers were aware that all patients received secukinumab, possibly introducing an observational bias.

In conclusion, these longitudinal results from MEASURE 1 demonstrate that secukinumab provides similar levels of improvement in the clinical signs and symptoms of AS at 2 years as those previously reported during the short-term placebo-controlled period and provide the first uncontrolled

Table 3 mSASSS in the X-ray completer cohort randomised to secukinumab at baseline

Variable	Secukinumab intravenous 150 mg	Secukinumab intravenous 75 mg	Secukinumab pooled
Overall population			
Patients (n)	86	82	168
Baseline	9.63 (16.63)	10.84 (16.69)	10.22 (16.62)
Change at week 104	0.30 (1.94)	0.31 (3.04)	0.30 (2.53)
Patients with syndesmophytes at baseline			
Patients (n)	51	53	104
Baseline	16.12 (19.09)	16.69 (18.32)	16.41 (18.61)
Change at week 104	0.49 (2.50)	0.45 (3.77)	0.47 (3.20)
Patients without syndesmophytes at baseline			
Patients (n)	35	29	64
Baseline	0.17 (0.56)	0.16 (0.40)	0.16 (0.49)
Change at week 104	0.01 (0.19)	0.03 (0.33)	0.02 (0.26)
Elevated hsCRP			
Patients (n)	55	50	105
Baseline	11.57 (17.69)	14.22 (19.16)	12.83 (18.36)
Change at week 104	0.48 (2.25)	0.46 (3.08)	0.47 (2.66)
Normal hsCRP			
Patients (n)	31	32	63
Baseline	6.18 (14.19)	5.56 (10.04)	5.87 (12.16)
Change at week 104	−0.03 (1.16)	0.06 (3.00)	0.02 (2.27)
Male			
Patients (n)	63	60	123
Baseline	11.91 (18.57)	13.70 (18.44)	12.78 (18.45)
Change at week 104	0.19 (2.01)	0.58 (3.44)	0.38 (2.79)
Female			
Patients (n)	23	22	45
Baseline	3.39 (6.44)	3.05 (5.68)	3.22 (6.02)
Change at week 104	0.59 (1.73)	−0.46 (1.23)	0.08 (1.58)
Smokers (at baseline)			
Patients (n)	25	17	42
Baseline	13.10 (19.45)	10.88 (9.20)	12.20 (16.00)
Change at week 104	−0.18 (1.71)	1.56 (4.00)	0.52 (2.95)
Non-smokers (at baseline)			
Patients (n)	61	65	126
Baseline	8.21 (15.28)	10.83 (18.21)	9.56 (16.84)
Change at week 104	0.49 (2.00)	−0.02 (2.67)	0.23 (2.38)

n indicates number of patients with evaluable paired X-ray data at both baseline and week 104 (X-ray completers). Data shown as mean (SD). mSASSS ranges from 0 to 72, with higher scores indicating greater radiographic damage. Patients received 10 mg/kg secukinumab at baseline and weeks 2 and 4, before receiving indicated dose of secukinumab subcutaneously every four weeks from week 8.
hsCRP, high-sensitivity C-reactive protein; mSASSS, modified Stoke Ankylosing Spondylitis Spine Score.

data on spinal radiographic progression in patients with AS under treatment with an IL-17A inhibitor. Long-term controlled studies are needed to evaluate whether secukinumab inhibits the progression of structural manifestations of AS.

Table 4 Incidence of treatment-emergent adverse events (AEs) during the entire treatment period through to week 104

Variable	Any secukinumab 150 mg (n=181)*	Any secukinumab 75 mg (n=179)*	Any secukinumab pooled (n=360)*
Exposure to study treatment (days)†	621.3 (187.5)	642.0 (180.9)	631.6 (184.3)
Number of patients with event (%)			
Any AE	157 (86.7)	144 (80.4)	301 (83.6)
Serious AE‡	22 (12.2)	24 (13.4)	46 (12.8)
Any AE leading to discontinuation§	17 (9.4)	8 (4.5)	25 (6.9)
Infection or infestation¶	110 (60.8)	100 (55.9)	210 (58.3)
Common AEs (seen in >5% of patients on secukinumab), n (%)			
Nasopharyngitis	44 (24.3)	35 (19.6)	79 (21.9)
Diarrhoea	25 (13.8)	22 (12.3)	47 (13.1)
Headache	22 (12.2)	20 (11.2)	42 (11.7)
Upper respiratory tract infection	17 (9.4)	21 (11.7)	38 (10.6)
Pharyngitis	21 (11.6)	12 (6.7)	33 (9.2)
Dyslipidaemia	14 (7.7)	16 (8.9)	30 (8.3)
Influenza	17 (9.4)	13 (7.3)	30 (8.3)
Oropharyngeal pain	16 (8.8)	13 (7.3)	29 (8.1)
Arthralgia	13 (7.2)	11 (6.1)	24 (6.7)
Back pain	13 (7.2)	7 (3.9)	20 (5.6)
Leucopenia	8 (4.4)	12 (6.7)	20 (5.6)
Cough	10 (5.5)	9 (5.0)	19 (5.3)
Nausea	10 (5.5)	9 (5.0)	19 (5.3)
AEs of special interest, n (exposure-adjusted incidence rate per 100 patient-years)			
Candida infections	2 (0.7)	2 (0.6)	4 (0.7)
Serious infections	3 (1.0)	3 (1.0)	6 (1.0)
Crohn's disease	1 (0.3)	4 (1.3)	5 (0.8)
Major adverse cardiac events (adjudicated)	2 (0.7)	2 (0.6)	4 (0.6)
Malignancy	3 (1.0)	1 (0.3)	4 (0.6)
Neutropenia (preferred term)	9 (3.1)	14 (4.8)	23 (3.9)

*Includes patients randomised to secukinumab at baseline and patients who were randomised to placebo who switched to secukinumab at weeks 16 or 24.
†Reported as mean (SD).
‡Serious AEs also include deaths.
§Up to week 104; an additional two patients discontinued secukinumab due to any AEs after week 104.
¶System organ class category.

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Patient consent Obtained.

Ethics approval The study was conducted in accordance with Good Clinical Practice and the principles of the Declaration of Helsinki.

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OPEN ACCESS

EXTENDED REPORT

Low immunogenicity of tocilizumab in patients with rheumatoid arthritis

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ABSTRACT

Objective Subcutaneous (SC) and intravenous formulations of tocilizumab (TCZ) are available for the treatment of patients with rheumatoid arthritis (RA), based on the efficacy and safety observed in clinical trials. Anti-TCZ antibody development and its impact on safety and efficacy were evaluated in adult patients with RA treated with intravenous TCZ (TCZ-IV) or TCZ-SC as monotherapy or in combination with conventional synthetic disease-modifying antirheumatic drugs (csDMARDs).

Methods Data from 5 TCZ-SC and 8 TCZ-IV phase III clinical trials and 1 TCZ-IV clinical pharmacology safety study (>50 000 samples) were pooled to assess the immunogenicity profile of TCZ-SC and TCZ-IV (8974 total patients). The analysis included antidrug antibody (ADA) measurement following TCZ-SC or TCZ-IV treatment as monotherapy or in combination with csDMARDs, after dosing interruptions or in TCZ-washout samples, and the correlation of ADAs with clinical response, adverse events or pharmacokinetics (PK).

Results The proportion of patients who developed ADAs following TCZ-SC or TCZ-IV treatment was 1.5% and 1.2%, respectively. ADA development was also comparable between patients who received TCZ monotherapy and those who received concomitant csDMARDs (0.7–2.0%). ADA development did not correlate with PK or safety events, including anaphylaxis, hypersensitivity or injection-site reactions, and no patients who developed ADAs had loss of efficacy.

Conclusions The immunogenicity risk of TCZ-SC and TCZ-IV treatment was low, either as monotherapy or in combination with csDMARDs. Anti-TCZ antibodies developed among the small proportion of patients had no evident impact on PK, efficacy or safety.

INTRODUCTION

For patients with rheumatoid arthritis (RA) who do not respond to or are intolerant of conventional synthetic disease-modifying antirheumatic drugs (csDMARDs), biologic DMARDs (bDMARDs) are recommended.[1] Often, a bDMARD and ≥1 csDMARD are used in combination, but bDMARDs can also be used as monotherapy. The currently approved bDMARDs include antitumour necrosis factor-α agents (aTNFs), anti-interleukin 6 receptor (IL-6R) therapy, anti-CD20 B cell targeted therapy and T cell co-stimulation inhibition. One safety concern of bDMARDs is the development of antidrug antibodies (ADAs).[2] Multiple factors may contribute to ADA development, including structure and idiotype,[3] route of administration,[3] mechanism of action,[4] concomitant csDMARD use,[5 6] disease activity,[7] genetic status,[3 8] patient immunocompetence,[3] treatment duration,[3] the disease itself[9] and drug dose/frequency.[8] ADAs can lead to loss of efficacy[10] and/or immune-mediated adverse reactions, including IgE-mediated or non-IgE-mediated events.[11]

Tocilizumab (TCZ) is a humanised monoclonal antibody (mAb) of the IgG_1 subclass that blocks IL-6 binding to the membrane-bound and soluble IL-6R, consequently inhibiting IL-6 activity. TCZ is approved for adult RA (as intravenous or subcutaneous (SC) formulations) and as intravenous for systemic-course and polyarticular-course juvenile idiopathic arthritis and Castleman disease (Japan only).[12] TCZ has demonstrated efficacy and a well characterised safety profile as monotherapy or in combination with csDMARDs.[13–19]

This study addresses important clinical and scientific questions: Is a therapeutic antibody by SC administration more immunogenic compared with intravenous administration? Is the immunogenic risk of TCZ monotherapy similar to that of co-therapy with methotrexate (MTX)? Here, the immunogenicity of TCZ is assessed in different clinical settings —ADA development following TCZ administration as SC or intravenous formulations as monotherapy or in combination with csDMARDs, after dosing interruptions and in TCZ-washout samples—as well as its correlation with adverse events (AEs), clinical response and pharmacokinetics (PK). Data were derived from five TCZ-SC and nine intravenous TCZ (TCZ-IV) RA trials plus their long-term extensions: SUMMACTA,[20 21] BREVACTA,[22 23] the TCZ-SC long-term extension rollover study of US patients from BREVACTA and SUMMACTA,[24] MUSASHI (Multi-Center Double-Blind Study of Tocilizumab Subcutaneous Injection in Patients Having Rheumatoid Arthritis to Verify Noninferiority Against Intravenous Infusion),[25 26] FUNCTION,[27] AMBITION (Actemra vs Methotrexate Double-Blind Investigative Trial in Monotherapy),[15] TOWARD (Tocilizumab in Combination With Traditional DMARD Therapy),[17] OPTION (Tocilizumab Pivotal Trial in Methotrexate Inadequate Responders),[13] LITHE (Tocilizumab Safety and the Prevention of Structural

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Joint Damage),[14] RADIATE (Research on Actemra Determining Efficacy After Anti-TNF Failures),[16] TOZURA global umbrella study (interim analysis) and a clinical pharmacology study.[28]

PATIENTS AND METHODS

Study designs

The study designs of the TCZ trials are summarised (see online supplementary table S1).[13–17 20 22 24 25 27]

Sampling

Blood samples for ADA detection and PK analysis were collected at baseline and regularly predose (TCZ trough level) throughout the studies and at the study completion or early withdrawal visit. Furthermore, patients who withdrew due to hypersensitivity reactions in five of the studies had additional samples for ADAs collected at the time of the event and at least 4–8 weeks after the last treatment.[20 22 24 27] To minimise potential TCZ interference in the immunogenicity assay, in the TCZ-IV versus TCZ-SC study, TCZ-washout samples (at least 4 weeks or 8 weeks after the last treatment, or predose samples after treatment interruptions during the study) were evaluated.[20]

Immunogenicity assessment strategy and assays

In all studies, consistent assay methodology was applied,[29] and a sequential testing strategy was adopted (figure 1). All samples were initially screened for antibodies, and positive samples were analysed by a confirmation assay for specificity. Characterisation of any samples with confirmed anti-TCZ antibodies was performed to detect neutralising potential and IgE isotype. In three studies, an IgE assay was also conducted in patients who withdrew because of hypersensitivity reactions, regardless of their confirmation assay status.[20 22 24 27] The IgE assay was not performed in the TCZ-IV studies consistently; therefore, results were not available. Clinical AEs and efficacy measures were evaluated in association with ADA development.

The screening assay employed a bridging ELISA and used biotinylated TCZ from different labelling preparations immobilised on streptavidin-coated microtitre plates. Anti-TCZ antibodies form a complex of TCZ-biotin/anti-TCZ antibody/TCZ-digoxigenin, captured by immobilised streptavidin and then detected by an antidigoxigenin-peroxidase antibody (figure 2A). An assay cut point was determined from serum samples from patients with RA, containing various levels of rheumatoid factor in order to minimise its interference. The confirmation assay was conducted the same as the screening assay except the preincubation of test or control samples with digoxigenylated TCZ was performed in parallel in the presence and absence of excess free TCZ, which competes with digoxigenylated TCZ and biotinylated TCZ for binding to anti-TCZ antibodies (figure 2B).

To detect neutralising potential of ADAs, an inhibition ELISA was performed for all studies except the Japanese study (figure 2C). The neutralising assay evaluates whether anti-TCZ antibodies competitively interfere with the binding of TCZ to immobilised soluble IL-6R. Blocking the binding of TCZ to IL-6R, resulting in a decrease in assay signal, is indicative that anti-TCZ antibodies can neutralise the therapeutic effect of TCZ. In the Japanese study, an antigen-binding fragment (Fab) assay in a bridging ELISA format that can detect anti-TCZ antibodies that bind to the Fab fragment of TCZ was applied as the neutralising assay.[25 26] IgE isotype antibodies were detected using the ImmunoCAP assay system (Quest Diagnostics) (figure 2D).[29] Anti-TCZ-IgE antibodies captured by immobilised TCZ were detected by an antihuman IgE-specific antibody.

PK assay

TCZ serum concentrations were determined by ELISA. The lower limit of quantitation was 100 ng/mL. The impact of ADAs on PK was formally evaluated in three intravenous studies and two SC studies.[13 16 17 20 22]

Analyses

In all studies except the Japanese study, hypersensitivity events were conservatively defined as all AEs (excluding injection-site reactions (ISRs)) that occurred during or within 24 hours of an infusion or injection and were not judged unrelated to

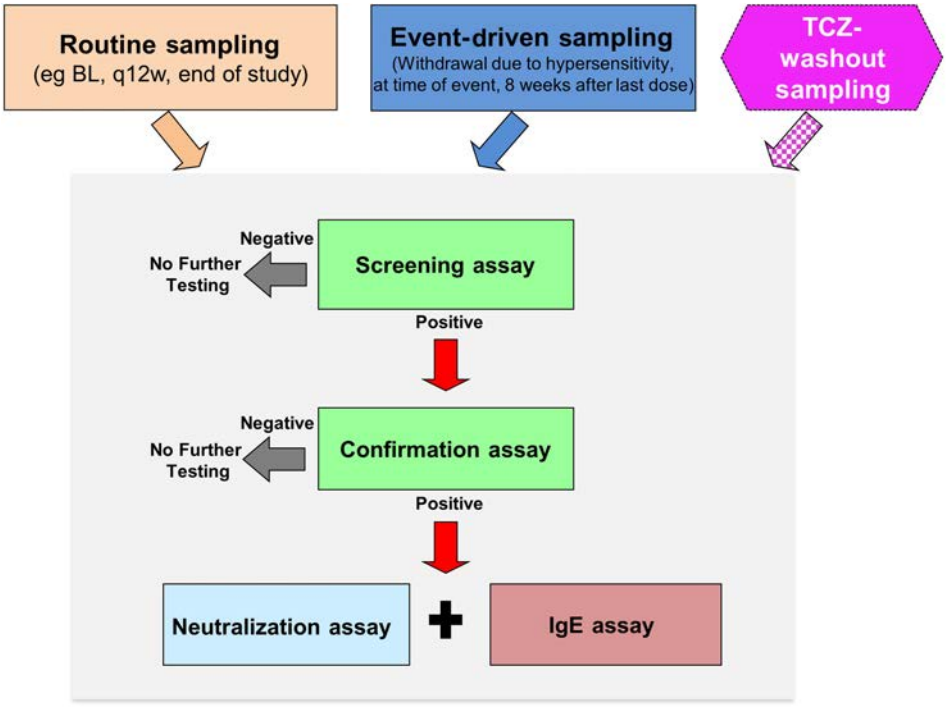

Figure 1 Tocilizumab (TCZ) immunogenicity assessment strategy*. *Blood samples were taken at baseline (BL) and regularly prior to dosing throughout the studies. q12w, every 12 weeks.

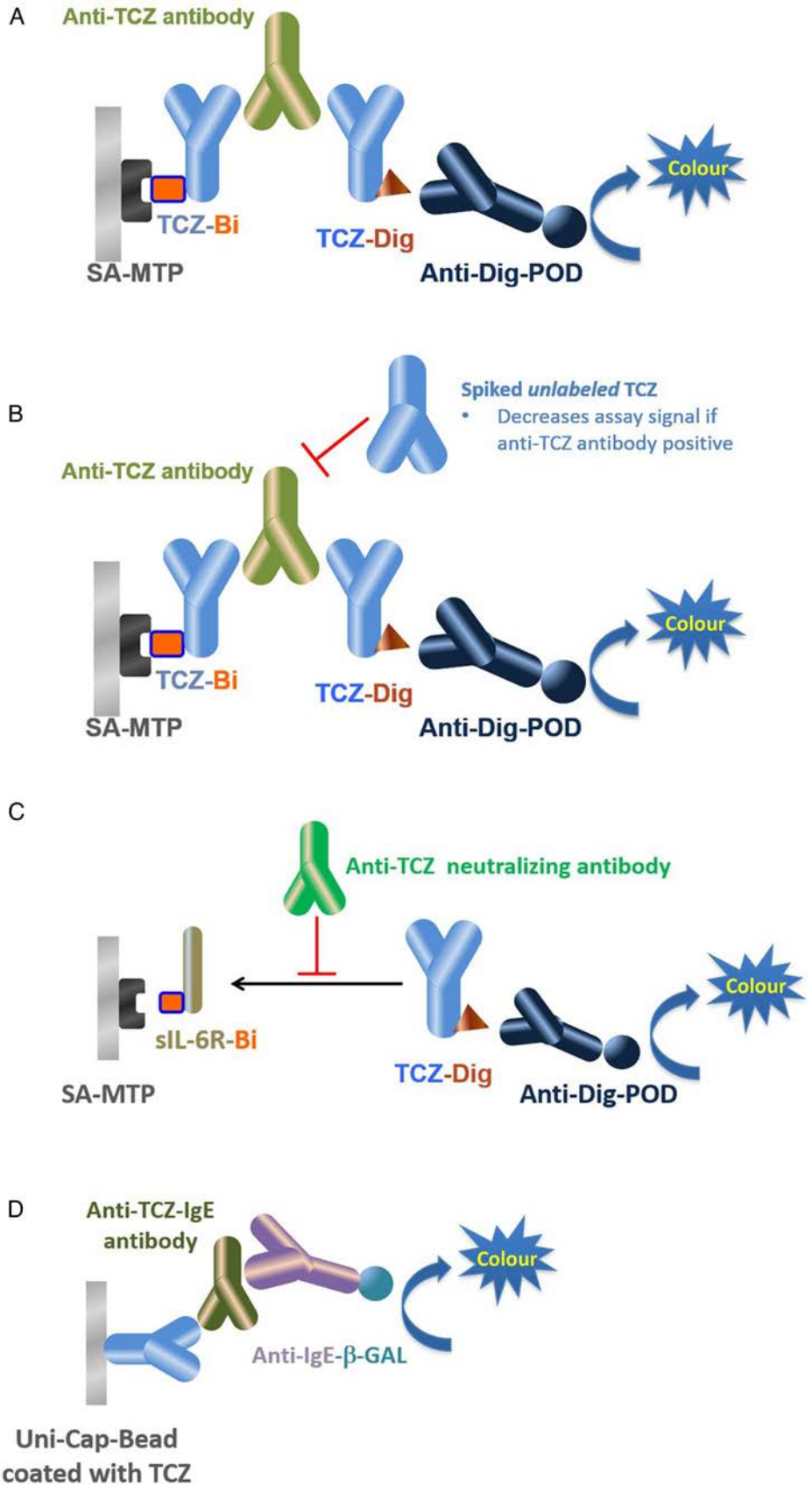

Figure 2 Anti-tocilizumab (anti-TCZ) antibody assay. (A) The screening assay employed bridging ELISAs and used biotinylated TCZ from different labelling preparations immobilised on streptavidin-coated microtitre plates. Bi, biotin; Dig, digoxigenin; SA-MTP, streptavidin-coated microtitre plate; POD, peroxidase. (B) For samples positive from the screening assay, an additional competitive displacement step was used for the confirmation assay, where unlabelled TCZ inhibited the formation of TCZ-Bi/anti-TCZ antibody/TCZ-Dig complexes. (C) An inhibition ELISA was adopted to detect the neutralising potential of anti-TCZ antibodies (whether anti-TCZ antibodies competitively interfere with the binding of TCZ to immobilised soluble IL-6 receptor (sIL-6R)). (D) IgE isotype antibodies were detected using the ImmunoCAP assay system (Quest Diagnostics).

treatment by the investigator; those events may or may not be consistent with hypersensitivity clinically. Anaphylactic reactions were events that occurred during or within 24 hours of an infusion or injection and met Sampson criteria.[30] Serious hypersensitivity events were hypersensitivity events that were also reported as serious AEs, and clinically significant hypersensitivity events were hypersensitivity events that led to study withdrawal. ISRs were AEs occurring at the local injection

sites following SC administration. In the Japanese study, hypersensitivity events were defined as AEs (excluding ISRs) that occurred during or within 24 hours of an infusion or injection and were also judged to be a hypersensitivity event by the clinical expert.[25 26]

Assay results were also evaluated for patients who met the criteria for loss of efficacy, defined as those who withdrew from the study prematurely due to insufficient therapeutic response after experiencing an American College of Rheumatology criteria for 50% improvement or a European League Against Rheumatism good response.

RESULTS

Patient population

The TCZ-SC all-exposure population consisted of 3099 patients from the clinical trials, including 616 patients who received TCZ-SC as monotherapy and 2483 who received TCZ-SC in combination with csDMARDs (figure 3A). TCZ-SC treatment was administered for up to 3.5 years. The TCZ-IV all-exposure population consisted of 5875 patients, with 753 patients who received TCZ-IV as monotherapy and 5122 who received TCZ-IV in combination with csDMARDs (figure 3B). TCZ-IV treatment was administered for up to 5 years.

Incidence of ADA development and effect on safety and efficacy following TCZ-SC or TCZ-IV all-exposure

Of the patients who received TCZ-SC or TCZ-IV and were screened for ADAs (99.8% and 98.8%, respectively), the proportion of patients who developed ADAs following either TCZ treatment was low and comparable (1.5% (47 patients) and 1.2% (69 patients), respectively; table 1). Among the patients who developed ADAs, 40 (85.1%) who received TCZ-SC and 54 (78.3%) who received TCZ-IV were also positive for the neutralising assay. Of the patients who were screened for ADAs, 9 (0.3%) who received TCZ-SC developed IgE antibodies; results for IgE antibodies were not available for TCZ-IV. In all studies, most detected ADAs were transient and did not occur at all time points (see online supplementary table S2).

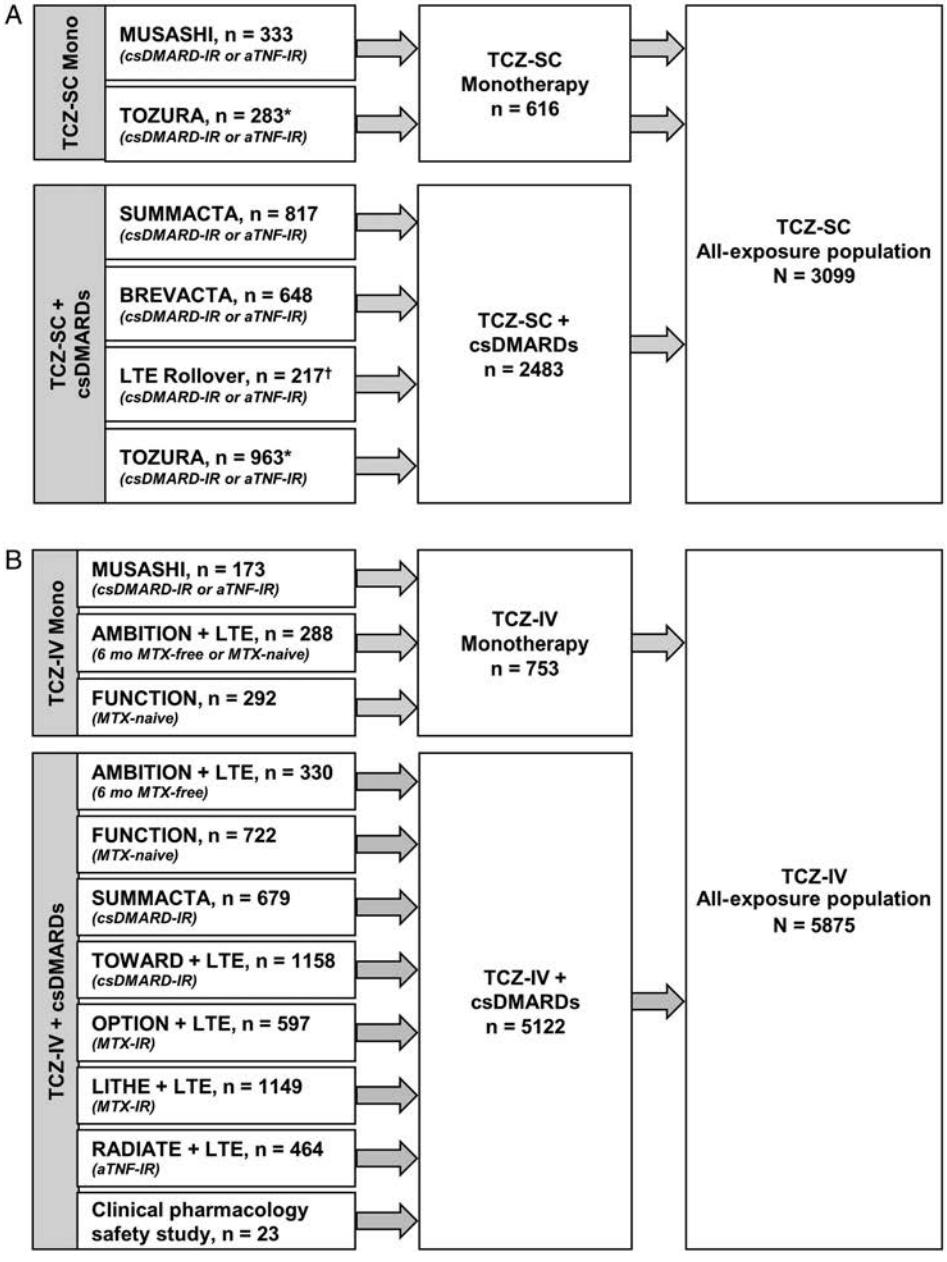

Figure 3 Patient disposition. Immunogenicity was assessed from the clinical trials following treatment in patients with rheumatoid arthritis. (A) Treatment with subcutaneous tocilizumab (TCZ-SC). Mono, monotherapy; csDMARD, conventional synthetic disease-modifying antirheumatic drug; IR. inadequate responder; aTNF, antitumour necrosis factor-α agent; LTE, long-term extension. (B) Treatment with intravenous tocilizumab (TCZ-IV). MTX, methotrexate.
*TOZURA is a multinational, open-label, single-arm global umbrella study comprising 11 protocols from different countries/regions.
†There were a total of 217 patients who received TCZ-SC treatment in the long-term extension (LTE) rollover study, including 55 patients who switched from TCZ-IV (in SUMMACTA) to TCZ-SC for the LTE rollover period.

Among the all-exposure safety populations, no patients who received TCZ-SC experienced anaphylaxis, whereas 10 patients (0.2%) who received TCZ-IV had anaphylaxis (table 1). Clinically significant hypersensitivity (leading to study withdrawal) occurred in 31 patients (1.0%) who received TCZ-SC and in 91 patients (1.5%) who received TCZ-IV; 10 patients (0.3%) in the TCZ-SC group and 51 (0.9%) in the TCZ-IV group had serious hypersensitivity (hypersensitivity events meeting seriousness criteria). Of the 47 patients who received TCZ-SC and developed ADAs, 1 (2.1%) experienced clinically significant hypersensitivity, but none had serious hypersensitivity. Of the 69 patients who received TCZ-IV and developed ADAs, 5 (7.2%) experienced anaphylaxis, 10 (14.5%) had clinically significant hypersensitivity, and 6 (8.7%) had serious hypersensitivity, including the 5 patients with anaphylaxis. Among the patients who received TCZ-SC, a total of 310 (10.0%) experienced ISRs. Of the 47 patients who received TCZ-SC and developed ADAs, 4 (8.5%) experienced ISRs; all events resolved without sequelae.

Among all patients who developed ADAs with neutralising potential following TCZ treatment, none experienced loss of efficacy, regardless of formulation (table 1).

Incidence of ADA development and effect on safety and efficacy following TCZ monotherapy or in combination with csDMARDs

The overall incidence of ADA development was low in the 1360 patients treated with TCZ monotherapy (intravenous: 0.7%; SC: 2.0%) and the 7540 patients treated with TCZ+csDMARDs (intravenous: 1.3%; SC: 1.4%), regardless of formulation (table 2).

Among the safety population, the incidences of hypersensitivity events were consistent between patients who received TCZ monotherapy or TCZ + csDMARDs (table 2). No patients experienced anaphylaxis with TCZ-SC compared with 1 patient (0.1%) who received TCZ-IV monotherapy and 9 patients (0.2%) who received TCZ-IV+csDMARDs. Clinically significant hypersensitivity occurred in 6 patients (1.0%) who received TCZ-SC monotherapy and in 25 patients (1.0%) who received TCZ-SC+csDMARDs. Serious hypersensitivity occurred in one patient (0.2%) in the TCZ-SC monotherapy group and in nine patients (0.4%) in the TCZ-SC+csDMARDs group. Twelve patients (1.6%) who received TCZ-IV monotherapy and 79 (1.5%) who received TCZ-IV+csDMARDs had clinically significant hypersensitivity events. Nine patients (1.2%) who received TCZ-IV monotherapy and 42 (0.8%) who received TCZ-IV +csDMARDs had serious hypersensitivity events.

There was no clear impact of ADA development on safety, regardless of TCZ administration as monotherapy or in combination with csDMARDs (table 2). Of the five patients who received TCZ-IV monotherapy and developed ADAs, one had clinically significant hypersensitivity and none had serious hypersensitivity or anaphylaxis. Of the 64 patients who received TCZ-IV+csDMARDs and developed ADAs, 9 experienced clinically significant hypersensitivity and 6 had serious hypersensitivity events, including the 5 anaphylaxis cases. Of the 12 patients who received TCZ-SC monotherapy and developed ADAs, 1 had clinically significant hypersensitivity and none had serious hypersensitivity or anaphylaxis. Of the 35 patients who received TCZ-SC+csDMARDs and developed ADAs, none experienced anaphylaxis, serious hypersensitivity or clinically significant hypersensitivity.

ISRs were reported in 81 patients (13.1%) who received TCZ-SC monotherapy compared with 229 (9.2%) who received TCZ-SC+csDMARDs (table 2). One patient (0.2%) who received TCZ-SC monotherapy and developed ADAs had an ISR; three (0.1%) of the patients who received TCZ-SC +csDMARDs and developed ADAs had ISRs.

Among all patients who developed ADAs with neutralising potential following TCZ treatment, none experienced loss of efficacy, regardless of whether it was administered as monotherapy or in combination with csDMARDs (table 2).

Table 1 Immunogenicity rates and safety and efficacy in patients who developed anti-TCZ antibodies following TCZ-SC or TCZ-IV treatment

	TCZ-SC 162 mg qw or q2w all-exposure (n=3099)	TCZ-IV 4 mg/kg or 8 mg/kg q4w all-exposure (n=5875)
Anaphylaxis, n (%)*	0	10 (0.2)
Clinically significant hypersensitivity (leading to withdrawal), n (%)†	31 (1.0)	91 (1.5)
Serious hypersensitivity (reported as SAE), n (%)‡	10 (0.3)	51 (0.9)
Injection-site reactions, n (%)	310 (10.0)	N/A
Total patients screened for ADAs, n (%)	3094 (99.8)	5806 (98.8)
Total patients who developed ADAs, n (%)§	47 (1.5)	69 (1.2)
Positive neutralisation assay, n (%)§¶	40 (1.3)	54 (0.9)
Positive IgE assay, n (%)§	9 (0.3)	N/A
Anaphylaxis, n (%)*§	0	5 (0.1)
Clinically significant hypersensitivity (leading to withdrawal), n (%)†§	1 (0.03)	10 (0.2)
Serious hypersensitivity (reported as SAE), n (%)‡§	0	6 (0.1)
Injection-site reactions, n (%)§	4 (0.1)	N/A
Loss of efficacy, n (%)§**	0	0

*Anaphylactic reactions were events that occurred during or within 24 hours of an infusion or injection and met Sampson criteria.
†Clinically significant hypersensitivity events were defined as any events that occurred during or within 24 hours of an infusion or injection and led to withdrawal from treatment.
‡Serious hypersensitivity events were defined as any events that occurred during or within 24 hours of an infusion or injection and were reported as SAEs.
§Denominator is total patients screened for ADAs.
¶The Fab assay was applied in the MUSASHI study to measure neutralisation potential.
**Loss of efficacy was defined as patients who withdrew from the study prematurely due to insufficient therapeutic response after experiencing an American College of Rheumatology criteria for 50% improvement (ACR50) or European League Against Rheumatism good response.
ADA, antidrug antibody; N/A, not available; q2w, every other week; q4w, every 4 weeks; qw, every week; SAE, serious adverse event; TCZ, tocilizumab; TCZ-IV, intravenous TCZ; TCZ-SC, subcutaneous TCZ.

Table 2 Safety, immunogenicity and effect of ADAs on safety and efficacy following TCZ as monotherapy or in combination with conventional synthetic disease-modifying antirheumatic drugs (csDMARDs)

	TCZ-SC mono 162 mg qw or q2w (n=616)	TCZ-SC+csDMARDs 162 mg qw or q2w (n=2483)	TCZ-IV mono 4 mg/kg or 8 mg/kg q4w (n=753)	TCZ-IV+csDMARDs 4 mg/kg or 8 mg/kg q4w (n=5122)
Anaphylaxis, n (%)*	0	0	1 (0.1)	9 (0.2)
Clinically significant hypersensitivity (leading to withdrawal), n (%)†	6 (1.0)	25 (1.0)	12 (1.6)	79 (1.5)
Serious hypersensitivity (reported as SAE), n (%)‡	1 (0.2)	9 (0.4)	9 (1.2)	42 (0.8)
Injection-site reactions, n (%)	81 (13.1)	229 (9.2)	N/A	N/A
Total patients screened for ADAs, n (%)	615 (99.8)	2479 (99.8)	745 (98.9)	5061 (98.8)
Total patients who developed ADAs, n (%)§	12 (2.0)	35 (1.4)	5 (0.7)	64 (1.3)
Positive neutralisation assay, n (%)§¶	7 (1.1)	33 (1.3)	4 (0.5)	50 (1.0)
Positive IgE assay, n (%)§	3 (0.5)	6 (0.2)	N/A	N/A
Anaphylaxis, n (%)*§	0	0	0	5 (0.1)
Clinically significant hypersensitivity (leading to withdrawal), n (%)†§	1 (0.2)	0	1 (0.1)	9 (0.2)
Serious hypersensitivity (reported as SAE), n (%)‡§	0	0	0	6 (0.1)
Injection-site reactions, n (%)§	1 (0.2)	3 (0.1)	N/A	N/A
Loss of efficacy, n (%)§**	0	0	0	0

*Anaphylactic reactions were events that occurred during or within 24 hours of an infusion or injection and met Sampson criteria.
†Clinically significant hypersensitivity events were defined as any events that occurred during or within 24 hours of an infusion or injection and led to withdrawal from treatment.
‡Serious hypersensitivity events were defined as any events that occurred during or within 24 hours of an infusion or injection and were reported as SAEs.
§Denominator is total patients screened for ADAs.
¶The Fab assay was applied in the MUSASHI study to measure neutralisation potential.
**Loss of efficacy was defined as patients who withdrew from the study prematurely due to insufficient therapeutic response after experiencing an American College of Rheumatology criteria for 50% improvement (ACR50) or European League Against Rheumatism good response.
ADA, antidrug antibody; N/A, not available; q2w, every other week; q4w, every 4 weeks; qw, every week; SAE, serious adverse event; TCZ, tocilizumab; TCZ-IV, intravenous TCZ; TCZ-SC, subcutaneous TCZ.

TCZ-washout samples

To minimise the potential TCZ interference in the immunogenicity assay, additional samples for ADA measurements were obtained from the TCZ-SC versus TCZ-IV study[20] at the follow-up visits after treatment completion or after dosing interruption. In total, 928 samples were collected from 879 patients (table 3). Among them, 549 samples (59.2%) from 503 patients were TCZ-free (TCZ serum levels below the limit of quantitation) and 239 samples (25.8%) from 238 patients had low TCZ concentration (<10 µg/mL). Of the 503 patients who provided TCZ-free samples, which allows for TCZ interference in the immunogenicity assay to be excluded, only one patient (0.2%) was positive for ADAs. Another two samples from two patients who were positive for ADAs had TCZ concentrations of 0.2 µg/mL and 18.1 µg/mL. All three patients who developed ADAs did not experience hypersensitivity reactions or ISRs and did not withdraw due to insufficient therapeutic response or meet the criteria for loss of efficacy. None of the three patients who were determined as ADA-positive in washout samples were positive at the regular sampling time points.

Table 3 TCZ-washout samples by TCZ concentration (SUMMACTA)

Total: 928 total samples from 879 patients	TCZ BLQ	TCZ <10 µg/mL	TCZ ≥10 µg/mL
Washout samples, n (%)*	549 (59.2)	239 (25.8)	140 (15.1)
Patients, n	503	238	138
Total patients who developed ADAs, n (%)†	1 (0.2)	1 (0.4)	1 (0.7)
Positive neutralisation assay†	0	0	1 (0.7)
Positive IgE assay	0	0	0

Note: Multiple samples (with different TCZ concentrations) could be provided by a single patient.
*Denominator is total sample number.
†Denominator is total number of patients who provided washout samples.
ADA, antidrug antibody; BLQ, below the lower limit of quantitation (TCZ concentration, 100 ng/mL); TCZ, tocilizumab.

Immunogenicity in patients who missed doses

ADA development after dose interruption was analysed in three TCZ-SC studies. In the TCZ-SC versus TCZ-IV study,[31] 179 patients from the TCZ-SC once-weekly (qw) group and 40 patients from the TCZ-IV-switch-to-TCZ-SC group missed ≥3 consecutive TCZ-SC qw injections, and 241 patients from the TCZ-IV every-4-weeks and TCZ-SC-switch-to-TCZ-IV groups missed ≥1 TCZ-IV infusion during the study; among these patients, two in the TCZ-SC arm and two in the TCZ-IV arm had negative screening assay results before the first missed dose and then were positive for confirmation and neutralising assays after dosing was resumed. In the TCZ-SC versus placebo study,[31] 188 patients in the TCZ-SC every-other-week group and 48 patients in the placebo-switch-to-TCZ-SC group missed ≥1 dose during the treatment period and had negative ADA assays before the missed injection. One patient in the placebo-switch-to-TCZ-SC arm was positive for ADAs by the confirmation and neutralising assays after dosing was resumed. In the Japanese study,[25 26] 247 patients in the safety population who received TCZ-SC had an injection interval of >21 days between doses and were negative for ADAs prior to the dosing interval. Among them, one patient in the TCZ-SC arm developed ADAs after reinitiating TCZ treatment. For all TCZ-SC studies, no impact of ADAs on efficacy or safety was observed in patients who developed ADAs after dose interruption.

Pharmacokinetics

There was no obvious trend of reduced serum TCZ levels in the patients who tested positive for ADAs, including those with neutralising potential. A graphical analysis of apparent clearance estimated by population PK analysis for patients with positive

ADA compared with patients with negative ADA status showed no differences in intravenous studies (see online supplementary figure S1) or SC studies (see online supplementary figure S2). Moreover, no correlation was observed between relative ADA concentration and TCZ values among ADA-positive patients in the intravenous versus SC study (see online supplementary figure S3).

In the TCZ-IV monotherapy versus TCZ-IV+MTX study in patients with early RA, no overall trends of decreasing concentrations were noted for up to 2 years of treatment[27] (in preparation/to be submitted). Similarly, in the TCZ-SC+csDMARDs versus TCZ-IV+csDMARDs study, once steady state was reached, mean TCZ concentrations in patients from both groups remained stable up to week 97.[21]

DISCUSSION

Our pooled results from 8974 patients treated with TCZ indicated that the incidence of ADA development was low, regardless of intravenous or SC formulation and whether it was administered as monotherapy or in combination with csDMARDs. In patients who did develop ADAs, ADAs were mostly transient and no correlation to PK, safety events or loss of efficacy was observed. The precise mechanism of the observed low immunogenicity in patients treated with TCZ has not been fully elucidated; the immunogenic potential of a biologic treatment is affected by several factors, including molecule-related factors (eg, mechanisms of action, molecular structure and manufacturing process) and patient characteristics. ADA incidence is also dependent on the assay itself (eg, assay sensitivity, specificity and methodology).

Immunogenicity assays are challenged and complicated by drug interference, and the observed low incidence of ADA might be a reflection of the assay used. To minimise TCZ interference, TCZ-washout samples were collected and evaluated in the TCZ-SC versus TCZ-IV study.[20] Among the 503 patients who provided TCZ-free samples, the proportion who developed ADAs was low (0.2%) across treatment arms, confirming a low incidence of ADA development when drug interference is ruled out. Moreover, the observed low incidence of ADA development is consistent with three independently published studies that examined the immunogenicity of TCZ using commercially available immunogenicity assays; in those studies, 0% to 3.3% of patients treated with TCZ developed ADAs.[32–34]

One possible mechanism of the observed low immunogenicity of TCZ might be related to the downregulation of B cell activities due to the blocking of IL-6 signalling (a different mechanism of action from that of aTNFs). Our findings and a recent study[33] indicate no increased risk of ADA development and no clear impact on TCZ trough level in either TCZ monotherapy or combination therapy settings[21 27] (in preparation/to be submitted). Consistently, similar efficacy has been observed with TCZ monotherapy compared with TCZ in combination with csDMARDs (either intravenous or SC).[35–38] In contrast, it has been reported that with two aTNFs (adalimumab and infliximab), concomitant administration of MTX suppresses immunogenicity and maximises efficacy.[7 39 40] Development of ADAs against adalimumab and infliximab may correlate with the disappearance of drug from the blood and may decrease efficacy by neutralising the drug or by creating immune complexes.[10 41] In this study, patients who were positive for neutralising assay did not experience a loss of efficacy; it is possible that while the neutralising antibodies were able to block TCZ in vitro, they may not function as such in vivo (eg, are not at sufficient concentration and/or affinity) to affect TCZ levels or efficacy. It is unclear why in three patients, ADA became present after drug washout, and the release of the inhibition of B cell activity after TCZ washout leading to ADA development might be a possible explanation; however, most of the detected anti-TCZ antibodies were transient in this study.

Other possible factors contributing to low immunogenicity might be molecule-related factors, including mAb structure (eg, a specific molecular structure with an idiotype of low immunogenic potential) and manufacturing processes. In general, it is not clear whether a humanised mAb treatment is more immunogenic than a fully human mAb. ADA development has been reported for fully human mAbs (eg, adalimumab and golimumab).[42] ADAs against the fully human adalimumab induced neutralising responses that varied by disease and therapy (5–89%), and ADAs correlate with a lack of efficacy in some adalimumab-treated patients.[7 41 43]

To our knowledge, this study, including data from >8900 patients, is the most robust and comprehensive clinical trial-based assessment addressing immunogenicity compared with published data for a biologic RA treatment. In the small proportion of patients who developed ADAs following administration of TCZ-SC or TCZ-IV, no clear correlation of ADA development to PK, clinical response or AEs was observed. Further, administration of TCZ as monotherapy did not increase the risk of immunogenicity and had no impact on the TCZ trough level. However, the limitation due to the low number of ADA-positive patients is acknowledged, especially between subgroups such as TCZ monotherapy versus TCZ in combination with MTX. Overall, our data suggest that routine ADA testing is unnecessary for the clinical use of TCZ in treating adult RA.

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EXTENDED REPORT

Presence of multiple spondyloarthritis (SpA) features is important but not sufficient for a diagnosis of axial spondyloarthritis: data from the SPondyloArthritis Caught Early (SPACE) cohort

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ABSTRACT

Objectives Concerns have been raised about overdiagnosis of axial spondyloarthritis (axSpA). We investigated whether patients with chronic back pain (CBP) of short duration and multiple SpA features are always diagnosed with axSpA by the rheumatologist, and to what extent fulfilment of the Assessment of SpondyloArthritis International Society (ASAS) axSpA criteria is associated with an axSpA diagnosis.

Methods Baseline data from 500 patients from the SPondyloArthritis Caught Early cohort which includes patients with CBP (≥3 months, ≤2 years, onset <45 years) were analysed. All patients underwent full diagnostic workup including MRI of the sacroiliac joints (MRI-SI) and radiograph of sacroiliac joints (X-SI). For each patient, the total number of SpA features excluding sacroiliac imaging and human leucocyte antigen B27 (HLA-B27) status was calculated.

Results Before sacroiliac imaging and HLA-B27 testing, 32% of patients had ≤1 SpA feature, 29% had 2 SpA features, 16% had 3 SpA features and 24% had ≥4 SpA features. A diagnosis of axSpA was made in 250 (50%) of the patients: 24% with ≤1 SpA feature, 43% with 2 SpA features, 62% with 3 SpA features and 85% with ≥4 SpA features. Of the 230 patients with a positive ASAS classification 40 (17.4%) did not have a diagnosis of axSpA. HLA-B27 positivity (OR 5.6; 95% CI 3.7 to 8.3) and any (MRI-SI and/or X-SI) positive imaging (OR 34.3; 95% CI 17.3 to 67.7) were strong determinants of an axSpA diagnosis.

Conclusions In this cohort of patients with CBP, neither the presence of numerous SpA features nor fulfilment of the ASAS classification criteria did automatically lead to a diagnosis axSpA. Positive imaging was considered particularly important in making a diagnosis of axSpA.

INTRODUCTION

Axial spondyloarthritis (axSpA) has a heterogeneous clinical presentation and does not have a single pathognomonic feature that distinguishes the disease from other conditions with similar symptoms.[1 2] Therefore, it is a challenge to identify axSpA early in patients with chronic back pain (CBP). In daily rheumatological practice, a diagnosis of axSpA is generally made in patients with CBP on the basis of a combination of symptoms from medical history, physical examination, laboratory investigations and findings on imaging.[3 4]

In 2009 the Assessment of SpondyloArthritis International Society (ASAS) developed classification criteria for axSpA. The criteria combine information from several sources such as medical history, physical examination, laboratory testing and imaging.[5] In a secondary or tertiary care setting the fulfilment of the ASAS criteria is strongly associated with a clinical diagnosis of axSpA at the group level, but the criteria cannot be used for diagnosing axSpA in individual patients.[6 7] Classification criteria can only be applied in patients in whom a diagnosis of axSpA has been established (not vice versa).[8–10] The recognition of axSpA therefore requires the physician's knowledge about SpA, as well as expertise in aggregating information obtained during the diagnostic workup and a differential diagnosis.

In order to assist physicians in the diagnosis of axSpA the ASAS modified Berlin algorithm has been developed, which can be applied in patients with CBP with age of onset <45 years (figure 1). As a first step the algorithm advises a radiograph of the sacroiliac joints (X-SI) in all patients. According to the algorithm patients with CBP with indisputable radiographic sacroiliitis may be readily diagnosed with axSpA. Patients without clear sacroiliitis on radiographs are subsequently stratified according to the number of SpA features they have after patient history, physical examination and measuring C reactive protein (CRP) and erythrocyte sedimentation rate (ESR). An important feature of the algorithm is that it allows a diagnosis of axSpA in patients with ≥ four SpA features without further imaging (MRI of the sacroiliac joints (MRI-SI)) or human leucocyte antigen B27 (HLA-B27) testing. Moreover, HLA-B27 positive patients with normal radiographs and two or three SpA features may also be diagnosed with axSpA without performing MRI-SI. Van den Berg *et al*[11] have already shown that an axSpA diagnosis according to the modified Berlin algorithm is not necessarily the same as an expert's (ie, rheumatologist's) clinical diagnosis, so false-positive and false-negative diagnoses may occur if the algorithm is followed blindly. Therefore, it should be stressed again that the ASAS modified Berlin algorithm is only a tool in aiding rheumatologists in diagnosing axSpA and

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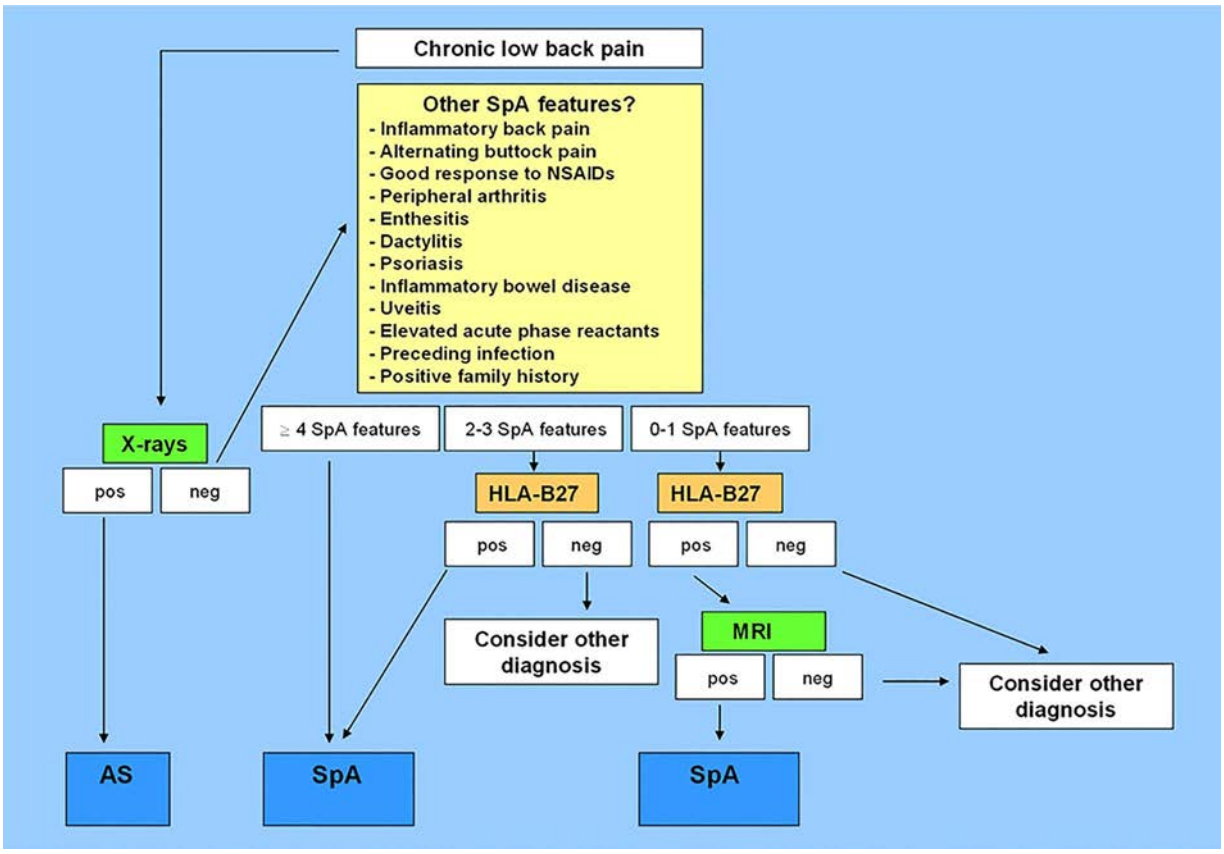

Figure 1 Assessment of SpondyloArthritis International Society (ASAS) modification of the Berlin algorithm* for diagnosing axial spondyloarthritis (adapted from van den Berg *et al* [11] * and Rudwaleit *et al* [2]). HLA-B27, human leucocyte antigen B27; NSAIDs, non-steroidal anti-inflammatory drugs; SpA, spondyloarthritis.

can and should not replace a differential diagnostic procedure in patients with CBP.

Nevertheless, several concerns have been raised about the risk of overdiagnosis of axSpA when the diagnosis is made by counting the number of SpA features without paying attention to an alternative diagnosis that may be more likely.[12] Similarly, the use of the ASAS classification criteria as diagnostic criteria may lead to misdiagnosis. These issues are of particular concern in patients with non-inflammatory conditions in whom overdiagnosis may inappropriately lead to the start of anti-inflammatory treatments that will not be effective but are associated with side effects and costs. Concerns like these have contributed to the US Food and Drug Administration formal disapproval of adalimumab and certolizumab for the treatment of non-radiographic axSpA in the USA, while both drugs have been approved by the European Medicines Agency for this indication in the European Union.[13]

The diagnostic process of early axSpA in patients presenting with CBP is not well studied. Cohort studies typically include patients with an established diagnosis of axSpA. The multicentre SPondyloArthritis Caught Early (SPACE) cohort is a study that has included patients presenting with CBP but without a formal diagnosis who have been referred to a rheumatologist. Consequently, the SPACE cohort contains patients with and without a diagnosis of axSpA.

The main objectives of our study were to investigate (1) which SpA features contribute most to a diagnosis of axSpA; (2) if the presence of multiple SpA features automatically leads to a diagnosis of axSpA in patients presenting with CBP; and (3) how positive classification according to the ASAS criteria relates to a diagnosis of axSpA.

METHODS

Study design and population

The SPACE cohort is a prospective multicentre study, which was initiated in January 2009. The study has been described elsewhere.[14] In brief, patients with CBP (≥3 months and ≤2 years) of unknown origin and age of onset <45 years were included. Patients were recruited for the study from five different rheumatology outpatient clinics in the Netherlands (Amsterdam, Gouda, Leiden), Norway (Oslo) and Italy (Padua).

Data of 157 patients from the Leiden University Medical Center (LUMC) in the Netherlands have previously been published as part of the validation of the modified Berlin algorithm.

Imaging of the sacroiliac joints

Plain radiographs of the pelvis (X-SI) were performed in anteroposterior view. MRI-SI were also performed: the acquired sequences were coronal oblique T1-weighted turbo spin echo and short tau inversion recovery with a slice thickness of 4 mm. Each centre interpreted the radiographs and MRI-SI on the presence of sacroiliitis using global assessment as part of routine clinical practice (local reading) with radiologists specifically being asked whether there was evidence of sacroiliitis.

Clinical measurements

Patients underwent a full diagnostic workup including the assessment of SpA features according to the ASAS criteria: CRP and ESR, HLA-B27, imaging (X-SI and MRI-SI), and the actual presence or a history of all other SpA features: inflammatory back pain (IBP), good response to non-steroidal anti-

inflammatory drugs (NSAIDs), positive family history of SpA, peripheral arthritis, dactylitis, enthesitis, acute anterior uveitis, inflammatory bowel disease, and psoriasis. Rheumatologists provided a diagnosis of axSpA based on all collected information, including imaging and HLA-B27 status. In case of 'no axSpA' rheumatologists were asked to provide a most likely alternative diagnosis. In addition, rheumatologists were requested to provide a level of confidence about the diagnosis on an 11-point numerical rating scale ranging from 0 (not confident at all) to 10 (very confident) after imaging was performed. Independently of the clinical diagnosis the ASAS axSpA classification criteria were used to classify patients using the local imaging results. The rheumatologists were not formally informed about the patients' classification status at the time of diagnosis.

Statistical analysis

For the present analyses baseline data were available (n=522). Patients with missing values for ≥1 SpA feature, including imaging and HLA-B27 status, and those with missing information on clinical diagnosis, were excluded from the analyses (n=22). The total number of SpA features was determined without taking HLA-B27 and imaging into account. Next, patients were stratified according to the number of SpA features present: ≤ one feature, two features, three features and ≥ four features. Patient characteristics are presented for the total patient group and for each subgroup as mean±SD or number (%). The rheumatologist's diagnosis was the main outcome. Sensitivity and specificity were calculated to assess the agreement between the clinical diagnosis and the ASAS axSpA classification criteria. Where zeroes caused problems with computation of ORs or their SEs, 0.5 was added to all cells. Multivariable logistic regression analysis was performed to assess independent determinants of clinical diagnosis.

Data analysis was performed using STATA SE V.14. p Values ≤0.05 were considered statistically significant.

RESULTS

A total of 500 patients with CBP of short duration and complete data was analysed. Of these patients 37% were male, mean age (SD) was 29.3 (8.3) years and mean symptom duration was 13.4 (7.4) months (table 1). Of all patients, 159 (32%) had less than or equal to one feature, 143 (29%) had two features, 79 (16%) had three features and 119 (24%) had four or more features. Age at onset of back pain, sex and disease duration were similar across subgroups. Of the 159 patients in the ≤ one SpA feature subgroup 24% was diagnosed with axSpA; for patients with two SpA features this was 43%, for patients with three SpA features 62% and for patients with ≥ four SpA features this was 85%. When stratifying for each participating centre the same trend—higher percentages of diagnosis with increasing numbers of features—in clinical diagnosis was observed (see online supplementary table S1).

In patients with ≤ one SpA feature 9/159 (6%) had radiographic sacroiliitis and 26/159 (16%) had a positive MRI-SI (table 2). Of the patients with normal radiographs 99/150 (66%) had neither a positive MRI-SI nor HLA-B27 and only 2/99 (2%) were diagnosed with axSpA (both patients with

Table 1 Baseline characteristics of patients with chronic back pain in the SPACE cohort and stratified by total number of SpA features after medical history taking, physical examination and measurement of acute phase reactants but before HLA-B27 testing and imaging

Characteristic	All patients, n=500	Patients with ≤1 feature, n=159	Patients with 2 features, n=143	Patients with 3 features, n=79	Patients with ≥4 features, n=119
Age, years	29.3 (8.3)	29.7 (8.8)	28.8 (8.3)	29.1 (8.0)	29.5 (7.9)
Symptom duration, months	13.4 (7.4)	12.9 (7.3)	14.6 (7.7)	13.3 (7.0)	12.7 (7.4)
Male	185 (37)	51 (32)	56 (39)	24 (30)	54 (45)
IBP	329 (66)	43 (27)	103 (72)	71 (90)	112 (94)
Good response to NSAIDs*	208 (42)	13 (8)	50 (35)	47 (60)	98 (82)
Positive family history SpA†	206 (41)	26 (16)	57 (40)	43 (54)	80 (67)
Peripheral arthritis‡	74 (15)	2 (1)	15 (11)	11 (14)	46 (39)
Dactylitis‡	26 (5)	0 (0)	1 (1)	3 (4)	22 (19)
Enthesitis‡	108 (22)	4 (3)	12 (8)	15 (19)	77 (65)
Anterior uveitis‡	38 (8)	2 (1)	9 (6)	6 (8)	21 (18)
IBD‡	35 (7)	8 (5)	7 (5)	7 (9)	13 (11)
Psoriasis‡	57 (11)	2 (1)	7 (5)	8 (10)	40 (34)
Elevated CRP (mg/L)/ESR (mm)§	132 (26)	12 (8)	25 (18)	26 (33)	69 (58)
HLA-B27 positive	198 (40)	36 (23)	65 (46)	41 (52)	56 (47)
Imaging¶					
X-SI positive	58 (12)	9 (6)	16 (11)	5 (6)	28 (24)
MRI-SI positive	146 (29)	33 (21)	37 (26)	29 (37)	47 (40)
Diagnosis of axSpA**	250 (50)	38 (24)	62 (43)	49 (62)	101 (85)

Results are presented as mean±SD or number (%).
*Back pain not present anymore or is much better 24–48 hours after a full dose of NSAID.
†Presence in first-degree or second-degree relatives of any of the following: ankylosing spondylitis, psoriasis, acute anterior uveitis, reactive arthritis or IBD.
‡Past or present condition, either confirmed or diagnosed by a physician.
§Values greater than the upper limit of normal.
¶According to global assessment radiologist (local reading).
**Diagnosis based on information after full diagnostic workup: medical history, physical examination, imaging and laboratory testing.
AxSpA, axial spondyloarthritis; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; HLA-B27, human leucocyte antigen B27; IBD, inflammatory bowel disease; IBP, inflammatory back pain; MRI-SI, MRI of sacroiliac joints; NSAIDs, non-steroidal anti-inflammatory drugs; SpA, spondyloarthritis; SPACE, SPondyloArthritis Caught Early; X-SI, radiograph of sacroiliac joints.

Table 2 Diagnosis and classification of patients (n=500) with ≤ one, two, three and ≥ four spondyloarthritis (SpA) features after medical history taking, physical examination and measurement of acute phase reactants, followed by sacroiliac imaging and HLA-B27 testing

Number of SpA features	X-SI status	HLA-B27/MRI status	Rheumatologist SpA diagnosis yes	Rheumatologist SpA diagnosis no	ASAS axSpA classification yes	ASAS axSpA classification no
0–1 n=159	X-SI+ n=9	HLA-B27+/MRI+	4		4	
		HLA-B27+/MRI−	1	1	2	
		HLA-B27−/MRI+	1		1	
		HLA-B27−/MRI−	2		2	
	X-SI− n=150	HLA-B27+/MRI+	6	1	7	
		HLA-B27+/MRI−	7	16		23
		HLA-B27−/MRI+	15	6	14	7
		HLA-B27−/MRI−	2	97		99
Mean level of confidence regarding diagnosis (SD)			6.9 (2.3)	7.5 (2.4)		
2 n=143	X-SI+ n=16	HLA-B27+/MRI+	14		14	
		HLA-B27+/MRI−	1		1	
		HLA-B27−/MRI+	1		1	
		HLA-B27−/MRI−				
	X-SI− n=127	HLA-B27+/MRI+	15		15	
		HLA-B27+/MRI−	15	20	35	
		HLA-B27−/MRI+	5	2	7	
		HLA-B27−/MRI−	11	59		70
Mean level of confidence regarding diagnosis (SD)			7.6 (1.9)	6.7 (2.3)		
3 n=79	X-SI+ n=5	HLA-B27+/MRI+	3		3	
		HLA-B27+/MRI−	1		1	
		HLA-B27−/MRI+	1		1	
		HLA-B27−/MRI−				
	X-SI− n=74	HLA-B27+/MRI+	17		17	
		HLA-B27+/MRI−	11	9	20	
		HLA-B27−/MRI+	8		8	
		HLA-B27−/MRI−	8	21		29
Mean level of confidence regarding diagnosis (SD)			8.0 (1.9)	7.1 (2.0)		
≥4 n=119	X-SI+ n=28	HLA-B27+/MRI+	15		15	
		HLA-B27+/MRI−				
		HLA-B27−/MRI+	8		8	
		HLA-B27−/MRI−	5		5	
	X-SI− n=91	HLA-B27+/MRI+	16		16	
		HLA-B27+/MRI−	21	4	25	
		HLA-B27−/MRI+	8		8	
		HLA-B27−/MRI−	28	14		42
Mean level of confidence regarding diagnosis (SD)			8.0 (2.0)	7.3 (1.7)		

Diagnosis based on information after full diagnostic workup: medical history, physical examination, imaging and laboratory testing. ASAS axSpA criteria, ASAS criteria for axial spondyloarthritis. Mean level of confidence regarding diagnosis: 0 (not confident at all) through 10 (very confident).
AxSpA, axial spondyloarthritis; ASAS, Assessment of SpondyloArthritis international Society; HLA-B27, human leucocyte antigen B27; X-SI, radiograph of sacroiliac joints; SpA, spondyloarthritis.

CBP had one SpA feature which were IBP and positive family history, respectively). In total, 38/159 (24%) patients were diagnosed with axSpA. One patient with radiographic sacroiliitis was not diagnosed with axSpA. When the ASAS axSpA classification criteria were applied, five patients without a diagnosis of axSpA fulfilled the ASAS criteria. In addition, 13 patients with an axSpA diagnosis did not fulfil the ASAS classification criteria.

In patients with two SpA features 16/143 (11%) had radiographic sacroiliitis and 35/143 (24.5%) patients had a positive MRI-SI. Of the patients with normal radiographs 70/127 (55%) had neither a positive MRI-SI nor HLA-B27 and 11/127 (9%) were diagnosed with axSpA. In total, 62/143 (43%) patients were diagnosed with axSpA. All patients with radiographic sacroiliitis were diagnosed with axSpA. When the ASAS axSpA classification criteria were applied, 22 patients without a diagnosis of axSpA fulfilled the ASAS-criteria and 11 patients with an axSpA diagnosis did not fulfil the ASAS-criteria.

In patients with three SpA features 5/79 (6%) had radiographic sacroiliitis and 29/79 (38%) had a positive MRI-SI. Of the patients with normal radiographs 29/74 (39%) had neither a positive MRI-SI nor HLA-B27 and 8/74 (11%) were diagnosed with axSpA. In total, 49/79 (62%) patients were diagnosed with axSpA. All patients with radiographic sacroiliitis were diagnosed with axSpA. When the ASAS axSpA classification criteria were applied, nine patients without a diagnosis of axSpA fulfilled

ASAS criteria and eight patients with an axSpA diagnosis did not fulfil the ASAS criteria.

In patients with ≥ four SpA features 28/119 (24%) had radiographic sacroiliitis and 47/119 (40%) had a positive MRI-SI. Of the 91 patients with normal radiographs 42 (46%) had neither a positive MRI-SI nor HLA-B27 and 28/91 (31%) were diagnosed with axSpA. In total, 101/119 (85%) patients were diagnosed with axSpA. Again, all patients with radiographic sacroiliitis (28/28) were diagnosed with axSpA. Remarkably, 18/119 patients (15%) with ≥ four SpA features but with negative imaging were not given the diagnosis of axSpA, four of whom were HLA-B27 positive. When the ASAS axSpA classification criteria were applied, 4 patients without a diagnosis of axSpA fulfilled the ASAS criteria and 28 patients with an axSpA diagnosis did not fulfil the ASAS criteria. Moreover, patients with ≥ four features not diagnosed with axSpA were mostly given the diagnosis non-specific back pain and degenerative disc disease (data not shown). In these patients the most common SpA features were a positive family history for SpA (67%), good response to NSAIDs (82%) and IBP (94%).

Overall, the mean levels of confidence (SD) regarding a diagnosis of axSpA and no axSpA were 7.7 (2.0) and 7.2 (2.3), respectively. Mean levels of confidence of axSpA diagnosis for the different patient subgroups rose with the presence of more SpA features; ≤ one feature, mean 6.9 (2.3); two features, mean 7.6 (1.9); three features, mean 8.0 (1.9); ≥ four features, mean 8.0 (2.0) (table 2).

With the clinical diagnosis of the rheumatologist as the gold standard, sensitivity and specificity of the ASAS classification criteria for axSpA were 76% (190/250) and 84% (210/250), respectively (table 3).

In univariable analysis, HLA-B27 positivity and any positive imaging were associated with an axSpA diagnosis (OR 5.6; 95% CI 3.7 to 8.3 and OR 34.3; 95% CI 17.3 to 67.7, respectively). These associations were similar across subgroups (tables 4 and 5). In multivariable logistic regression analysis with clinical diagnosis as the dependent variable and SpA features from the ASAS criteria as independent variables HLA-B27 and positive imaging were both independent determinants of diagnosis (data not shown).

DISCUSSION

Prompted by concerns regarding overdiagnosis of axSpA we investigated whether in patients referred with recent onset CBP and a suspicion of axSpA, the presence of several SpA features suffices for a diagnosis of axSpA. While, as expected, an increasing number of SpA features was associated with an increased likelihood of axSpA diagnosis this association was not absolute.

Table 3 Concordance between clinical axSpA diagnosis and meeting the ASAS classification criteria for axSpA in patients with CBP with the physician's diagnosis as the gold standard in the SPACE cohort (n=500)

	Clinical axSpA diagnosis		
ASAS classification criteria	**Yes**	**No**	**Total**
Yes	190	40	230
No	60	210	270
Total	250	250	500

Sensitivity 76% (190/250) and specificity 84% (210/250). Positive predictive value: 190/230 (83%), negative predictive value: 210/270 (78%).
AxSpA, axial spondyloarthritis; ASAS, Assessment of SpondyloArthritis international Society; CBP, chronic back pain; SPACE, SPondyloArthritis Caught Early.

Table 4 Concordance between clinical axSpA diagnosis and presence of HLA-B27 for all patients and stratified for total number of SpA features

	Clinical axSpA diagnosis		
	Yes	**No**	**Total**
All patients			
HLA-B27 positive			
Yes	147	51	198
No	103	199	302
Total	250	250	500
OR (95% CI)	5.6 (3.7 to 8.3)		
≤1 feature			
HLA-B27 positive			
Yes	18	18	36
No	20	103	123
Total	38	121	159
OR (95% CI)	5.2 (2.3 to 11.6)		
2 features			
HLA-B27 positive			
Yes	45	20	45
No	17	61	78
Total	62	81	143
OR (95% CI)	8.1 (3.8 to 17.1)		
3 features			
HLA-B27 positive			
Yes	32	9	41
No	17	21	38
Total	49	30	79
OR (95% CI)	4.4 (1.7 to 11.7)		
≥4 features			
HLA-B27 positive			
Yes	52	4	56
No	49	14	63
Total	101	18	119
OR (95% CI)	3.7 (1.1 to 12.1)		

axSpA, axial spondyloarthritis; HLA-B27, human leucocyte antigen B27; SpA, spondyloarthritis.

Numerous patients with multiple SpA features did not get a diagnosis of axSpA. Among them are half of the HLA-B27 positive patients with three SpA features but without imaging abnormalities. This example clearly shows that a clinical diagnosis is based on more than simply a sum of features.

In this cohort the ASAS classification criteria had an overall sensitivity and specificity of 76% and 84%, respectively. This is comparable to those found in the original ASAS cohort. In line with the finding that patients with multiple SpA features are not always diagnosed with axSpA 17% of patients that on paper met the ASAS classification criteria, which requires presence of at least two SpA features, were not diagnosed with axSpA.

An important finding is the prominent—if not dominant—role of imaging and HLA-B27 testing in diagnosing axSpA in rheumatology clinics. The statistically stronger association between positive imaging and axSpA diagnosis as compared with HLA-B27 and axSpA diagnosis (or any other SpA feature) should be interpreted with caution. The prevalence of axSpA in this cohort of patients specifically referred to the rheumatologist (50%) is much higher than the prevalence of axSpA in unselected patients with CBP, and we do not know which screening

Table 5 Concordance between clinical axSpA diagnosis and any positive imaging (MRI-SI and/or X-SI) for all patients and stratified for total number of SpA features

	Clinical axSpA diagnosis		
	Yes	No	Total
All patients			
Any positive imaging			
Yes	147	10	157
No	103	240	343
Total	250	250	500
OR (95% CI)	34.3 (17.3 to 67.7)		
≤1 feature			
Any positive imaging			
Yes	29	8	37
No	9	113	122
Total	38	121	159
OR (95% CI)	45.5 (16.1 to 128.3)		
2 features			
Any positive imaging			
Yes	36	2	38
No	26	79	105
Total	62	81	143
OR (95% CI)	54.7 (12.3 to 243)		
3 features			
Any positive imaging			
Yes	30	0*	30
No	19	30	49
Total	49	30	79
OR (95% CI)	95.4 (5.5 to 1652.2)		
≥4 features			
Any positive imaging			
Yes	52	0*	52
No	49	18	67
Total	101	18	119
OR (95% CI)	39.2 (2.3 to 668.8)		

*For computation of ORs in case of zeroes, 0.5 were added to all cells.
axSpA, axial spondyloarthritis; MRI-SI, MRI of sacroiliac joints; X-SI, radiograph of sacroiliac joints; SpA, spondyloarthritis.

tools were applied to select patients for referral. In our cohort X-SI was positive in only a minority of patients while an analysis of 204 referral letters indicated that HLA-B27 positivity was mentioned four times more often than a positive MRI-SI as a reason for referral (unpublished data). This difference in absolute prevalence implies that the impact of different ORs (OR=5.6 for HLA-B27 and OR=35 for imaging) may be far more similar than the ORs suggest.

Nevertheless, our findings stress the dominance of imaging in establishing an axSpA diagnosis and add to the importance of a proper interpretation of the images.[15–17]

At first sight, some of the diagnoses may raise suspicion. For instance, a diagnosis of axSpA may not be expected in HLA-B27 negative patients that have normal imaging tests, and only a few other SpA features. In such patients, a diagnosis may still be justifiable because of features or symptoms that are not part of the ASAS criteria, for example, buttock pain, IBP according to Calin or Berlin criteria, presence of structural (but not active) lesions on MRI-SI or spinal inflammatory lesions, even though the latter two manifestations are rare in the absence of bone marrow oedema on MRI-SI.[18]

Furthermore, differences in the interpretation of imaging may also have contributed to unexpected diagnoses. Even though the assessment of the radiologist was used for the analyses, the rheumatologist has provided the diagnosis and may—based on the clinical symptoms—have overruled the radiologist's report, for instance by taking structural lesions or spinal inflammatory lesions into account.[18 19]

A possible limitation of this study is that the clinical diagnosis —as is usual in clinical practice—was provided by only one rheumatologist. Each rheumatologist may consider different features, apart from positive imaging and presence of HLA-B27, being most informative for axSpA diagnosis. Even though this was not assessed it is conceivable this might have influenced the diagnosis. Future studies should definitely assess interobserver variance in clinical diagnosis.

The ASAS modified Berlin algorithm can be used by rheumatologists in the clinical decision making process when diagnosing patients with CBP. But blindly applying the ASAS modified Berlin algorithm will also result in false-positive and false-negative diagnoses. As has become clear in our study, in patients without radiographic sacroiliitis but with multiple SpA features (and/or presence of HLA-B27), the algorithm immediately leads to an axSpA diagnosis, while in clinical practice this is not always clear. In 15% of the patients with ≥ four SpA features and 13% of the HLA-B27 positive patients with two to three SpA features that should have a clinical diagnosis of axSpA according to the algorithm, such a diagnosis was not confirmed by the clinician.

While the SPACE cohort is running in different countries and settings (academic and non-academic), we did not find an important centre effect. In all centres the likelihood of axSpA diagnosis similarly increased by an increasing number of SpA features, which adds to the credibility of our data. Nevertheless, patients were diagnosed by hospital-based rheumatologists with an expertise in diagnosing patients with axSpA, and results of this study cannot be extrapolated to different clinical settings such as primary care and common rheumatology practices or those of other medical specialties.

In conclusion, in clinical practice the mere presence of SpA features does not automatically result in a clinical diagnosis of axSpA. Furthermore, this study confirms that the ASAS modified Berlin algorithm could be used as a guidance tool but that a thorough diagnostic workup with ample consideration for alternative diagnoses is still mandatory. Preferably, all information including imaging of sacroiliac joints and presence of HLA-B27 should be available to the rheumatologist to come to a final diagnosis.

Contributors ZEZ designed the study, performed the statistical analyses, interpreted findings, and drafted and revised the manuscript. FvG and DvdH designed the study, interpreted the data, and drafted the manuscript. All authors contributed to the acquisition and interpretation of data, and read and approved the final manuscript.

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CONCISE REPORT

Safety of synthetic and biological DMARDs: a systematic literature review informing the 2016 update of the EULAR recommendations for management of rheumatoid arthritis

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ABSTRACT

Objectives To assess the safety of synthetic (s) and biological (b) disease-modifying antirheumatic drugs (DMARDs) for the management of rheumatoid arthritis (RA) to inform the European League Against Rheumatism recommendations for the management of RA.

Methods Systematic literature review (SLR) of observational studies comparing any DMARD with another intervention for the management of patients with RA. All safety outcomes were included. A comparator group was required for the study to be included. Risk of bias was assessed with the Hayden's tool.

Results Twenty-six observational studies addressing diverse safety outcomes of therapy with bDMARDs met eligibility criteria (15 on serious infections, 4 on malignancies). Substantial heterogeneity precluded meta-analysis. Together with the evidence from the 2013 SLR, based on 15 studies, 7 at low risk of bias, patients on bDMARDs compared with patients on conventional sDMARDs had a higher risk of serious infections (adjusted HR (aHR) 1.1 to 1.8)—without differences across bDMARDs—a higher risk of tuberculosis (aHR 2.7 to 12.5), but no increased risk of infection by herpes zoster. Patients on bDMARDs did not have an increased risk of malignancies in general, lymphoma or non-melanoma skin cancer, but the risk of melanoma may be slightly increased (aHR 1.5).

Conclusions These findings confirm the known safety pattern of bDMARDs, including both tumour necrosis factor-α inhibitor (TNFi) and non-TNFi, for the treatment of RA.

INTRODUCTION

The armamentarium nowadays available for the treatment of patients with rheumatoid arthritis (RA) is impressive and has substantially expanded in the last decades. A plethora of conventional synthetic disease-modifying antirheumatic drugs (csDMARDs), biological DMARDs (bDMARDs) and more recently also targeted synthetic DMARDs (tsDMARDs), which can be used in different sequences and/or combinations, is at the disposal of rheumatologists to offer to patients. This, naturally, also implies choices to be made when deciding on the best treatment for a particular patient.

Treatment decisions, particularly in the case of patients with RA with insufficient response to a first csDMARD, are mainly made based on the expected efficacy of a drug.[1] However, there are no important differences in efficacy across bDMARDs and tsDMARDs.[2 3] Therefore, other aspects among which safety may have a more prominent place in decision-making.[1] While short-term safety is addressed in clinical trials, it is long-term safety that we are primarily interested in when making our decisions. Observational studies (eg, cohort studies, registries) provide us with more relevant information since, unlike clinical trials, they include a non-selected group of patients, are representative of daily clinical practice and cover a longer period of time.[4]

In order to inform the task force responsible for the 2016 update of the European League Against Rheumatism (EULAR) RA management recommendations, we performed a systematic literature review (SLR) to update the evidence for the safety of csDMARDs, tsDMARDs and bDMARDs in patients with RA.[5] This SLR is an update of the SLR performed previously for the corresponding 2013 update of the RA management recommendations.[6] The results of this and two other SLRs[2 3] provided the task force with the current state of evidence.

METHODS

Literature search

The search was performed in MEDLINE, EMBASE and The Cochrane CENTRAL Register of Controlled Trials (Central), until 9 March 2016, without language restrictions. All newly included studies were published from 2013 onwards, as an update of the previous SLR.[6] As this SLR is an update of the 2013 SLR,[6] results are shown together to give a more complete overview on the safety of DMARDs. Details on complete search strategies are provided in online supplementary material. References from included studies were also screened.

The literature search addressed the safety of DMARDs. The research questions were structured

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according to a PICO format (Patients, Intervention, Comparator and Outcomes) and eligible study types were defined.[7] Participants were adults (aged ≥18 years) with a clinical diagnosis of RA. Studies including patients with other diagnoses were eligible only if the results from patients with RA were presented separately. The intervention was any DMARD (csDMARD, bDMARD—including biosimilars—or tsDMARD), including all drugs (methotrexate, leflunomide, hydroxychloroquine, sulfasalazine, gold/auranofin, azathioprine, chlorambucil, chloroquine, ciclosporin, cyclophosphamide, mycophenolate, minocycline, penicillamine, tacrolimus, anakinra, infliximab, etanercept, adalimumab, rituximab (RTX), abatacept, tocilizumab, golimumab, certolizumab pegol or tofacitinib), formulations and duration). Glucocorticoids were also included. The comparator was a(nother) bDMARD, sDMARD, glucocorticoid, combination therapy or the general population. Studies were only eligible if they included a comparator group, as a formal comparison is the only insightful manner to take any conclusions about safety. All safety outcomes were considered, namely infections (including serious infections, opportunistic infections such as tuberculosis and herpes zoster), malignancies, mortality, cardiovascular disease, change in lipid levels, impairment in renal function, elevation of liver enzymes, haematological abnormalities, gastrointestinal effects, demyelinating disease, induction of autoimmune disease and teratogenicity. Only observational studies were included, namely cohort studies/registries and study series with >30 cases.

Selection of studies, data extraction and assessment of risk of bias

Two reviewers (SR and AS) independently screened titles and abstracts, and if necessary the full-text, for eligibility. In cases of disagreement, a third reviewer (RBML) was involved. Data from eligible studies were extracted regarding study and population characteristics, inclusion and exclusion criteria, follow-up time, interventions, outcome definition and outcome measures using a standardised data extraction form.

The two reviewers independently assessed the risk of bias of each included study using the 'Hayden-tool', which evaluates the following items: participation, attrition, prognostic factor measurement, outcome measurement, confounding and analysis.[8]

RESULTS

In total and after de-duplication, 4436 references were obtained, of which 26 studies were included (flowchart in online supplementary figure S1). All studies included patients on bDMARDs and only one study also addressed a comparison between csDMARDs.[9] There were no eligible studies on tsDMARDs or glucocorticoids. Of the included studies, 15 studies focused on infections,[10–24] 4 on malignancies,[9 25–27] 1 on mortality,[28] 4 on cardiovascular events[29–32] and 2 on interstitial lung disease.[33 34] Details can be found in tables 1–3 and online supplementary tables S1–S57.

Studies were very heterogeneous in every single item of the PICO, thus precluding data-pooling (meta-analysis), and results are presented descriptively.

Of the newly included 11 studies addressing serious infections, 6 compared patients on bDMARDs with those on csDMARDs or with the general population,[10–15] whereas 8 studies[10–12 16–20] addressed a comparison between different bDMARDs (3 studies addressed both comparisons)[10–12]—tables 1 and 2. In total, and considering the previous evidence from 2013,[6] 15 studies, 7 at low risk of bias, compared the risk of serious infections between bDMARDs and csDMARDs and overall found a significantly increased risk with adjusted HRs (aHR) between 1.0 and 1.8 per study.[10–15 35–43] More recent studies at low risk of bias did not show an increased risk.[10 14] One study comparing bDMARDs with the general population reported standardised incidence rates of 16–20 for tumour necrosis factor-α inhibitor (TNFi) and even higher for RTX (table 1).[15] In total, six studies,[21 44–48] performed in European and American datasets, of which four were at low risk of bias, focused on the occurrence of herpes zoster, most of them reporting no increased risk for this type of infection in patients on TNFi (no studies for other bDMARDs), particularly the studies at low risk of bias and/or those that had been adjusted for dropouts.[21 44–46]

Seven studies addressing tuberculosis, most of them being at moderate or high risk of bias, showed an increased risk of tuberculosis in patients on TNFi (no studies for other sDMARDS), both compared with the general population and to patients on csDMARDs (aHR 2.7 to 12.5 per study).[11 22 23 49 50]

One study at moderate risk of bias did not show an increased risk of skin infections in patients on TNFi compared with patients on csDMARDs.[24] One study at moderate risk of bias reported no increased risk of non-viral opportunistic infections in patients on TNFi versus csDMARDs.[23]

Concerning comparisons across bDMARDs, eight studies, only one of them being at low risk of bias, compared the risk of serious infections across bDMARDs and in general did not show differences between several drugs.[10–12 16–20] Comparisons included TNFi and non-TNFi, both aggregated in classes and as individual drugs. One of the studies found a signal for a higher risk of serious infections with infliximab compared with etanercept[17] and another for infliximab, etanercept and RTX compared with abatacept[20] (table 2). No differences were found between TNFi and non-TNFi on the risk of herpes zoster.[21]

The overall risk of malignancies was investigated in a total of nine studies, six of them being at low risk of bias (table 3).[9 10 14 25 51–55] Both in comparison to the general population and to patients on csDMARDs, patients on bDMARDs did not show an increased risk for malignancies. In a few more recent studies, patients on non-TNFi were also included.[9 10] Similarly, no increased risk for solid cancers has been found for patients on bDMARDs compared with csDMARDs (two studies were at low risk of bias).[9 26] The same was true for the analysis of the individual solid cancers (eg, breast cancer, lung cancer, colorectal cancer—online supplementary table S37.2). Patients on bDMARDs (five studies, three at low risk of bias, all with TNFi), as compared with the general population, had a higher risk of lymphoma, with adjusted aHRs ranging from 2.3 to 5.9, but in comparison to patients on csDMARDs (three studies, two at low risk of bias), no increased risk was found. In patients on bDMARDs, non-melanoma skin cancer may occur more frequently than in the general population (aHR 1.7; one study at low risk of bias), but compared with csDMARDs, there was no increased risk (four studies, two at low risk of bias). A 'safety alarm signal' was shown for abatacept compared with csDMARDs: a higher risk for its occurrence, with an aHR of 15.3 (95% CI 2.1 to 114), but this 'signal' was only based on two cases.[9] One study at low risk of bias has shown that patients on bDMARDs may have an increased risk for melanoma compared with csDMARDs (aHR 1.5 (95% CI 1.0 to 2.2)).[56]

For the remaining outcomes, the scarcity of data precluded definitive conclusions, but new safety signals were absent (see online supplementary tables S39–S57).

Table 1 Serious infections in patients on bDMARDs compared with patients on csDMARDs or general population (observational studies)

Year of publication	Study ID	Registry	Intervention	Control	aHR (intervention vs comparator/control)	Risk of bias
Serious infections						
≤2013	Galloway 2011 Rheumatology(a)[35]	BSRBR	3 TNFi	csDMARDs	**1.2 (1.1 to 1.5)**	Low
	Greenberg 2010 ARD[36]	CORRONA	3 TNFi+MTX	MTX	**1.1 (1.0 to 1.3)**	Low
	Grijalva 2011 JAMA[37]	Claim database	3 TNFi	csDMARDs	1.1 (0.9 to 1.2)	Moderate
	Grijalva 2010 Rheumatology[38]	Claim database	3 TNFi	MTX	1.3 (0.8 to 2.2)	Moderate
	Komano 2011 J Rheum[39]	REAL	ETA/IFX	csDMARDs	RR **2.4 (1.1 to 5.1)**	Moderate
	Sakai 2012 AC&R[40]	REAL	ETA/IFX	csDMARDs	RR **2.0 (1.3 to 3.2)**	Moderate
	Strangfeld 2011 ARD[41]	RABBIT	3 TNFi	csDMARDs	**1.8 (1.2 to 2.7)**	Low
	Lane 2011 Medicine (Baltimore)[42]	Claim database	3 TNFi	csDMARDs	**1.2 (1.0 to 1.5)** vs HCQ, SSZ, gold	Moderate
	Galloway 2011 Rheumatology (b)[43]	BSRBR	Anakinra	csDMARDs	1.6 (0.9 to 2.7)	Low
2013–2016	Aaltonen 2015 J Rheum[10]	National Register for Biologic Treatment in Finland (ROB-FIN)	3 TNFi	csDMARDs	0.9 (0.6 to 1.4)	Low
			RTX		1.1 (0.6 to 1.9)	
	Chiu 2014 Int J Rheum Dis[11]	Taiwan's National Health Insurance Research Database	TNFi (ETA/ADA)	csDMARDs	1.0 (0.9 to 1.2)*	High
	Lampropoulos 2015 Clin Exp Rheumatol[12]	Files Laiko University Hospital	bDMARDs (9?)	csDMARDs	**6.9 (3.1 to 15.4)**	High
	Miranda 2014 Rev Colom Rheumatol[13]	Files Colombian Hospital	bDMARDs†	csDMARDs	**2.7 (1.1 to 6.3)**	High
	Morgan 2014 Rheumatology[14]	BSRBR	ETA	csDMARDs	1.0 (0.8 to 1.3)	Low
	Cobo Ibanez 2014 Rheumatol Int[15]	BIOBADASER	3 TNFi	General population	♂ 16 (13–20); ‡♀21 (19–24)	Low
			RTX		♂ 32 (1–179); ‡♀186 (106–302)	
Herpes zoster						
≤2013	Galloway ARD 2013[44]	BSRBR	3 TNFi	csDMARDs	**1.7 (1.1 to 2.7)**; adjusted for drop-outs 1.5 (1.0 to 2.4)	Low
	McDonald 2009 Clin Inf Diseases[47]	Claim Database	3 TNFi	csDMARDs	**1.4 (1.1 to 1.8)**	Moderate
	Strangfeld 2009 JAMA[45]	RABBIT	3 TNFi	csDMARDs	1.6 (1.0 to 2.7)	Low
	Garcia-Doval 2010 ARD[48]	BIOBADASER	TNFi (3?)	General population	**10 (3 to 26)**	Low
	Winthrop 2013 JAMA[46]	Claim Dabatase	3 TNFi	csDMARDs	1.0 (0.8 to 1.3)	Moderate
2013–2016	Pappas 2015 AC&R[21]	CORRONA	csDMARDs	TNFi	1.4 (0.8 to 2.3)	Low
Tuberculosis						
≤2013	Dixon 2010 ARD(a)[66]	BSRBR	3 TNFi	csDMARDs	NR	Low
	Tam 2010 Clin Exp Rheumatol[49]	Hong Kong Cohort	3 TNFi	General population csDMARDS	**34.9 (8.9 to 137.2)** **12.5 (3.5 to 44.7)**	Moderate
	Tubach 2009 A&R[50]	RATIO	3 TNFi	General population	**12.4 (9.1 to 16.9)**	Low
	Winthrop ARD[67]	Claim database	3 TNFi	General population	NR	Moderate
2013–2016	Ke 2013 Tuberc Lung Dis[22]	Taiwan's National Health Insurance Research Database	TNFi (ADA/ETA)	csDMARDs	**4.9 (2.1 to 11.1)**	Moderate
	Baddley 2014 ARD[23]	4 US insurance datasets—SABER study (claims dataset)	3 TNFi	csDMARDs	4.2 (0.5 to 33.5)	Moderate
	Chiu 2014 Int J Rheum Dis[11]	Taiwan's National Health Insurance Research Database	TNFi (ADA/ETA)	csDMARDs	**2.7 (2.1 to 3.3)**	High
Skin infections						
2013–2016	Wasson 2013 BMC Infect Dis[24]	US Veterans (Claims database)	3 TNFi	csDMARDs	1.1 (0.6 to 2.0)§	Moderate

Continued

Table 1 Continued

Year of publication	Study ID	Registry	Intervention	Control	aHR (intervention vs comparator/control)	Risk of bias
Non-viral opportunistic infections¶						
2013–2016	Baddley 2014 ARD[23]	4 US insurance datasets (SABER study (claims dataset)	3 TNFi	csDMARDs	1.6 (0.9 to 3.1)	Moderate
			ADA		1.8 (0.6 to 5.3)	
			ETA		0.8 (0.4 to 1.8)	
			IFX		**2.6 (1.2 to 5.6)**	

Estimates in bold reflect a risk/ratio statistically significantly different from 1, ie association is statistically significant.
More details are found in online supplementary tables S1–S31.
*Unadjusted estimate; no adjusted estimate reported.
†ADA, ABA, ETA, RTX, TCZ, IFX.
‡Standardised incidence rates are reported, with the general population as the reference.
§Adjusted OR reported.
¶Non-viral opportunistic infections included fungal infections, tuberculosis, pneumocystosis, nocardiosis/actinomycosis, non-tuberculous mycobacteria, salmonellosis, listeriosis and legionellosis.
ADA, adalimumab; aHR, adjusted Hazard Ratio; bDMARDs, biological disease-modifying antirheumatic drugs; BIOBADASER, Spanish Biologics Register; BSRBR, British Society of Rheumatology Biologics Register; CORRONA, Consortium of Rheumatology Researchers of North America; csDMARDs, conventional synthetic disease-modifying antirheumatic drugs; CZP, certolizumab; ETA, etanercept; HCQ, hydroxychloroquine; IFX, infliximab; MTX, methotrexate; NR, not reported; RABBIT, Rheumatoid Arthritis Observation of Biologic Therapy (in German); RATIO, French Biologics Register; REAL, Registry of Japanese Rheumatoid Arthritis Patients for Long-term Safety; RR, relative risk; RTX, rituximab; SSZ, sulfasalazine; TCZ, tocilizumab; TNFi, tumour necrosis factor α inhibitor.

DISCUSSION

Existing literature has further confirmed that patients on bDMARDs (both TNFi and non-TNFi) have an increased risk of serious infections compared with patients on csDMARDs and that in general there are no differences across bDMARDs. There is an increased risk for tuberculosis with TNFi, whereas this has not been studied well for non-TNFi. There does not seem to be an increased risk of herpes zoster with bDMARDs. In addition, bDMARDs are not associated with an increased risk of malignancies, with the potential exception of melanoma, based on one study only.

Interestingly, more recent studies addressing serious infections, and especially those at low risk of bias, did not show an increased risk of infections anymore.[10 14] This contrasts with earlier studies addressing the same outcome, in which a higher risk of infections had been reported consistently even in those at low risk of bias.[35 36 41] This effect may reflect a change in the attitude of physicians who now more carefully screen and monitor patients (including infection prophylaxis, when indicated) and treat infections in patients on bDMARDs appropriately.

In general, our conclusions are in line with those drawn in 2013,[6] which is reassuring. The accumulating body of evidence related to bDMARDs is consistently showing us that patients with RA can be treated in a relatively safe way with these drugs. This SLR extends these conclusions also to non-TNFi bDMARDs, which was not possible in the previous SLR.[6] Still, most literature on safety pertains to TNFi, and we need more studies including non-TNFi bDMARDs and tsDMARDs in future.

This SLR also highlights the importance of observational studies in addressing safety aspects of treatment, particularly those studies that include a comparator and truly allow us to assign risks to patients on a particular intervention (eg, bDMARDs). Without a proper comparison, it is impossible to truly judge risks. In addition, these are studies that include all types of patients and follow them up for a long period of time, directly reflecting daily clinical practice, which increases their generalisability.[4] This is what we need to get better insight into safety aspects of treatments as it complements the limited information derived from clinical trials. Admittedly, conducting this type of analysis in observational studies properly is challenging.[57] Several confounders can influence the relationships of interest, and they need to be carefully considered. Even though this is done, even the 'best comparator' that we at the moment have to contrast safety of bDMARDs with, namely csDMARDs, also implies challenges and limitations, as we know that patients on csDMARDs have less severe disease, or sometimes historical data are used for comparison purposes, which also introduces some sources of bias. Increasingly complex analyses are being undertaken to circumvent the known challenges, for example, analysis adjusted for propensity score.[58] Collaborations between registries are important in order to homogenise procedures, raise the overall quality and allow comparisons, and these should be encouraged.[59] This will lead to better information for clinicians and better care to patients. Over and above the current data from observational studies, other information previously obtained through randomised clinical trials (RCTs) or addressed in package inserts should be taken into account. The labels of each drug, including adverse events and lab monitoring, remain undisputed and it is good practice to follow them.

Although this SLR aimed at including all DMARDs, the eligible studies were only on bDMARDs. This points to the need

Table 2 Serious infections in patients on bDMARDs, comparison between different bDMARDs (observational studies)

Year of publication	Study ID	Registry	Intervention	Control	aHR (intervention vs comparator/control)	Risk of bias
Serious Infections						
2013–2016	Aaltonen 2015 J Rheum[10]	National Register for Biologic Treatment in Finland (ROB-FIN)	RTX	TNFi	1.4 (0.8 to 2.6)	Low
	Chiang 2014 Comp methods[16]	Taiwan's National Health Insurance Research Database	ETA	ADA	2.0 (1.1 to 3.6)*	High
	Chiu 2014 Int J Rheum Dis[11]		ADA	ETA	1.8 (1.2 to 2.8)	High
	Curtis 2014 AC&R[17]	US Veterans (claims dataset)	ABA	ETA	1.1 (0.6 to 2.1)	Moderate
			ADA		1.4 (0.9 to 2.2)	
			IFX		**2.3 (1.3 to 4.0)**	
			RTX		1.4 (0.8 to 2.6)	
	Johnston 2013 Semin Arthr Rheum[18]	MarketScan (claims dataset)	ABA	RTX	1.2 (0.8 to †)	Moderate
			ADA		1.1 (0.7 to 1.7)	
			ETA		1.3 (0.8 to 2.0)	
			IFX		1.6 (1.0 to 2.6)	
	Lampropoulos 2015 Clin Exp Rheumatol[12]	Files Laiko University Hospital	ADA	IFX	1.1 (p=0.819)	High
			ETA		0.7 (p=0.559)	
	Sakai 2015 AR&T[19]	REAL	TCZ	TNFi	2.2 (0.9 to 5.4)	Moderate
	Yun 2016 A&R[20]	Medicare claims dataset	ADA	ABA	1.1 (0.9 to 1.3)	
			CZP		1.1 (0.9 to 1.3)	
			ETA		**1.2 (1.1 to 1.5)**	
			IFX		**1.4 (1.2 to 1.6)**	
			GOL		1.1 (0.9 to 1.4)	
			RTX		**1.4 (1.2 to 1.5)**	
			TCZ		1.1 (0.9 to 1.3)	
Herpes zoster						
2013–2016	Pappas 2015 AC&R[21]	CORRONA	Non-TNFi	TNFi	0.8 (0.5 to 1.4)	Low
Tuberculosis						
2013–2016	Chiang 2014 Comp methods[16]	Taiwan's National Health Insurance Research Database	ETA	ADA	2.4 (0.3 to 19.0)	High
	Chiu 2014 Int J Rheum Dis[11]		ADA	ETA	**2.4 (1.3 to 4.2)**	High
Non-viral opportunistic infections‡						
2013–2016	Baddley 2014 ARD[23]	4 US insurance datasets (SABER study (claims dataset))	ADA	ETA	1.8 (0.8 to 4.0)	Moderate
			IFX		**2.9 (1.5 to 5.4)**	

Estimates in bold reflect a risk/ratio statistically significantly different from 1, ie association is statistically significant.
More details are found in online supplementary tables S1–S31.
*Unadjusted estimate; no adjusted estimate reported.
†No upper border of CI given.
‡Non-viral opportunistic infections included fungal infections, tuberculosis, pneumocystosis, nocardiosis/actinomycosis, non-tuberculous mycobacteria, salmonellosis, listeriosis and legionellosis.
ABA, abatacept; ADA, adalimumab; aHR, adjusted adjusted Hazard Ratio; CORRONA, Consortium of Rheumatology Researchers of North America; CZP, certolizumab pegol; ETA, etanercept; GOL, golimumab; IFX, infliximab; REAL, Registry of Japanese Rheumatoid Arthritis Patients for Long-term Safety; RTX, rituximab; TCZ, tocilizumab; TNFi, tumour necrosis factor α inhibitor.

for good quality safety studies addressing the remaining DMARDs. Only one study included a comparison between csDMARDs and the focus of that study was still on bDMARDs.[9] Among the studies on bDMARDs, none of them included patients on biosimilars (yet). In addition, observational studies addressing tsDMARDs (Jak inhibitor(s)) have not yet been found. However, RCT data point towards a higher risk of serious infections, infections caused by herpes zoster and tuberculosis, risks that should not be ignored and that warrant further research.[2 60] Finally, while glucocorticoids are gaining importance as bridging treatment for RA, no single study meeting the eligibility criteria could be found. Nevertheless, concerns regarding the long-term safety of glucocorticoids remain,[2] and recent studies, even though some of them are uncontrolled or may suffer from confounding by indication, point towards a higher cardiovascular risk, a higher risk of infections and higher mortality in patients taking glucocorticoids.[28 61–64] These are all questions that should be addressed, likely in registries, and with the use of analytical techniques that have previously been used with success in safety studies with bDMARDs.

In line with the frequent updates of the EULAR recommendations for the management of RA, it is to expect that an update of this SLR will soon deserve careful attention, particularly if the above-mentioned unmet needs are fulfilled and more good quality safety registry data, and covering more interventions, become available. An example is the recent study from Strangfeld *et al*[65] showing a higher risk of lower intestinal perforation in patients taking tocilizumab compared with patients on csDMARDs, which has no longer been included in this SLR because it was accepted for publication after the update of the search for this SLR and when the task force meeting for the EULAR recommendations on the management of RA had already taken place. This and other

Table 3 Malignancies in patients on bDMARDs compared with patients on csDMARDs or general population (observational studies)

Year of publication	Study ID	Registry	Intervention	Control csDMARDs	Control general population	aHR (intervention vs comparator/control)	aHR (intervention vs general population)	Risk of bias
All types of cancer								
≤2013	Askling 2009 A&R[51]	ARTIS	3 TNFi	csDMARDs	General population	TNFi vs pts starting MTX: 1.0 (0.8 to 1.2); TNFi vs csDMARDs combination therapy 1.0 (0.7 to 1.4)	1.1 (1.0 to 1.3)	Low
	Carmona 2011 Semin Arthritis Rheum[52]	BIOBADASER	3 TNFi	csDMARDs	General population	0.5 (0.1 to 2.5)	**0.7 (0.5 to 0.9)**	Low
	Hayes 2013 A&R[53]	Claim database	3 TNFi	csDMARDs	NR	0.8 (0.6 to 1.1) Ever-analysis 0.9 (0.8 to 1.1)	NR	Moderate
	Pallavicini 2010 Autoimmunity Reviews[54]	LORHEN	3 TNFi	NR	General population	NR	Milan*: 0.9 (0.6 to 1.5) to Varese 1.1 (0.6 to 1.7)	Moderate
	Strangfeld 2010 AR&T[55]	RABBIT	3 TNFi	csDMARDs	General population	0.7 (0.4 to 1.1)	0.8 (0.5 to 1.0)	Low
			ANA			1.4 (0.6 to 3.5)	NR	
2013–2016	Berghen 2015 Clin Rheumatol[25]	Cases from the Leuven University Hospital	3 TNFi	NR	General population	NR	♂**163.5 (156.8 to 170.6)**† ♀**145.5 (137.2 to 154.3)**	Moderate
	Aaltonen 2015 J Rheum[10]	National Register for Biologic Treatment in Finland (ROB-FIN)	3 TNFi	csDMARDs	NR	1.2 (0.6 to 2.2)	NR	Low
			ADA			1.1 (0.5 to 2.2)		
			ETA			1.3 (0.7 to 2.6)		
			IFX			1.2 (0.4 to 3.1)		
			RTX			1.2 (0.5 to 3.2)		
	Morgan 2014 Rheumatology[14]	BSRBR	ETA	csDMARDs	NR	0.8 (0.7 to 1.0)	NR	Low
	Solomon 2014 Semin Arthr Rheum[9]	CORRONA	3 TNFi	MTX	NR	**0.3 (0.1 to 0.6)**	NR	Low
			RTX			0.4 (0.1 to 2.6)		
			ABA			1.6 (0.4 to 6.0)		
Patients with history of cancer								
≤2013	Dixon 2010 AC&R[68]	BSRBR	3 TNFi	csDMARDs	NR	0.5 (0.1 to 2.2); censoring after first cancer 0.5 (0.1 to 2.2)	NR	Low
Solid cancers								
2013–2016	Mercer 2015 ARD[26]	BSRBR	3 TNFi	csDMARDs	NR	0.8 (0.6 to 1.1)	NR	Low
			ADA			0.8 (0.6 to 1.1)		
			ETA			0.9 (0.7 to 1.2)		
			IFX			0.8 (0.6 to 1.1)		
	Solomon 2014 Semin Arthr Rheum[9]	CORRONA	3 TNFi	MTX	NR	0.2 (0.1 to 0.6)	NR	Low
			RTX			0.3 (0.0 to 3.4)		
			ABA			0.4 (0.1 to 2.2)		
Lymphoma								
≤2013	Askling 2009 ARD[69]	ARTIS	3 TNFi	csDMARDs	Gen population	1.4 (0.8 to 2.1)	**2.7 (1.8 to 4.1)**	Low
	Mariette 2010 ARD[70]	RATIO	3 TNFi	NR	Gen population	NR	**2.3 (1.6 to 3.3)**	Low
	Carmona 2011 Semin Arthritis Rheum[52]	BIOBADASER	3 TNFi	csDMARDs	General population	NR	Hodgkin's lymphoma 5.3 (0.1 to 29.5); non-Hodgkin's lymphoma 1.5 (0.31 to 4.4)	Low

Continued

Table 3 Continued

Year of publication	Study ID	Registry	Intervention	Control csDMARDs	Control general population	aHR (intervention vs comparator/control)	aHR (intervention vs general population)	Risk of bias
	Haynes 2013 A&R[53]	Claim database	3 TNFi	csDMARDs	NR	0.8 (0.3 to 2.1) Ever-analysis 1.3 (0.7 to 2.2)	NR	Moderate
	Pallavicini 2010 Autoimmunity Reviews[54]	LOHREN	3 TNFi	NR	General population	NR	**Milan 6.0 (1.6 to 15.4) to Varese 5.0 (1.3 to 12.7);**	Moderate
2013–2016	Berghen 2015 Clin Rheumatol[25]	Cases from the Leuven University Hospital	3 TNFi	NR	General population	NR	♂ 423.6 (361.9-492.8)† ♀1135 (1003.1-1279.5)	Moderate
Non-melanoma skin cancer								
≤2013	Amari 2011 Rheumatology[71]	Claim database	3 TNFi	csDMARDs	NR	**1.4 (1.2 to 1.6);** **TNFi vs MTX 1.4 (1.2 to 1.7)**	NR	Moderate
	Mercer 2012 ARD[72]	BSRBR	3 TNFi	csDMARDs	General population	BCC 1.0 (0.5 to 1.7), SCC 1.2 (0.4 to 3.8); first cancer per subject BCC 0.8 (0. 5 to 1.5)	**1.7 (1.4 to 2.0)**	Low
	Haynes 2013 A&R[53]	Claim database	3 TNFi	csDMARDs	NR	0.8 (0.5 to 1.4) Ever-analysis 1.1 (0.8 to 1.5)	NR	Moderate
2013–2016	Solomon 2014 Semin Arthr Rheum[9]	CORRONA	3 TNFi	MTX	NR	0.4 (0.1 to 1.2)	NR	Low
			RTX			0.7 (0.0 to 13.6)		
			ABA			**15.3 (2.1 to 114.0)‡**		
Melanoma								
≤2013	Raaschou 2013 BMJ[56]	ARTIS	5 TNFi	csDMARDs	NR	**1.5 (1.0 to 2.2)**	NR	Low

Estimates in bold reflect a risk/ratio statistically significantly different from 1, ie association is statistically significant.
More details are found in online supplementary tables S32–S38.
*Milan refers the Milan Cancer Report and Varese to the Varese Cancer Registry database. These were the general population comparators used.
†Standardised incidence rates are reported, with the general population as the reference; SIR of 100 means no difference between incidence rates.
‡n=2 cases.
ABA, abatacept; ADA, adalimumab; aHR, adjusted HR; ANA, anakinra; ARTIS, Swedish Biologics Register; BCC, basal cell carcinoma; bDMARDs, biological disease-modifying antirheumatic drugs; BIOBADASER, Spanish Biologics Register; BSRBR, British Society of Rheumatology Biologics Register; CORRONA, Consortium of Rheumatology Researchers of North America; csDMARDs, conventional synthetic disease-modifying antirheumatic drugs; ETA, etanercept; IFX, infliximab; LOHREN, Lombardy Rheumatology Network (Italian Biologics Register); MTX, methotrexate; NA, not available (not mentioned in the original article); NR, not reported; pts, patients; RABBIT, Rheumatoid Arthritis Observation of Biologic Therapy (in German); RATIO, French Biologics Register; RTX, rituximab; SCC, squamous cell carcinoma; TNFi, tumour necrosis factor α inhibitor.

relevant studies should be considered in a future update of this SLR.

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CONCISE REPORT

Efficacy of glucocorticoids, conventional and targeted synthetic disease-modifying antirheumatic drugs: a systematic literature review informing the 2016 update of The EULAR recommendations for the management of rheumatoid arthritis

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ABSTRACT

Objectives To perform a systematic literature review (SLR) informing the 2016 update of the recommendations for the management of rheumatoid arthritis (RA).

Methods An SLR for the period between 2013 and 2016 was undertaken to assess the efficacy of glucocorticoids (GCs), conventional synthetic disease-modifying antirheumatic drugs (csDMARDs) and targeted synthetic DMARDs (tsDMARDs) (tofacitinib and baricitinib) in randomised clinical trials.

Results For GCs, four studies were included in the SLR. Patients without poor prognostic factors experienced benefit when GCs were added to methotrexate (MTX). Lower doses of GCs were similar to higher doses. For csDMARDs, two new studies comparing MTX monotherapy with combination csDMARD were included in the SLR. In the tREACH trial at the end of 12 months no difference between the groups in disease activity, functional ability and radiographic progression was seen, using principles of tight control (treat-to-target). In the CareRA trial, combination therapy with csDMARDs was not superior to MTX monotherapy and monotherapy was better tolerated.

For tsDMARDs, tofacitinib and baricitinib were shown to be more effective than placebo (MTX) in different patient populations.

Conclusions Addition of GCs to csDMARD therapy may be beneficial but the benefits should be balanced against the risk of toxicity. Under tight control conditions MTX monotherapy is not less effective than combination csDMARDs, but better tolerated. Tofacitinib and baricitinib are efficacious in patients with RA, including those with refractory disease.

INTRODUCTION

The landscape of rheumatoid arthritis (RA) treatment has unquestionably changed dramatically during the last decade. The development and introduction to daily clinical practice of disease modifying antirheumatic drugs (DMARDs) as well as earlier diagnosis and treatment, and well defined goals of treatment, have contributed to this treatment revolution. Despite this progress, there are still unmet needs, and a better application of the currently available treatments as well as better treatment strategies are needed. Practical recommendations based on the existing evidence are appropriate tools for the rheumatologists. In 2013 a European League Against Rheumatism (EULAR) task force has revised the previous recommendations on RA treatment.[1] A revision of the 2013 recommendations was now undertaken.

The aim of this review was to inform the new EULAR recommendations[2] on the management of RA on efficacy of glucocorticoids (GCs), conventional synthetic DMARDs (csDMARDs) and two targeted synthetic DMARDs (tsDMARDs), tofacitinib and baricitinib based on new evidence accrued since 2013.[3] The results of this and two other systematic literature reviews (SLRs)[4 5] provided the task force with the current state of evidence.

METHODS

An SLR using MEDLINE, EMBASE and the Cochrane CENTRAL library was performed from January 2013 until February 2016, based on a prespecified PICOS statement: P=population, I=interventions, C=comparators, O=outcomes and S=study design. The population was 'adult RA patients'; the intervention was (1) GCs, (2) csDMARDs (methotrexate (MTX), leflunomide, sulfasalazine, hydroxychloroquine, intramuscular gold, auranofin, azathioprine, ciclosporine, minocycline, D-penicillamine, cyclophosphamide, chlorambucil, mycophenolate, tacrolimus), (as monotherapy or combination therapy) and (3) tsDMARDs (tofacitinib and baricitinib); the comparator was patients not receiving the abovementioned treatments; the outcome pertained to efficacy on disease activity, function, patient reported outcomes (PROs) and structural damage; and the study design always was 'randomised controlled trials' (RCTs). Risk of bias (RoB) was assessed using the Cochrane RoB assessment tool (Cochrane Handbook for Systematic Reviews of Interventions V.5.1.0 March 2011 (cited September 2016); available from: http://handbook.cochrane.org/). ORs for dichotomous measures were

determined to assess the magnitude of treatment effect. The DerSimonian and Laird random-effects model was used to pool the data when possible, allowing for both within-study and between-study variations. Statistical heterogeneity among studies was evaluated using the I^2 statistic and χ^2 test where a p value <0.10 was considered to be statistically significant. A value of above 50% for I^2 was considered to be high. Details about the search and the studies included can be found in the online supplementary material. The selected group of patients included in RCTs as well as the relatively short duration of RCTs, makes addressing long-term safety of drugs in RCTs difficult. For this reason, safety aspects of GCs and csDMARDs were addressed in a separate SLR based on observational studies coming from registries.[5] Some safety issues regarding tsDMARDs will be discussed here, since real life data of tsDMARDs are still lacking.

RESULTS

Efficacy of addition of GCs to csDMARDs

Of 348 hits, 4 studies were included in the analysis (table 1). The selection of articles is shown in online supplementary figure S1. A small study by Menon *et al*[6] showed greater efficacy of a combination of csDMARDs with intra-articular GCs than with csDMARDs alone in patients with RA with less than 2 years disease duration, but this was an open label study with high RoB. In the CareRA trial patients with early RA, but without poor prognostic factors, benefited from the addition of GCs (COBRA-slim) to MTX with no differences in safety observed.[7] The primary end point of this study was not met, since the percentage of patients achieving remission at week 16 was only numerically but not significantly higher in the GC group (65.1% vs 46.8%, p=0.08). However, this substudy analysis did not have sufficient statistical power and had a high RoB, primarily due to lack of blinding.

A non-inferiority trial compared two different GC strategies; the COBRA-light strategy (prednisolone at 30 mg/day, tapered to 7.5 mg/day in 9 weeks) in combination with MTX; and the COBRA strategy, using prednisolone at 60 mg/day (tapered to7.5 mg/day in 6 weeks) in combination with both MTX and sulfasalazine. The lower dose of GCs was efficacious in suppressing clinical disease activity and improving functional ability, but non-inferiority could not be claimed formally.[8 9] The degree of radiographic progression was similar in the two groups (COBRA and COBRA-light). However, this study also had a high RoB (open design), and no comparison with application of conventional GCs was performed.

In a double-blind RCT with patients with established RA, low-dose prednisone with modified release ('chronotherapy') added to existing DMARD treatment in patients with active disease had a significant effect on disease activity and health-related quality of life compared with placebo.[10]

A pooled analysis could not be performed because of significant heterogeneity of the studies regarding designs, patient populations, doses and routes of administration of GCs, and outcome measures. The results of the newer RCTs are in accordance with the previously formulated standpoint that GC when added to csDMARD therapy may have beneficial effects. Safety aspects, as addressed in a separate SLR, have to be taken into consideration.[5] Level of evidence (LOE): 1a.

Efficacy of csDMARDs and csDMARD combinations

In total 518 studies were screened. The selection of articles is shown in online supplementary figure S2. Only two new studies comparing MTX monotherapy with MTX in combination with another csDMARD without differences in GC usage were

Table 1 Randomised controlled trials of glucocorticoids (GCs) added to csDMARDs in RA

Study	Study design	RoB	Disease duration (years)	N. patients	GC regimen	Control group	Primary outcome	Result in GC group	Result in control group	p Value
Menon *et al*[6]	Superiority, open	High	<2	56	i.a. triamcinolone acetonide	No i.a. GCs	DAS28 at week 12 ACR 20/50/70 at week 12*	3.39 100/60/36	4.99 84/20/0	0.001 <0.05
CareRA, Verschueren *et al*[7]	Superiority, open	High	≤1	90	p.o. Prednisolone (step-down from 30 mg/day)	No oral GCs	DAS28 (CRP) <2.6 at week 16	65.1%	46.8%	0.08
CAPRA-2. Buttgereit *et al*[10]	Superiority, double-blind	Low	Mean 8	350	MR prednisone (5 mg/day)	Placebo	ACR20 at week 12	48%	29%	<0.001
den Uyl *et al*[8]† ter Wee *et al*[9]†	Non-inferiority, open	High	≤2	164	COBRA light (step-down from 30 mg/day)	COBRA (step-down from 60 mg/day)	mean ΔDAS44 at week 26	−2.18 (SD 1.1) in COBRA light	−2.50 (SD 1.21) in COBRA	0.08

*Composite primary end point.
†Two separate publications based on the same study.
ACR, American College of Rheumatology; CRP, C reactive protein; csDMARD, conventional synthetic disease-modifying antirheumatic drug; DAS, Disease Activity Score; i.a., intra-articular; MR, modified release; p.o., per os; RA, rheumatoid arthritis; RoB, risk of bias.

included in the SLR. In the tREACH trial, that applied tight control principles, at 12 months, disease activity, functional ability and radiographic progression were similar in the two groups who received csDMARD combination therapy (MTX, sulfasalazine and hydroxychloroquine) with either oral GCs or intramuscular GCs and the group that received MTX monotherapy (see online supplementary table S1). GCs were given either intramuscularly (methylprednisolone 120 mg or triamcinolone 80 mg) or in an oral tapering scheme (weeks 1–4: 15 mg/day, weeks 5–6: 10 mg/day, weeks 7–8: 5 mg/day and weeks 9–10: 2.5 mg/day). In addition, a higher number of medication adjustments due to adverse events (AEs) were applied in the combination group.[11 12] Interestingly, for the two groups on combination therapy, intramuscular and oral GCs were similarly effective as modes of bridging therapy.

In the CareRA trial (in a different subpopulation than the one described above in a different part of the CareRA trial) patients with early RA and risk factors for more aggressive disease did not benefit from combination of MTX with other csDMARDs in comparison to MTX monotherapy (both combined with GCs) (see online supplementary table S1). In these arms GCs were dosed orally using a weekly step-down scheme (30–20–12.5–10–7.5–5 mg prednisone). Monotherapy with MTX was better tolerated.[13] The CareRA trial has a high RoB (open label).

The results of the newer RCTs are in accordance with the previously formulated standpoint that combination of csDMARDs is not better than monotherapy with MTX. The need for more optimal use of csDMARDs, particularly regarding the dose of csDMARDs, however, is obvious. One double-blind RCT failed to show differences between two starting doses of MTX, namely 7.5 mg and 15 mg weekly.[14] In the CONCERTO trial initiating adalimumab+MTX combination therapy, the efficacy of 10 mg/week and 20 mg/week MTX was not statistically different in patients with early RA.[15] One study compared a loading dose of leflunomide (100 mg×1 for 3 days) with a fixed dose of 20 mg daily and did not show differences in efficacy but a better safety profile for the fixed dose.[16] A weekly dose of 50 mg leflunomide showed similar benefits to a daily dose of 10 mg leflunomide for the treatment of mild-to-moderate early RA.[17] The latter however was an open superiority study with a high RoB and 10 mg leflunomide daily is considered a suboptimal dose.

Efficacy of tsDMARDs (tofacitinib and baricitinib)

From the 134 hits on tofacitinib 9 were identified as RCTs (table 2).[18–26] Efficacy of tofacitinib, both as monotherapy and in combination with MTX, was formally proven in different patient populations (MTX-naïve, csDMARD and biological DMARD (bDMARD) inadequate responders) compared with placebo (background MTX). For baricitinib the literature search yielded eight new RCTs (two of them had PROs as main study outcomes) (table 3).[27–34] Similar clinical efficacy of baricitinib in monotherapy and in combination with MTX has been suggested, but only the combination (baricitinib+MTX) significantly inhibited radiographic progression.[29] In the MTX-IR (inadequate responder) RA-BEAM study, comparing adalimumab+MTX versus baricitinib+MTX versus placebo+MTX, showed small but significantly lower responses for adalimumab +MTX versus baricitinib+MTX, but both were higher than placebo+MTX (Disease Activity Score 28-C reactive protein <2.6 19% vs 24% vs 4%) at week 12.[30]

Importantly, baricitinib has now shown efficacy in a refractory RA population after failure of both antitumour necrosis factor (anti-TNF) and non-anti-TNF bDMARDs.[16] All studies had low RoB. The selection of articles for tofacitinib and baricitinib is shown in online supplementary figures S3 and S4, respectively.

No meta-analysis could be performed due to the heterogeneity between the studies. The most commonly found laboratory abnormalities with tofacitinib were mild decreases in neutrophil and lymphocyte counts and mild increases in aminotransferase and creatinine levels, while baricitinib was associated with reductions in haemoglobin levels. The relative risks for serious AEs with tofacitinib and baricitinib compared with placebo were 0.8 (95% CI 0.5 to 1.3) and 1.0 (95% CI 0.6 to 1.7), respectively. However, a significantly increased risk of herpes infection was seen (RR=3.1, 95% CI 1.1 to 8.5) with tofacitinib.

The results of the newer RCTs are in accordance with the previously formulated standpoint that the tsDMARDs (tofacitinib and baricitinib) are effective and safe in the short term. (LOE: 1A)

DISCUSSION

Overall, the results of this review confirmed the previous SLR and expanded the overall insights. Although the evidence on efficacy of short-term GCs when added to csDMARDs is robust and undisputed, there are still concerns regarding long-term safety (such as infections, diabetes, osteoporosis, and gastrointestinal and cardiovascular events). Preliminary long-term results of the CAMERA II trial showed a low occurrence of AEs but suggested for the first time an increased cardiovascular risk for the patients with early RA treated with 10 mg/day prednisone for at least 2 years.[35] These results are still unpublished (abstract in American College of Rheumatology 2015). A separate SLR focusing on the safety of GCs has been performed in order to inform the task force and enable the formation of the recommendations.[5] GC safety aspects have also been addressed in a separate paper prior EULAR activity.[36] Clear consensus regarding the dose and tapering of GCs is still lacking. New data have suggested that short-term lower doses of GCs (starting at 30 mg prednisone per day with rapid tapering), as in the COBRA-light regimen, might be a feasible alternative to the higher doses (starting at 60 mg/day) as in the COBRA regimen, although formal non-inferiority was not proven. In fact, this trial did not fulfil the inclusion criteria for the SLR, since there was no comparator group (group without GCs according to the PICO). However, we decided to include it in the SLR since the question posed is highly clinically relevant.

Interestingly, the tREACH trial has suggested that the efficacy of oral GCs as bridging treatments was not superior to intramuscular GCs. Two new studies were published regarding chronotherapy and intra-articular GC therapy, thus answering one of the research questions posed in 2013. The latter however was a high RoB study.

Regarding the choice of csDMARD combination therapy over monotherapy, again—and in contradiction with the perception of many clinicians—we could not substantiate clear evidence in favour of combination therapy with csDMARDs. Neither the 1-year results of the tREACH, nor those of the CARERA study, showed clear evidence that MTX monotherapy is inferior to combination therapy with csDMARDs when used in combination with GCs and when a tight treat-to-target approach is employed. Importantly, monotherapy was generally better tolerated than combination therapy in these studies. Generally, the complexity of the design of pragmatic trials and certain methodological issues, such as high dropout rates and change of primary end point, make the interpretation of the results challenging.

Table 2 Randomised controlled trials of tofacitinib in RA

Study	Study design	N patients	Patient population	Primary outcome	Tofacitinib monotherapy or in combination with csDMARDs	Comparator arm	Result in tofacitinib arm (5 mg×2)	Result in tofacitinib arm (10 mg×2)	Result in comparator arm	p Value
Lee *et al*[23] ORAL start	Superiority, double-blind	958	DMARD-naive	ACR70 and mean change from baseline SHS at month 6	Monotherapy	MTX	25.5% least-squares mean (±SE)=0.2±0.1	37.7% least-squares mean (±SE)=<0.1 ±0.1	12% least-squares mean (±SE)=0.8 ±0.2	p<0.001 for either dose vs MTX p<0.001 (both tofacitinib groups vs placebo)
Kremer *et al*[22] ORAL sync	Superiority, double-blind	795	csDMARDs and/or bDMARDs IR	ACR20 at month 6 Change in HAQ at month 3 Remission at month 6	Combination with csDMARDs	Placebo with csDMARDs	52.1% −0.44 8.5%	56.6% −0.53 12.5%	30.8% −0.16 2.6%	<0.001 <0.001 <0.005
Van der Heijde *et al*[20] ORAL scan	Superiority, double-blind	800	MTX IR§	ACR20 at month 6	Combination with MTX	Placebo with MTX	51.5%	61.8%	25.3%	<0.001 for both tofacitinib doses vs placebo
				Change in SHS at month 6			0.12	0.06	0.47	<0.05 only for 10 mg tofacitinib vs placebo
				ΔHAQ at month 3			−0.4	−0.54	−0.15	<0.0001 (10 mg)*
				Remission at month 6			7.2%	16%	1.6%	<0.001 (10 mg)*
Burmester *et al*[21] ORAL step	Superiority, double-blind	399	TNFis IR+##	ACR20 at month 3 ΔHAQ at month 3 Remission at month 3	Combination with MTX	Placebo with MTX	41.7% −0.43 6.7%	48.1% −0.46 8.8%	24.4% −0.18 1.7%	<0.001† <0.0001† <0.05†
Tanaka *et al*[24]	Superiority, double-blind	318	csDMARDs and/or bDMARs IR	ACR20 at month 3	Monotherapy	Placebo	73.1%	84.9%	15.4%	<0.0001 both groups vs placebo
Strand *et al*[18] ORAL solo	Superiority, double-blind	611	csDMARDs and/or bDMARDs IR	Multiple PROs	Monotherapy	Placebo	Tofacitinib > placebo			
Strand *et al*[19] ORAL step	Superiority, double-blind	399	≥1 TNFi*	Multiple PROs	Combination with MTX	Placebo with MTX	Tofacitinib > placebo			
Wallenstein *et al*[25]	2 Phase-IIb studies, double-blind	507 (combination with MTX) 384 (monotherapy)	MTX IR	Multiple PROs	Both in combination with MTX and in monotherapy	Placebo	Tofacitinib > placebo (both as monotherapy and in combination with MTX)			
Strand *et al*[26] ORAL standard	Superiority, double-blind	717	MTX IR*	Multiple PROs	Combination with csDMARDs	Placebo	Tofacitinib+MTX > control group			

*Since tofacitinib at 5 mg twice daily failed to be statistically significant for radiographic progression, and due to the step-down procedure applied to primary efficacy end points, significance was not declared for the HAQ DI score or remission (DAS28-ESR <2.6) for tofacitinib at 5 mg twice daily.
†For both groups versus placebo.
ACR, American College of Rheumatology; bDMARD, biological DMARD; csDMARD, conventional synthetic DMARD; DAS, Disease Activity Score; DMARD, disease-modifying antirheumatic drug; HAQ, Health Assessment Questionnaire; IR, inadequate responder; MTX, methotrexate; PROs, patient reported outcomes; RA, rheumatoid arthritis; SHS, van der Heijde modification of the total Sharp Score; TNFi, tumour necrosis factor inhibitor.

Table 3 Randomised controlled trials of baricitinib in RA

Study	Study design	N patients	Patient population	Primary outcome	Baricitinib arm(s)	Comparator arm	Result in baricitinib arm (4 mg)	Result in comparator arm	p Value
Fleischmann *et al*[29] RA-begin*	Phase III, non-inferiority	584	DMARD naive	ACR20 at week 24	Monotherapy or in combination with MTX	MTX	77% (both as monotherapy or in comb with MTX)	62%	<0.01
Keystone *et al*[27]	Phase II, superiority	301	MTX IR	ACR20 at week 12	Baricitinib+MTX	Placebo+MTX	76%	41%	<0.001
Tanaka *et al*[29]	Phase II, superiority	145	MTX IR	ACR20 at week 12	Baricitinib+MTX	Placebo+MTX	77%	31%	<0.001
Taylor *et al*[30] RA-beam	Phase IIII, (Superiority design for baricitinib vs placebo)	1305	MTX IR	ACR2o at week 12	Baricitinib+MTX	Placebo+MTX ADA+MTX	70%	40% in the placebo arm 61% in the ADA arm	<0.001 vs placebo <0.05 vs ADA
Genovese *et al*[31] Smolen *et al*[33] RA-beacon‡	Phase III, superiority	527	bDMARDs IR	ACR20 at week 12 Several PROs	Baricitinib+MTX	Placebo+MTX	55% Baricitinib+MTX>placebo+MTX	27%	<0.001
Dougados *et al*[34] Emery *et al*[32] RA-build	Phase III, superiority	684	csDMARDs IR	ACR20 at week 12 Several PROs	Baricitinib+csDMARD	Placebo +csDMARD	62% Bari>placebo	39%	<0.001

The primary objective evaluated non-inferiority of baricitinib 4 mg monotherapy to MTX on ACR20.
†38% of the patients had a history of treatment with at least one biologic DMARD that was not a TNFi.
ACR, American College of Rheumatology; ADA, adalimumab; bDMARD, biological DMARD; csDMARD, conventional synthetic DMARD; DMARD, disease-modifying antirheumatic drug; IR, inadequate responder; MTX, methotrexate; PROs, patient-reported outcomes; RA, rheumatoid arthritis; TNFi, tumour necrosis factor inhibitor.

There is a clear need for studies addressing the optimal use of csDMARDs. No new studies fulfilling the inclusion criteria regarding dose and route of administration of MTX were identified. A previous SLR by Visser and van der Heijde[37] had addressed this issue.

Tofacitinib is the first JAK inhibitor approved for the treatment of RA in many countries and baricitinib is under regulatory evaluation. This SLR confirmed that tofacitinib has beneficial effects on disease activity, physical function, radiographic progression and PROs, both in patients with early RA who are DMARD-naive and in patients with established disease who have failed csDMARDs and/or bDMARDs. Baricitinib was found to be effective in MTX-naïve patients and also after failure of drugs with multiple modes of action. Data on long-term safety of this new class of DMARDs from real life observational studies are needed. Until then, rheumatologists are advised to take into account safety data obtained through RCTs and follow the labels of each drug, including AEs and lab monitoring.

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CONCISE REPORT

Efficacy of biological disease-modifying antirheumatic drugs: a systematic literature review informing the 2016 update of the EULAR recommendations for the management of rheumatoid arthritis

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ABSTRACT

Objectives To update the evidence for the efficacy of biological disease-modifying antirheumatic drugs (bDMARDs) in patients with rheumatoid arthritis (RA) to inform European League Against Rheumatism (EULAR) Task Force treatment recommendations.

Methods MEDLINE, EMBASE and Cochrane databases were searched for phase III or IV (or phase II, if these studies were lacking) randomised controlled trials (RCTs) published between January 2013 and February 2016. Abstracts from the American College of Rheumatology and EULAR conferences were obtained.

Results The RCTs confirmed greater efficacy with a bDMARD+conventional synthetic DMARD (csDMARD) versus a csDMARDs alone (level 1A evidence). Using a treat-to-target strategy approach, commencing and escalating csDMARD therapy and adding a bDMARD in cases of non-response, is an effective approach (1B). If a bDMARD had failed, improvements in clinical response were seen on switching to another bDMARD (1A), but no clear advantage was seen for switching to an agent with another mode of action. Maintenance of clinical response in patients in remission or low disease activity was best when continuing rather than stopping a bDMARD, but bDMARD dose reduction or 'spacing' was possible, with a substantial proportion of patients achieving bDMARD-free remission (2B). RCTs have also demonstrated efficacy of several new bDMARDs and biosimilar DMARDs (1B).

Conclusions This systematic literature review consistently confirmed the previously reported efficacy of bDMARDs in RA and provided additional information on bDMARD switching and dose reduction.

INTRODUCTION

Since the 2013 systematic literature review (SLR) on biological disease-modifying antirheumatic drugs (bDMARDs) in rheumatoid arthritis (RA),[1] there have been several trials addressing efficacy and safety of various established bDMARDs, looking at different aspects of therapy including induction, switching, tapering and stopping of bDMARDs. There have also been publications on new bDMARDs, including some with new modes of action, as well as on a number of biosimilar DMARDs (bsDMARDs).

Many clinical trials provide direct comparisons between a bDMARD and a conventional synthetic DMARD (csDMARD). The use of treat-to-target strategies,[2] however, better reflects real-life treatment approaches and therefore provides additional evidence for the use of these therapies in clinical practice. This SLR therefore also sought to provide an update on bDMARD strategy studies, previously defined as 'clinical trial(s) of any treatment of RA in which at least one arm consists of medication adjustment according to protocol, based on clinical outcomes aiming at a specific target'.[3]

This SLR aimed to update the body of evidence with information that has emerged since 2013 regarding the use of bDMARDs in RA. The results of this SLR and two others[4 5] provided the task force with the current state of evidence.

METHODS

The updated standard operating procedures by European League Against Rheumatism (EULAR) were followed.[6] As before,[1 7] studies on the following nine bDMARDs were included: adalimumab (ADA), certolizumab-pegol (CZP), etanercept (ETN), golimumab (GLM), infliximab (IFX), anakinra (ANA), abatacept (ABT), rituximab (RTX) and tocilizumab (TCZ).[1 7] Information was also sought on new bDMARDs, including bsDMARDs. The search was performed using MEDLINE, EMBASE and Cochrane CENTRAL databases between January 2013 and February 2016. Relevant abstracts were sought from the 2013–2015 American College of Rheumatology (ACR) and 2014–2016 EULAR conferences.

The study selection criteria were the same as those in previous EULAR bDMARD SLRs.[1 7] The Cochrane risk of bias assessment tool for RevMan 5.1[8] was used to assess the quality of published studies and the Oxford Centre for Evidence-based Medicine levels of evidence[9] was used to assign

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levels of evidence. Details on the search strategy can be found in the online supplementary material.

RESULTS

Of 10 187 articles from the database search, together with additional ACR and EULAR conference abstracts and articles found after the database search, 51 published papers and 35 abstracts met the inclusion criteria. Risk of bias was considered 'low' for most but not all studies. Open-label trials were assigned 'high risk of bias' for the category 'blinding of participants and personnel' (see online supplementary material).

Efficacy data are presented in five sections: (1) bDMARD efficacy trials (in combination with a csDMARD or as monotherapy); (2) bDMARD strategy trials; (3) bDMARD switching trials; (4) bDMARD stopping or dose reduction trials and (4) trials with new therapies (new bDMARDs and bsDMARDs, and bDMARDs versus a targeted synthetic DMARD (tsDMARD)).

Patients with RA were grouped as follows: (1) DMARD-naive, (2) methotrexate (MTX)-naive, (3) MTX-inadequate response (IR), (4) csDMARD-IR (mixed DMARD-IR) and (5) tumor necrosis factor inhibitor (TNFi) TNFi-IR. This is highlighted for each study and studies are divided accordingly in the online supplementary section.

Biological DMARD efficacy

The focus of the results was on the primary outcomes. Other efficacy outcomes are presented in the online supplementary section.

Existing biological DMARD+csDMARD combination versus csDMARD

Nine new studies have been published after 2013 confirming evidence for the efficacy of a bDMARD+csDMARD versus a csDMARD.[10–18] In DMARD-naive RA (2010 ACR/EULAR[19]), the C-EARLY[10] study met its primary endpoint of sustained Disease Activity Score using a 28 joint count (DAS28)<2.6 between weeks 40 and 52 (CZP+MTX vs placebo+MTX: 29% vs 15%). In MTX-naive RA, C-OPERA confirmed better efficacy of CZP+MTX compared with MTX alone.[14] CARDERA-2 failed to demonstrate radiological superiority of ANA+MTX versus MTX monotherapy.[13] In mixed DMARD-IR patients, subcutaneous TCZ+MTX was superior to background MTX in the BREVACTA study (ACR 20 at week 24: 61% vs 32% at week 24),[17] and RTX+background leflunomide to leflunomide in the AMARA study.[18]

The results of these new randomised controlled trials (RCTs) are in accordance with the previously formulated standpoint that a combination of a bDMARD and a csDMARD is more effective than a csDMARD alone. Level of evidence (LOE) as in the previous SLRs:[1] 1A.

Biological DMARD+MTX combination versus biological DMARD monotherapy

In the MTX-naive RA AVERT study, a status of DAS28<2.6 was more often achieved with ABT+MTX than with MTX monotherapy or ABT monotherapy at 12 months (60.9% vs 45.2% vs 42.5%). The FUNCTION study also showed higher proportions of patients with DAS remission and ACR responses—and less radiographic progression—with TCZ 8 mg/kg+MTX compared with TCZ monotherapy.[20 21] TCZ monotherapy had more DAS28<2.6 and less radiographic progression than MTX monotherapy, but most other secondary endpoints, including physical function, were not different.

In MTX- IR RA patients, the SURPRISE study showed at week 24, the time of the primary endpoint, that a status of DAS28<2.6 was more often achieved when adding TCZ to MTX versus switching from MTX to TCZ (70% vs 55%).[22] This modest benefit had disappeared at week 52 (72% vs 70%). Clinically relevant radiographic progression was lower with TCZ+MTX combination therapy than with TCZ monotherapy (van der Heijde-Sharp score ≥3: 7% vs 15%).

The results of the newer RCTs are in accordance with the previously formulated standpoint that a combination of any bDMARD and a csDMARD is more effective than bDMARD monotherapy (LOE as in the previous SLRs:[1] 1B).

Biological strategy-type studies

In the U-Act-Early RCT, MTX-naive patients were randomised to TCZ+MTX, TCZ monotherapy or MTX monotherapy using a treat-to-target approach.[23] The primary analysis (number of people achieving sustained DAS28<2.6 by the originally assigned treatment) was higher in the TCZ+MTX or TCZ monotherapy groups than the MTX monotherapy group (86% vs 84% vs 44%). In the clinically more relevant second analysis, and co-primary endpoint, which addresses the entire study period, the initial differences between the groups were no longer seen with the addition of TCZ in the MTX monotherapy group following a treat-to-target approach (86% vs 88% vs 77%).

In TACIT, a non-inferiority RCT in MTX-IR RA who had failed MTX and another csDMARD,[24] patients were randomised to either a strategy of TNFi-start, followed by a switch to a second bDMARD in case of no response, or to a strategy of combination csDMARD therapy, followed by the start of a bDMARD in case of non-response. The change in Health Assessment Questionnaire (HAQ) score after 12 months (primary endpoint) was not inferior for the strategy starting with combination csDMARD versus the strategy starting with TNFi (−0.45 vs −0.3). While earlier clinical responses were seen in the TNFi strategy, a status of DAS28<2.6 at 12 months was met by slightly more patients in the TNF start group than in the csDMARD group (44% vs 35%). Of note this was an open-label study with a (too) large non-inferiority margin that importantly limits its interpretability. Thus, the study had a high risk of bias. Adverse events were more frequently found in the csDMARD combination group.

Ten-year data from all four arms of the BeSt trial suggested that a high proportion of patients (53%) maintain long-term remission, either on drugs or drug free, and had very limited 10-year radiographic progression, confirming the effectiveness of early DMARD treatment together with a treat-to-target approach.[25]

The results of the newer RCTs are therefore in accordance with the previously formulated standpoint that strategies aiming at benchmarking disease activity and intensifying treatment when clinical remission or low disease activity is not yet reached may lead to favourable outcomes (LOE: 1B).

Switching between bDMARDs in TNFi-IR RA

Previous meta-analyses of RCTs had already demonstrated efficacy of all bDMARD classes in patients failing a TNFi (TNFi-IR) (LOE: 1A).[7 26] To date, new bDMARD switching trials of this type could not be found.

Patients from the DREAM cohort, who had failed a first TNFi and had DAS28≥3.2, were randomised to receive ABT or RTX, or a second TNFi in a trial with a non-inferiority design. The mean (SD) 12-month DAS28 were 3.8 (1.2) versus 3.4 (1.2) versus 3.5 (1.5) in the ABT, RTX and TNFi groups,

respectively.[27] In the ROC trial, patients who failed their first TNFi were randomised to either a second TNFi or to another mode of action bDMARD (ABT, RTX or TCZ).[28] At week 48, EULAR good response was 60% with a non-TNFi bDMARD versus 43.2% for a second TNFi.

The results of the newer RCTs are in accordance with the previously formulated standpoint that patients who have failed their first TNFi may expect benefit from a second TNFi or from a non-TNFi biological (LOE: 1A). There is insufficient evidence to prioritise either strategy.

Biological DMARD stopping or dose reduction

Biological DMARD stopping

In patients with MTX-naive RA, the AVERT trial[11] showed that patients with DAS28<3.2 on ABT+MTX, ABT or MTX maintained their drug-free status (DAS28<2.6, both at 12 and 18 months) in only 14.8% after stopping ABT+MTX, 12.4% after stopping ABT and 7.8% after stopping MTX.

In patients with MTX-IR RA, in the ENCOURAGE study, patients with DAS28<2.6 on ETN+MTX at 6 and 12 months were randomly assigned to strategies stopping or continuing their treatment. There were higher proportions of patients with DAS28<2.6 when continuing medication (88%) versus withdrawing ETN and continuing MTX (54%).[29]

In patients with MTX-IR RA that had participated in the ACT-RAY study, a follow-up study showed that in those with sustained DAS28<2.6 and discontinued TCZ only 38.4% of the TCZ+MTX group and 35.1% of the TCZ monotherapy group maintained that state for an average of 3 months.[30] The majority of those who lost response (84%) responded well to TCZ reintroduction, but 16% did not.

The results of the newer RCTs are in accordance with the previously formulated standpoint that a variable but relatively low proportion of patients who have sustained low disease activity or remission on a strategy with a bDMARD can *stop* that bDMARD (and continue MTX) without losing their status of low disease activity/remission (LOE: 2B).

Biological DMARD dose reduction

In MTX-naive RA patients, in a substudy of AGREE, patients with a DAS28 erythrocyte sedimentation rate (DAS28-ESR) <2.6 at year 2 on ABT 10 mg/kg+MTX were randomised to ABT 10 mg/kg (full dose)+MTX versus ABT 5 mg/kg (half dose)+MTX.[31] Similar relapse rates were seen in both groups (31% in the ABT 10 mg/kg and 34% in the 5 mg/kg groups).

The open-label non-inferiority DRESS RCT, in which patients in stable low disease activity on ADA or ETN were randomised to usual care or a dose reduction strategy (stepwise increase in injection intervals), showed that continuation versus dose reduction led to similar rates of 'major flare' (10% vs 12%).[32]

In the OPTIRRA RCT, patients in stable (3 months) low disease activity (DAS28<3.2) on ADA or ETN were randomised to continue ADA or ETN, taper ADA or ETN by 33% or taper ADA or ETN by 66%.[33] Similar flare rates were seen in the continuation and ADA or ETN 33% tapering group (14% vs 13%), but a higher rate in the ADA or ETN 66% tapering group (37%).

The SMART[34] study, in which TNFi-IR RA patients who achieved a EULAR (moderate or good) response on standard dose RTX were randomised to receive RTX 1000 mg once or RTX 1000 mg twice, suggested non-inferiority of both strategies (adjusted mean difference in DAS28-C reactive protein (DAS28-CRP) area under the curve 51.4 (95% CI −13.2 to 234)).

The results of the newer RCTs are in accordance with the previously formulated standpoint that a significant proportion of patients who have sustained low disease activity on a strategy with a bDMARD can *taper* that bDMARD (and continue MTX) without losing their status of low disease activity and that reducing the dose of the bDMARD by up to 50% or increasing the interval between doses accordingly conveys similar results as continuing full dose (LOE: 2B).

bDMARDs in comparison to new therapies

Existing bDMARDs versus new targeted synthetic DMARDs

In the MTX-IR RA-BEAM study, comparing ADA+MTX versus the tsDMARD baricitinib+MTX versus placebo+MTX, showed small but significantly lower responses for ADA+MTX versus baricitinib+MTX, but both were higher than placebo+MTX (DAS28-CRP<2.6 19% vs 24% vs 4%) at week 12.[35]

New biological DMARDs

Several new bDMARDs targeting well-known targets have undergone phase II or III clinical trials in MTX-IR or mixed-DMARD-IR RA patients and have consistently shown superiority in clinical responses versus placebo. These include the human interleukin (IL)-6-receptor-inhibitor sarilumab,[36] the humanised anti-IL6 clazakizumab,[37] the human anti-IL6 sirukumab[38] and also the granulocyte-monocyte colony stimulating factor receptor alpha inhibitor (GMCSFrα-i) mavrilimumab.[39] On the other hand, bDMARDs targeting the IL12/23p40-pathway (ustekinumab),[40] the IL23p19-pathway (guselkumab)[40] and the B-cell-activating factor (tabalumab)[41–43] have not demonstrated clinical efficacy over placebo in RA.

Studies have also formally demonstrated efficacy for sirukumab[38] and sarilumab[44] in patients previously exposed to other bDMARDs.

Biosimilar DMARDs

The long-term observational study of the PLANETRA trial has suggested sustained efficacy of those treated with the bsDMARD IFX CT-P13.[45] IFX CT-P13 also demonstrated clinical efficacy in another RCT of MTX-IR RA.[46] Efficacy was also formally proven in placebo-controlled RCTs with the ADA bsDMARDs ABP501[47] and SB5,[48] with the ETN bsDMARDs HD203[49] and SB4,[50] with the IFX bsDMARD SB2[51] and with the RTX bsDMARD BCD-020.[52]

The results of the newer RCTs are in accordance with the previously formulated standpoint that targeting the IL6-pathway, now including also the IL-6 ligand, may provide benefits to patients, that targeting the cytokine GMCSF is potentially beneficial to patients and that bsDMARDs are as effective biologicals as the originator bDMARDs in the treatment of patients with RA.

DISCUSSION

This review on bDMARDs in RA aimed to provide a systematic update of the body of evidence available for the treatment of patients with RA with bDMARDs. It only includes new data from 2013 onwards. These data were presented to the expert committee that convened to discuss the 2016 update of the EULAR recommendations on the (drug) management of patients with RA.[53]

The results of this SLR confirmed the efficacy of bDMARDs in combination with a csDMARD (ADA, CZP, ETN, GLM, IFX, ABT, RTX and TCZ but not ANA).[13] Combination therapy (bDMARD+csDMARD) was in general again found to be superior to bDMARD monotherapy.

Remarkably, we did not find any new 'head-to-head' trial with bDMARDs published after 2013 in this highly competitive field of high-cost drug treatment in RA. Investigators of

sponsored trials usually sought (reconfirmation of) superiority over placebo or engaged in low-commercial-risk strategy trials that reconfirmed the already inarguable efficacy of their bDMARD over placebo.

What is needed in the field of RA, known for its high number of very effective but costly treatments, is a proper evidence-based prioritisation of the drugs we have available. Guideline committees such as ours have to base their consensus on solid data stemming from direct comparisons of treatments. In the absence of high-quality *direct* comparisons, methodologists and (company) statisticians find escape routes in *indirect* comparisons and network meta-analyses. We will not dispute the modest merits of network meta-analyses, but warn against the careless interpretation of their results, since no (network) meta-analysis is methodologically better than the weakest trial contributing to it.

The most important findings in this update SLR were as follows: patients on MTX monotherapy achieved sustained remission when following a treat-to-target strategy.[23] Results from new strategy studies[23 24] in this regard support those from previous RCTs[1 3] and allow a firm conclusion: a treat-to-target approach, escalating csDMARD therapy and adding a bDMARD in cases of non-response, is an effective approach.

New trials in patients who have failed their first TNFi show that switching to a second bDMARD 'makes sense'. However, the current RCTs do not help us in deciding if this second bDMARD should be a TNFi DMARD or a non-TNFi DMARD. Sparse data that are currently available are not convincing. It may, for instance, be relevant that a patient has not had any response to bDMARD from its initiation (primary non-response) or that an initial response was lost over time (secondary non-response). Evidence from RCTs that may help answering such questions is still lacking.

Recently we have faced the advent of several bsDMARDs. Many of these have passed the hurdle of regulatory 'biosimilarity' and have entered the market or will do this soon. To date, there is no scientific indication that these bsDMARDs, which are already less expensive in some countries than their originator counterparts, are inferior to their 'parents' in efficacy or safety. In the absence of tangible distinctions between originator bDMARDs and their bsDMARDs, future guideline committees will likely base their priority on non-scientific arguments such as drug costs.

In general, patients with RA that have achieved low disease activity or remission are better off with continuation of their treatment than with stopping, but many of the patients can successfully apply bDMARD dose reduction or interval increase, and if a flare occurs most of them will regain disease control upon restarting their bDMARD. Prognostic factors that may help determining which patient subgroups are able to de-escalate therapy and achieve drug-free remission are needed.[54] Several studies have addressed these[55–58] but we could not find RCTs in which patients had been stratified according to prognostic factors for tapering.

Comparative data with the tsDMARD baricitinib suggested superior efficacy of baricitinib over ADA, but it remains to be seen if this short-term gain remains over time. Obviously, long-term data on safety still have to be awaited before a proper valuation can take place. There were also several RCTs demonstrating efficacy of new mode-of-action bDMARDs and bsDMARDs in RA.

As before, the sole source of efficacy studies in this SLR was RCTs. While registry data may provide real-life efficacy data, these are prone to bias and have not been included in this SLR. Registry data, however, are crucial to evaluate long-term drug safety and have been used in the EULAR SLR addressing DMARD safety.[4]

In conclusion, this literature review consistently confirmed the efficacy of bDMARDs in RA. It provides some evidence for bDMARD stopping and dose reduction, addressed the important topic of bDMARD switching in TNFi-IR RA and highlighted the advent of some new biological therapies.

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OPEN ACCESS

EXTENDED REPORT

Mitochondrial DNA haplogroups influence the risk of incident knee osteoarthritis in OAI and CHECK cohorts. A meta-analysis and functional study

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ABSTRACT

Objective To evaluate the influence of the mitochondrial DNA (mtDNA) haplogroups in the risk of incident knee osteoarthritis (OA) and to explain the functional consequences of this association to identify potential diagnostic biomarkers and therapeutic targets.

Methods Two prospective cohorts contributed participants. The osteoarthritis initiative (OAI) included 2579 subjects of the incidence subcohort, and the cohort hip and cohort knee (CHECK) included 635, both with 8-year follow-up. The analysis included the association of mtDNA haplogroups with the rate of incident knee OA in subjects from both cohorts followed by a subsequent meta-analysis. Transmitochondrial cybrids harbouring haplogroup J or H were constructed to detect differences between them in relation to physiological features including specific mitochondrial metabolic parameters, reactive oxygen species production, oxidative stress and apoptosis.

Results Compared with H, the haplogroup J associates with decreased risk of incident knee OA in subjects from OAI (HR=0.680; 95% CI 0.470 to 0.968; p<0.05) and CHECK (HR=0.728; 95% CI 0.469 to 0.998; p<0.05). The subsequent meta-analysis including 3214 cases showed that the haplogroup J associates with a lower risk of incident knee OA (HR=0.702; 95% CI 0.541 to 0.912; p=0.008). J cybrids show a lower free radical production, higher cell survival under oxidative stress conditions, lower grade of apoptosis as well as lower expression of the mitochondrially related pro-apoptotic gene BCL2 binding component 3 (BBC3). In addition, J cybrids also show a lower mitochondrial respiration and glycolysis leading to decreased ATP production.

Conclusions The physiological effects of the haplogroup J are beneficial to have a lower rate of incident knee OA over time. Potential drugs to treat OA could focus on emulating the mitochondrial behaviour of this haplogroup.

INTRODUCTION

Osteoarthritis (OA) is a chronic progressive disorder involving movable joints characterised by cell stress and extracellular matrix degradation initiated by micro-injury and macro-injury that activates maladaptive repair responses including pro-inflammatory pathways of innate immunity. The disease manifests first as a molecular derangement (abnormal tissue metabolism) followed by anatomical and/or physiological derangements (characterised by cartilage degradation, bone remodelling, osteophyte formation, joint inflammation and loss of normal joint function) that can culminate in illness.[1]

OA is the most common form of arthritis, occurring in 10%–20% of the population aged over 50 years, and it is estimated that the population with OA will double in the next 30 years.[2] Thus, the identification of risk factors that accelerate disease progression is critical since these factors could become potential targets for disease modification.[3] To achieve this objective, the use of well-characterised study cohorts to identify prognostic factors that predict the course of OA, as well as to identify markers for the (early) diagnosis and course of joint damage, becomes imperative. In this sense, both osteoarthritis initiative (OAI) and cohort hip and cohort knee (CHECK) stand out.

OA is a heterogeneous disease in which a combination of modifiable (ie, body mass index (BMI), joint injury) and non-modifiable factors (ie, age, gender, genetics) interact. Thus, the pathogenesis of OA is still unclear; however, it is widely accepted that genetics plays a main role in the prevalence and progression of this disease;[4] even prediction tools for knee OA based on genetic and clinical information have been developed.[5 6] Besides, during the last years, increasing evidence points to the implication of the mitochondria and the mitochondrial DNA (mtDNA) haplogroups in the pathogenesis of the disease.[2 7 8]

The mtDNA haplogroups are the result of maternally inherited mutations in the mtDNA acquired throughout the human history and shaped by the climate selection when humans migrated into colder climates.[9 10] Each of the mtDNA haplogroups harbours specific single nucleotide polymorphisms (SNPs) that influence the behaviour of the mitochondria[11] and interact with the nuclear genome,[12] influencing our health today.[10] Some of these genetic variants have been associated with degenerative disorders,[13] metabolic alterations[14] or even increased longevity in humans.[15]

Regarding OA, several studies also reported the association of specific haplogroups with a lower prevalence of the disease,[16–19] while others did not.[20] Moreover, recent studies showed significant

associations of specific haplogroups with a lower rate of radiographic progression and cartilage loss over time in different study cohorts worldwide such as the OAI[21] and one Spanish cohort.[22]

In an attempt to ascertain the correlation of mtDNA haplogroups and the rate of incident knee OA, in this work we performed a replication study and meta-analysis of two individual studies involving subjects from CHECK and OAI cohorts. In addition, functional studies using transmitochondrial cybrids were performed to ascertain the cellular mechanisms underlying the association of mtDNA variants with the pathogenesis of the disease.

METHODS

Incident knee OA study in subjects from the OAI and CHECK cohorts

Participants

The description of CHECK and OAI cohorts is included in the online supplementary methods section. For this study, we included longitudinal data, in terms of Kellgren and Lawrence (KL) grade, of 2579 participants of the incidence subcohort of the OAI and 635 CHECK participants that met the eligibility criteria for an incident knee OA study monitored at predefined intervals for a period of 8 years.

Incident knee OA criteria

The incidence of radiographic knee OA was defined on joint level (knees separately) following the appropriate proposed criteria for each of the two cohorts. In OAI subjects, we followed the definition of incident knee OA proposed by Felson *et al*[23] consisted in a new-onset KL grade ≥2 from baseline to follow-up. In CHECK subjects, we followed the proposed definition of incident knee OA for this cohort[24 25] consisted in a new-onset KL grade ≥1 from baseline to follow-up in accordance with the very early stage OA in CHECK subjects.

mtDNA haplogroups genotyping

The mtDNA haplogroups were assigned in 2579 DNA samples belonging to the OAI and 635 from CHECK following a previously described assay[16] (see online supplementary methods).

Statistical analysis

All the statistical analyses were performed using IBM-SPSS software V.19 (IBM, Armonk, New York, USA) and R software V.3.1.2 (The R Foundation for Statistical Computing). All comparisons were two-sided, with $p<0.05$ defined as statistically significant.

To avoid potential biases associated with the use of standard survival analysis in this context, interval-censored data analysis methods were used (see supplementary methods section). The multivariate analysis was performed by comparisons between haplogroups considering the most common haplogroup H as the reference group. Since there was no interest in all possible pairwise comparisons, no additional adjusting for multiple comparisons was done.

Meta-analysis

In this work, we performed a meta-analysis of incident knee OA including data from CHECK and OAI. Following the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines, we conducted a previous computerised search strategy to find other possible relevant studies to include in the meta-analysis (see supplementary methods).

The random-effects model described by DerSimonian and Laird[26] was used to calculate a summary statistic and its 95% CI. Adjusted HRs were used as the effect size measure for the association between mtDNA haplogroups and OA incidence. Meta-analysis results were presented on a forest plot graph. To explore heterogeneity, the I^2 index was computed. Meta-analysis was carried out using the R software programme (V.3.2.2), using the meta-package. A two-tailed p value <0.05 was considered to be significant. Meta-analysis was planned to be performed on $k=2$ studies, with an estimated statistical power of 91.9% to detect as statistically significant an HR ≤ 0.5 associated with each haplogroup, with a $p=0.05$ two-tailed significance level.

We only selected studies that met the following inclusion criteria: (i) evaluating the association between mtDNA haplogroups and the rate of incident OA over time; (ii) with sufficient data provided to calculate HRs with their corresponding 95% CI. On the contrary, those studies analysing the correlation between mtDNA haplogroups and the prevalence or risk of OA as well as those studies analysing other mtDNA mutations with incidence were excluded.

Functional studies using transmitochondrial cybrids

An extended version of the methodology described herein is available in the online supplementary methods.

Cybrid cultures and culture conditions

The human osteosarcoma cell line 143B.TK$^-$ Rho-0 was used to generate the transmitochondrial cybrids by fusing them with platelets from healthy donors carrying the haplogroups J or H following the protocol described by Chomyn,[27] such that we finally obtained two types of cybrids: cybrids harbouring haplogroup J and cybrids harbouring haplogroup H.

Flux assay measurements

Extracellular acidification rate (ECAR) (largely result of glycolysis) and oxygen consumption rate (OCR) (indicator of mitochondrial respiration) was determined by direct measurement in a SeaHorse XFp extracellular flux analyser instrument (Seahorse Bioscience, Agilent Technologies) following manufacturer's instructions.

Mitochondrial reactive oxygen species production assay

Mitochondrial peroxide and peroxynitrite were evaluated with dihydrorhodamine 123 (Sigma) and mitochondrial superoxide anion production was assessed with MitoSox Red (Thermo Fisher Scientific).

Oxidative stress response assay: viability assay

After incubation of cells with 300 μM H_2O_2, the cell viability was measured using CellTiter 96 Aqueous Assay (Promega) following manufacturer's recommendations.

Apoptosis assay

Cells were cultured in presence of staurosporine at 0.2 μM during 2 hours to induce apoptosis and were subsequently washed and resuspended in 1X Annexin-binding buffer; then 5 μL of the Annexin V-fluorescein isothiocyanate and 5 μL of propidium iodide (ImmunoStep) were added to each 100 μL of cell suspension.

In addition, the basal expression of the mitochondrial apoptotic-related genes B-cell CLL/lymphoma 2-like 13 (BCL2L13) and BCL2 binding component 3 (BBC3) in H and J cybrids was also quantified.

Statistical analysis

Results were expressed as the mean of three independent experiments (mean±SD) using two cybrids (two J cybrids and two H cybrids) from two individuals (two different J individuals and two different H individuals) and two clones from each cybrid. Statistically significant differences between the two groups were determined with t-test; p values <0.05 were considered to be significant.

RESULTS

mtDNA haplogroups and incident knee OA in subjects of the OAI cohort

No significant differences were detected in the distribution of age, BMI, Western Ontario and McMaster Universities Arthritis Index (WOMAC) and contralateral OA at baseline among patients with different haplogroups; however, a significantly different gender distribution was identified, both being haplogroups H and J that showed an increased percentage of females than the rest of haplogroups (table 1).

Cumulative probability of incident knee OA at 8 years was 25.1%. Subjects with the haplogroup J showed the lowest rate of incident knee OA over time, 20.1% (adjusted HR=0.680; 95% CI 0.470 to 0.968; p<0.05). In addition, males had a decreased risk too (adjusted HR=0.590; 95% CI 0.491 to 0.712; p<0.05), meanwhile BMI (adjusted HR=1.066; 95% CI 1.047 to 1.085; p<0.05), total WOMAC (adjusted HR=1.018; 95% CI 1.010 to 1.026;p<0.05) and contralateral knee OA (adjusted HR=1.593; 95% CI 1.328 to 1.900; p<0.05) at baseline were risk factors for a higher risk of incident knee OA (table 2).

mtDNA haplogroups and incident knee OA in subjects of CHECK cohort

No significant differences were detected in the distribution of age, gender, BMI, WOMAC and contralateral OA at baseline among patients with different haplogroups (table 1).

Global cumulative probability of incident knee OA at 8 years was 89.7%. The cumulative knee OA incidence was significantly lower in carriers of the haplogroup J, with 82.1% (adjusted HR=0.728; 95% CI 0.469 to 0.998; p<0.05). In addition, the model also showed that total WOMAC (adjusted HR=1.006; 95% CI 1.001 to 1.012; p<0.05) and contralateral knee OA (adjusted HR=1.313; 95% CI 1.087 to 1.576; p<0.05) at baseline were risk factors to develop incident knee OA (table 2).

Meta-analysis

The search process identified a total of seven non-duplicated articles and no unpublished article[16 18 22 28–31] (see online supplementary table S1). However, after reading the titles and abstracts none of these seven articles was subsequently selected for meta-analysis because they did not follow the inclusion criteria; namely, they did not evaluate the association between mtDNA haplogroups and the rate of incident knee OA over time but analysed the association between these mtDNA variants, or any other mtDNA mutations, and the prevalence of OA in cross-sectional studies. In conclusion, only the two association studies performed herein were selected for subsequent meta-analysis reaching a total of 3214 subjects (2579 patients from the OAI and 635 patients from CHECK) (table 3).

No between-study heterogeneity was detected for the haplogroup J (I^2=0%; p=0.798), however, the random-effects model was evenly used. Both studies showed a similar contribution or relative weight (W), being slightly higher in the OAI

Table 1 Demographic and clinical characteristics at baseline of the incidence subcohort of the OAI and CHECK cohorts grouped by mtDNA haplogroups

	OAI						CHECK					
mtDNA haplogroups	H (N=1042, 40.4%)	UK (N=612, 23.7%)	T (N=275, 10.7%)	J (N=236, 9.2%)	Others* (N=414, 16.1%)	p Value	Total (N=2579)	H (N=269, 42.4%)	UK (N=144, 22.7%)	T (N=68, 10.7%)	J (N=56, 8.8%)	Others* (N=98, 15.4%)	p Value	Total (N=635)
Age at baseline (years)	60.8±9.4	61.1±9.1	60.4±9.0	60.1±9.4	60.6±9.2	0.597†	60.7±9.3	55.2±5.3	56.1±5.2	55.6±5.5	57.0±5.1	55.2±5.2	0.110†	55.6±5.3
Gender						**0.024‡**							0.247‡	
Male	437 (41.9)	297 (48.5)	134 (48.7)	100 (42.4)	201 (48.6)		1169 (45.3)	57 (21.2)	25 (17.4)	21 (30.9)	12 (21.4)	24 (24.5)		139 (21.9)
Female	605 (58.1)	315 (51.5)	141 (51.3)	136 (57.6)	213 (51.4)		1410 (54.7)	212 (78.8)	119 (82.6)	47 (69.1)	44 (78.6)	74 (75.5)		496 (78.1)
BMI (kg/m^2)	27.4±4.4	27.5±4.6	27.9±4.6	27.5±4.4	27.7±4.4	0.500†	27.5±4.5	25.8±3.9	25.8±3.6	26.1±3.4	26.0±3.4	25.9±4.1	0.892†	25.9±3.8
WOMAC														
Pain	3.2±4.3	3.2±4.2	2.9±3.4	2.8±3.3	3.6±4.5	0.675†	3.2±4.2	4.4±3.2	5.1±3.5	4.8±3.0	4.8±3.4	4.8±3.6	0.489†	4.7±3.3
Stiffness	2.2±2.4	2.2±2.4	2.2±2.4	2.2±2.1	2.4±2.5	0.805†	2.2±2.4	2.5±1.6	2.5±1.7	2.7±1.7	2.8±1.9	2.7±1.7	0.754†	2.6±1.7
Disability	10.2±14.4	11.0±15.5	9.3±12.6	9.5±11.9	11.3±14.7	0.573†	10.4±14.3	14.1±10.8	15.5±11.3	15.0±10.9	16.8±12.3	15.3±11.8	0.627†	14.9±11.2
Total	15.6±20.1	16.5±21.1	14.4±17.3	14.6±16.1	17.3±20.5	0.540†	15.8±19.8	20.9±14.6	23.2±15.5	22.6±14.7	23.6±16.4	22.9±16.3	0.673†	22.2±15.2
Contralateral OA at baseline	402 (38.6)	234 (38.2)	110 (40.0)	89 (37.7)	139 (33.6)	0.402‡	974 (37.8)	88 (32.7)	40 (27.8)	26 (38.2)	24 (42.9)	29 (29.6)	0.232‡	207 (32.6)

Values are mean±SD or number of patients with percentage in parentheses.
*The group 'others' include mtDNA variants with a frequency below 5%.
†Kruskal-Wallis non-parametric test for comparison between mtDNA haplogroups.
‡χ^2 test; statistical significance declared at p≤0.05 (in bold).
BMI, body mass index; CHECK, cohort hip and cohort knee; mtDNA, mitochondrial DNA; OA, osteoarthritis; OAI, osteoarthritis initiative, WOMAC, Western Ontario and McMaster Universities Arthritis Index.

Table 2 Cumulative probability of incident knee OA at 8 years in subjects from the OAI and CHECK cohorts according to mtDNA haplogroups and results of the extended Cox proportional hazard model

Variables	Cumulative knee OA incidence at 8 years n (%)*	Adjusted HR	95% CI†
OAI			
Gender (male)		**0.590**	**0.491 to 0.712‡**
Age		1.007	0.997 to 1.017
BMI		**1.066**	**1.047 to 1.085‡**
WOMAC (total)		**1.018**	**1.010 to 1.026‡**
Contralateral OA at baseline		**1.593**	**1.328 to 1.900‡**
mtDNA haplogroups (N=2579)			
H (n=1042)	278 (26.7%)	1	
UK (n=612)	154 (25.1%)	0.908	0.727 to 1.131
T (n=275)	67 (24.3%)	0.896	0.658 to 1.221
J (n=236)	**47 (20.1%)**	**0.680**	**0.470 to 0.968‡**
Others§ (n=414)	102 (24.6%)	0.895	0.698 to 1.153
CHECK			
Gender (male)		0.869	0.698 to 1.043
Age		1.007	0.990 to 1.026
BMI		1.005	0.987 to 1.025
WOMAC (total)		**1.006**	**1.001 to 1.012‡**
Contralateral OA at baseline		**1.313**	**1.087 to 1.576‡**
mtDNA haplogroups (N=635)			
H (n=269)	248 (92.2%)	1	
UK (n=144)	126 (87.5%)	0.849	0.682 to 1.035
T (n=68)	64 (94.1%)	1.133	0.850 to 1.447
J (n=56)	**46 (82.1%)**	**0.728**	**0.469 to 0.998‡**
Others§ (n=98)	86 (87.7%)	0.810	0.627 to 1.032

*Cumulative incident knee OA rate from baseline to follow-up.
†CIs for the HRs obtained using the bootstrap methodology by the improved percentile method.
‡Statistical significance declared at $p \leq 0.05$.
§The group 'others' include mtDNA variants with a frequency below 5%.
Bold refers to parameters that reached the statistical significance ($p \leq 0.05$).
BMI, body mass index; CHECK, cohort hip and cohort knee; mtDNA, mitochondrial DNA; OA, osteoarthritis; OAI, osteoarthritis initiative, WOMAC, Western Ontario and McMaster Universities Arthritis Index.

Table 3 Characteristics of studies included in the meta-analysis

Cohort	Year	Sample size	Country	Ethnicity	Mean age	Type of study	Type of OA	Incident knee OA criteria	Genotyping method	Controlled confounder variables	Conclusion
CHECK	2016	635	The Netherlands	Caucasian	56	Incidence, prospective at 8 years	Knee OA	New-onset KL grade 1	SBE and PCR/RFLP	Gender, age, BMI, WOMAC, contralateral OA	Haplogroup J associates with decreased risk
OAI	2016	2579	USA	Caucasian	61	Incidence, Prospective at eight years	Knee OA	New-onset KL grade 2	SBE and PCR/RFLP	Gender, age, BMI, WOMAC, contralateral OA	Haplogroup J associates with decreased risk

BMI, body mass index; CHECK, cohort hip and cohort knee; KL, Kellgren and Lawrence; OA, osteoarthritis; SBE, single base extension; OAI, osteoarthritis initiative; RFLP, restriction fragment length polymorphism; WOMAC, Western Ontario and McMaster Universities Arthritis Index.

study (52.2%) compared with CHECK (47.8%). The results showed that haplogroup J significantly reduces the risk of incident knee OA (combined HR=0.702; 95% CI 0.541 to 0.912; p=0.008) (figure 1).

Functional study using transmitochondrial cybrids

Establishment of basal conditions

At 25 passages, the mtDNA copy number between H and J cybrids acquired the steady state levels (data not shown), meanwhile at 48 hours the doubling time (DT) showed no significant differences between H and J cybrids (data not shown). Based on these setting, all the experiments were performed starting from 25 passages and after 48 hours of cell culture.

Mitochondrial respiration and glycolytic capacity

To measure real-time glycolytic and mitochondrial respiration rates, cybrids were placed in an extracellular flux analyser (Seahorse Biosciences). The analyser measures ECAR and OCR in a transient microchamber, representing glycolysis and mitochondrial respiration, respectively. Glycolysis rate in H and J cybrids were 87.98±11.4 and 69.45±4.69 mpH/min, respectively, indicating a 21.06% decrease in J cybrids (p=0.0004);

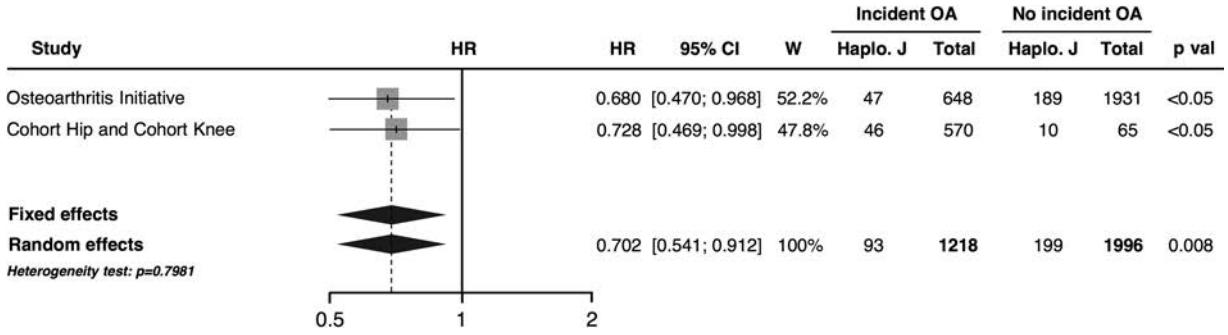

Figure 1 Forest plot of the associations analysed separately in this work involving the mitochondrial DNA haplogroup J and the risk of incident knee OA. OA, osteoarthritis; OAI, osteoarthritis initiative; CHECK, cohort hip and cohort knee; W, relative weight; Haplo. J, number of subjects with the haplogroup J that developed and did not develop incident knee OA during the follow-up; p Val: p Value.

glycolytic capacity rates in H and J cybrids were 116.3±12.76 and 91.69±6.48 mph/min, respectively, indicating a 21.16% decrease in J cybrids ($p<0.0001$) (figure 2A). Similarly, mitochondrial basal respiration rate was significantly lower in J cybrids (96.58±60.01 pmol/min) compared with H cybrids (209.08±55.7 pmol/min, $p=0.007$); as a result, H cybrids showed an increased ATP production (149.95±23.29 vs 65.94 ±25.72 pmol/min in J cybrids; $p<0.0001$) (figure 2B).

Mitochondrial reactive oxygen species production and oxidative stress response

Cybrids carrying the haplogroup H had a significantly higher production of peroxide and peroxynitrite than cybrids carrying the haplogroup J (52.51±11.34 vs 41.26±7.48; $p<0.05$) (figure 2C). Moreover, the analysis of mitochondrial anion superoxide revealed the same significant trend by which H cybrids showed higher levels than J cybrids (8.58±3.0 vs 4.25 ±0.9; $p<0.05$) (figure 2D).

In relation to oxidative stress response, the results showed that the percentage of survival cells in presence of H_2O_2 in H cybrids was significantly lower than in J cybrids (29.63±3.3 vs 56.45±7.36; $p<0.05$) (figure 2E).

Analysis of apoptosis

The analysis of apoptosis assessed in basal conditions revealed no significant differences between H and J cybrids (3.69±2 and 4.17±1.1, respectively; $p>0.05$) (data not shown). However, after incubation with staurosporine, cybrids carrying the haplogroup H showed a significantly increased number of apoptotic cells in comparison with J cybrids (7.35±3.78 vs 4.69±1.68; $p<0.05$) (figure 2F).

Further analysis of the pro-apoptotic gene BBC3 and the anti-apoptotic gene BCL2L13 showed a significantly increased expression of BBC3 in H cybrids compared with J cybrids (3.3-fold; $p<0.05$). On the contrary, no significant differences were detected in the expression of BCL2L13 (figure 2G).

DISCUSSION

Here, we report the first replication study followed by a meta-analysis addressing the association between mtDNA haplogroups and the risk of incident knee OA over time using new haplogroup data from two well-characterised cohorts of patients; a previous meta-analysis involving the study of the mtDNA haplogroups consisted in the analysis of their association with the prevalence of the disease.[28] In addition, we provide data supporting the possible explanation for these associations using transmitochondrial cybrids.

We assessed, for the first time, the influence of the mtDNA haplogroups in the rate of incident knee OA in subjects of the incidence subcohort of the OAI followed by a replication study in patients from CHECK. Despite OAI and CHECK are geographically different cohorts, the frequency distribution of the mtDNA haplogroups was very similar between both cohorts (χ^2 test; $p=0.924$, data not shown), ruling out a potential confusion due to ethnic origin. Compared with the incidence subgroup of the OAI, CHECK participants experienced an increased radiographic change at follow-up (89.7% vs 25.1%) mainly because of the different incident knee OA criteria and mean age at baseline between both cohorts, as previously described.[32] Although both cohorts focus on the early phase of OA, the CHECK cohort represents participants in an even earlier state of the disease[33] and the incident knee OA criteria slightly differs from the proposed by Felson *et al*[23] in subjects of the OAI. Notwithstanding, the results obtained reveal that subjects with haplogroup J show a lower rate of incident knee OA in both cohorts.

To date, different associations involving the haplogroup J have been described in the context of OA. Subjects carrying this haplogroup have a lower prevalence of knee and/or hip OA,[16 17 19 28] besides this mtDNA variant also associates with lower serum levels of catabolic type II collagen biomarkers[34] and metalloproteinases (MMPs)[35] and has been correlated with higher telomere length and lower nitric oxide production in articular chondrocytes.[36] However, a work by Hudson *et al* found no evidence of associations between mtDNA variants and the risk of OA.[20] Although the study by Hudson *et al* is a prevalence study, and not an incident knee OA analysis as is the case of the study presented herein, some points could clarify this; control samples used in their study are population-based controls with symptomatic information and without radiographic data. Up to 50% of patients without joint symptoms may have radiographic changes related to OA,[37] therefore, the selection of adequate healthy controls is crucial to draw consistent conclusions in case-control studies; this point could be one of the causes by which one study performed by the arcOGEN consortium also failed to replicate previous associations at genome-wide significance level ($p\leq5.0\times10^{-8}$), such as GDF5, chromosome 7q22 or MCF2L gene polymorphisms.[38] Finally, as postulated by these authors, the relative contribution of specific mtDNA variants could vary in different ethnic groups by means of homoplasy and/or geographic differences in the finer details of subhaplogroup structures of mtDNA.[20]

The results of the meta-analysis indicate that the associations are robust and reveal a strong association with the haplogroup J. The haplogroup J is characterised by a set of uncoupling

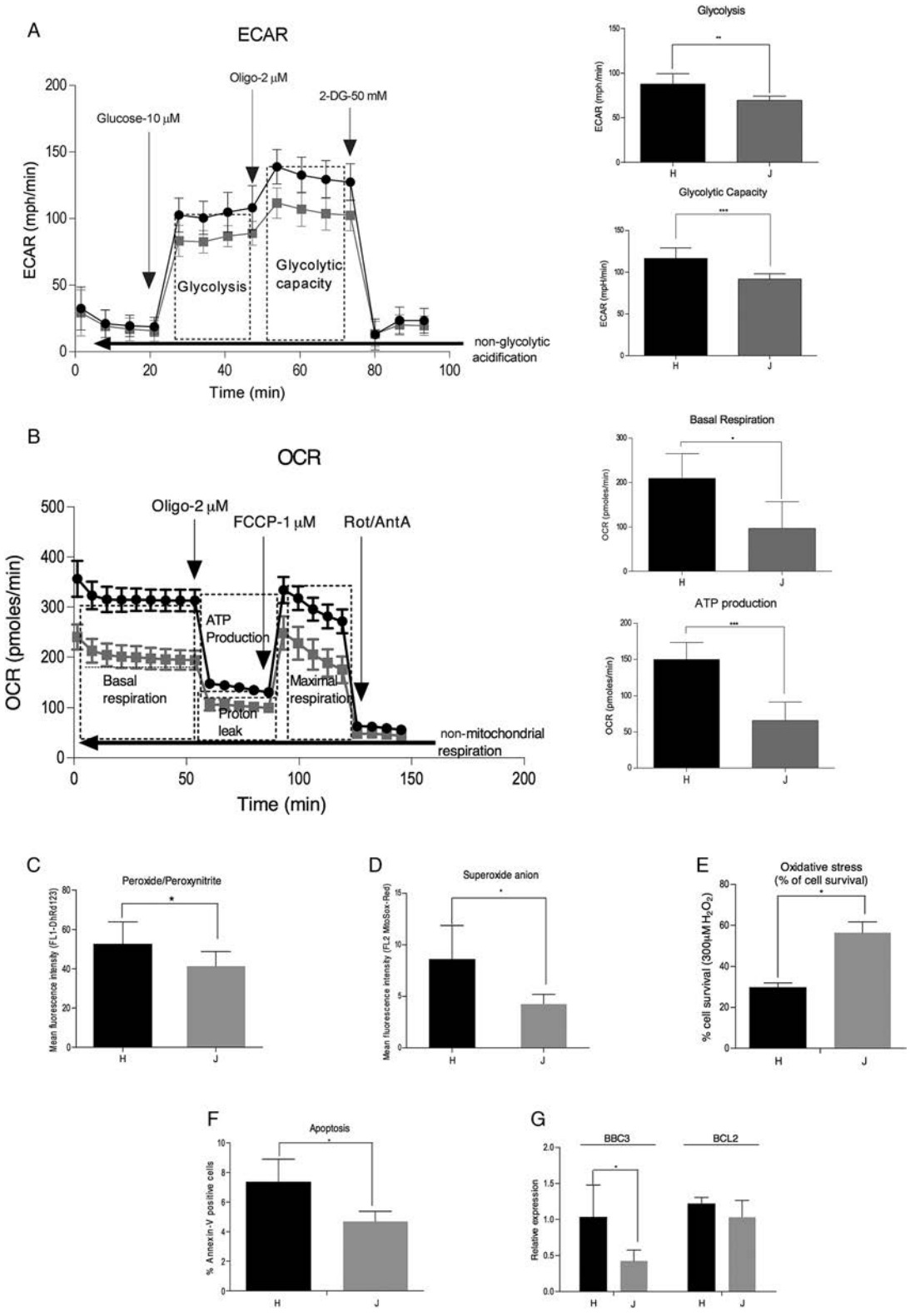

Figure 2 (A) Glycolysis stress test by extracellular acidification rate (ECAR) traces and bar graphs showing the glycolytic response of H and J cybrids in response to glucose, oligomycin (oligo) and 2-deoxy-glucose (2-DG) injection where indicated. Bar graphs show glycolysis and glycolytic capacity as calculated from trace and compared between groups. (B) Mitochondrial stress test by OCR traces and bar graphs showing the mitochondrial behaviour in H and J cybrids in response to oligomycin, FCCP and rotenone/antimycin (Rot/AntA) injection where indicated. Bar graphs show basal respiration and ATP production, calculated and compared between groups; (C) mitochondrial peroxide and peroxynitrite production; (D) mitochondrial anion superoxide production; (E) susceptibility to oxidative stress after incubation with 300 μM H_2O_2; (F) apoptosis measure with Annexin-V-fluorescein isothiocyanate: data are expressed as percentage of positive cells under stress induction with staurosporine at 0.2 μM; (G) gene expression of the apoptotic genes BBC3 and BCL2. The values represented were a mean±SD of three independent experiments using two cybrids from two different individuals and two clones from each cybrid. Black bars corresponds to H cybrids, while grey bars correspond to J cybrids; BBC3, BCL2 binding component; FCCP, carbonyl cyanide p-trifluoromethoxyphenylhydrazone; ***p<0.0001; **p<0.01; *p<0.05.

mitochondrial polymorphisms,[9 10] preferentially non-synonymous SNPs,[39] acquired during its evolutionary history. These aspects make this haplogroup to be biochemically different from other mitochondrial variants, specially the haplogroup H.[40 41]

Increasing evidence suggests that low-grade chronic inflammation in the joint promotes OA progression, and changes in cellular bioenergy metabolism can reprogramme inflammatory response, leading to the disturbance of cellular energy balance and increase cell stress.[42] This evidence implies that mitochondria, as the regulators of cell metabolism, as well as the mtDNA haplogroups, as one of the main modulators of cellular bioenergetics,[43] are involved in the development of OA as previously proposed.[2 44] Nevertheless, in an attempt to find out the possible cellular mechanisms underlying the associations described herein, we performed a functional study of the mtDNA haplogroups using transmitochondrial cybrids.

Transmitochondrial cybrids are cell lines consisting of mitochondria from different sources in a defined and uniform nuclear background. They constitute an interesting model and allow the study of the real role of different mtDNA polymorphisms under identical nuclei, and they also provide a window into early stages of disease pathogenesis, which is not available from pathological specimens. Because of that, these cell models have been proposed and widely used to explore the contribution of mitochondrial dysfunction and mtDNA mutations to the pathogenesis of human diseases, such as Parkinson or cancer.[45 46] Through the use of this cellular model, recent studies showed that J variant associates with decreased expression of specific genes related to inflammatory response, complement and apoptosis when compared with the haplogroup H.[12] The functional analysis described herein included specific metabolic measurements using an extracellular flux analyser as well as specific aspects proposed to be related to OA, such as mitochondrial reactive oxygen species (ROS) production, oxidative stress and apoptosis.[7 44 47] The results obtained reveal that cells harbouring the haplogroup J show a physiological behaviour that seems to be protective against the development of OA.

This physiological behaviour includes a significantly lower production of mitochondrial superoxide anion and peroxynitrite, as well as a higher ability to cope with oxidative stress. It has been demonstrated that mitochondria-derived ROS and nitrogen radicals lead to an upregulation of MMPs[44] as well as an overproduction of pro-inflammatory cytokines,[48] cellular damage or, in some cases, apoptosis. Interestingly, cybrids harbouring the haplogroup J show a significant lower rate of apoptosis under stress conditions, and a lower expression of the pro-apoptotic gene BBC3, which induces apoptosis through mitochondrial dysfunction.

From a metabolic point of view, haplogroups H and J have a different behaviour too. H cybrids show higher mitochondrial respiration rate and glycolytic capacity, which is reflected in an increased ATP generation compared with cybrids carrying the haplogroup J. The haplogroup H presents the highest levels of conserved amino acids,[9] which could determine its Oxidative Phosphorylation System (OXPHOS) coupling efficiency and ATP production[41]; however, this high efficiency would be accompanied by an increased ROS generation and a higher oxygen consumption, as demonstrated herein and proposed elsewhere.[10 41]

In recent years, several studies supported a key role of the mitochondria in the pathogenesis of OA and the study of this organelle in the context of this disease attracted much attention.[2 49 50] The mechanisms underlying, at least in part, the association described herein are related to different functional consequences characteristic of specific mitochondrial polymorphisms; thus, ROS production (both peroxynitrite and superoxide anion), oxidative stress and apoptosis are downregulated in cells harbouring mitochondrial polymorphisms characteristic of the mtDNA haplogroup J.

Although a direct functional link between haplogroups and cartilage biology has not been made in this study, a recent work in conplastic mice (mice with constant nuclear background but different mtDNA variants) shows that mtDNA haplotype profoundly influences in health longevity through mitochondrial proteostasis and ROS generation, insulin signalling, obesity, telomere shortening or mitochondrial dysfunction.[51] Because most of these aspects are also involved in OA pathogenesis, a functional link between mtDNA variation and cartilage biology could really exist.

Despite only two studies were combined, the results of the meta-analysis are consistent enough to support a real association of haplogroup J with a lower risk of incident knee OA. Furthermore, data from both cohorts were analysed in a similar way, and effect size measures were adjusted for baseline characteristics in order to minimise residual confounding. Both OAI and CHECK are well-characterised cohorts, constructed with rigorous methodology in which patients are evaluated by objective methods in spaced visits. This should contribute to minimise information biases and strengthen the conclusions of this study.

In summary, the results of this work have a special clinical relevance. On the one hand, they claim that polymorphisms of the mtDNA haplogroup J alter metabolism and cell physiology predisposing the cell to a less favourable environment to develop incident OA, which allows the design of potential drugs that emulate the physiological effects related to this haplogroup, as well as the consideration of the mtDNA haplogroups as candidate diagnostic biomarkers in OA; among these therapeutic strategies, potential drugs that modulate the activity of the mitochondrial respiratory chain in a similar way to that of the haplogroup J, or even the development of a cellular therapy using cells with mitochondria harbouring the haplogroup J, could be interesting. On the other hand, the results obtained permit to select patients with OA not harbouring the haplogroup J (ie, haplogroup H) as ideal candidates for clinical trials because they are more likely to suffer a higher rate of incident knee OA.

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Contributors FJB and IR-P contributed equally in the design and coordination of the study; both conceived the study, participated in its design and helped to draft the final version of the manuscript; MF-M carried out the cybrid experiments and helped to draft the manuscript and data interpretation; AS-H carried out the experimental procedures of mitochondrial haplogroup assignment; MEV-M, EC-P, SR and TH-G helped to carry out the cybrids cell culture, the subsequent experimental procedures and the haplogroup assignment; SP supervised the statistical procedures; NO-V and CF-L helped the understanding of the clinical and radiological variables included in the different OAI and CHECK datasets; RG provided the necessary infrastructure for the development of the transmitochondrial cybrids. All the authors approved the final version of the manuscript.

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Competing interests None declared.

Patient consent Obtained.

Ethics approval National Institutes of Health and Dutch Arthritis Association.

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EXTENDED REPORT

Dysbiosis and zonulin upregulation alter gut epithelial and vascular barriers in patients with ankylosing spondylitis

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ABSTRACT

Background Dysbiosis has been recently demonstrated in patients with ankylosing spondylitis (AS) but its implications in the modulation of intestinal immune responses have never been studied. The aim of this study was to investigate the role of ileal bacteria in modulating local and systemic immune responses in AS.

Methods Ileal biopsies were obtained from 50 HLA-B27$^+$ patients with AS and 20 normal subjects. Silver stain was used to visualise bacteria. Ileal expression of tight and adherens junction proteins was investigated by TaqMan real-time (RT)-PCR and immunohistochemistry. Serum levels of lipopolysaccharide (LPS), LPS-binding protein (LPS-BP), intestinal fatty acid-BP (iFABP) and zonulin were assayed by ELISA. Monocyte immunological functions were studied in in vitro experiments. In addition the effects of antibiotics on tight junctions in human leukocyte antigen (HLA)-B27 transgenic (TG) rats were assessed.

Results Adherent and invasive bacteria were observed in the gut of patients with AS with the bacterial scores significantly correlated with gut inflammation. Impairment of the gut vascular barrier (GVB) was also present in AS, accompanied by significant upregulation of zonulin, and associated with high serum levels of LPS, LPS-BP, iFABP and zonulin. In in vitro studies zonulin altered endothelial tight junctions while its epithelial release was modulated by isolated AS ileal bacteria. AS circulating monocytes displayed an anergic phenotype partially restored by ex vivo stimulation with LPS+sCD14 and their stimulation with recombinant zonulin induced a clear M2 phenotype. Antibiotics restored tight junction function in HLA-B27 TG rats.

Conclusions Bacterial ileitis, increased zonulin expression and damaged intestinal mucosal barrier and GVB, characterises the gut of patients with AS and are associated with increased blood levels of zonulin, and bacterial products. Bacterial products and zonulin influence monocyte behaviour.

INTRODUCTION

In healthy subjects, the gastrointestinal tract is colonised by a broad range of microbes, termed the gut microbiota.[1] In heathy individuals a gut epithelial barrier[2] and a gut vascular barrier (GVB)[3] control the translocation of bacteria and bacterial antigens into the bloodstream. The homoeostasis of normal microbial flora in the gut is essential for intestinal health and its altered balance, termed dysbiosis, may influence intestinal permeability through the release of zonulin,[4] a protein that modulates the permeability of epithelial tight junctions of the digestive tract.

Dysbiosis has been recently demonstrated in the terminal ileum of patients with ankylosing spondylitis (AS) together with the presence of subclinical gut inflammation.[5 6] It is unclear, however, whether this dysbiosis is a cause or consequence of the inflammation and whether dysbiosis modulates immune responses in AS. The aim of the present study was to study the tissue localisation of bacteria in the gut of patients with AS and the eventual changes in gut-epithelial barrier and GVB integrity. We also assessed the role of zonulin in modulating intestinal permeability and monocyte activation. Finally, we analysed whether alterations in gut permeability and microbiota composition are associated with systemic immune responses.

METHODS

For more details about patients and controls see supplemental methods and online supplementary table S1.

Histomorphological grading and immunohistochemistry

One hundred and sixty-five biopsies were obtained from the 50 patients with AS enrolled. Gut specimens from patients with AS were histologically divided as previously described in: normal gut histology, acute and chronic inflammation.[7 8] The degree of gut inflammation was also evaluated by using interleukin (IL)-8 as a general marker of inflammation.[9] For more details about bacteria characterisation and immunohistochemistry see supplemental methods.

Isolation of bacteria

Ileal biopsy specimens from patients and controls enrolled at the University of Palermo, were immediately processed for bacteriological study in the Microbiology Laboratory, Azienda Ospedaliera Villa Sofia Cervello, Palermo, Italy according to Conte *et al*.[10] For more information, see supplemental methods.

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Cultures for aerobic and facultative anaerobic bacteria

For bacterial cultures only ileal biopsies obtained from patients with AS and controls enrolled at the University of Palermo were used. For more information, see online supplementary methods.

RNA extraction and quantitative TaqMan real-time (RT)-PCR

Total RNA was extracted using the Qiagen RNeasy Mini kit, with on-column DNase I digestion as previously described.[8] For more information, see online supplementary methods.

Flow cytometry analysis of surface and intracellular antigens

Peripheral blood mononuclear cells (PBMCs) were isolated from the peripheral blood of 20 patients with AS and 10 healthy controls as previously described.[8] A list of the antibodies used is provided in online supplementary table S2.

ELISA for circulating LPS, iFABP and zonulin

Levels of lipopolysaccharide (LPS), LPS-binding protein (BP), intestinal fatty acid-BP (iFABP) and zonulin proteins were analysed in sera of all patients with AS and controls. For more information, see online supplementary methods.

Cell cultures

In order to evaluate the role of intestinal bacteria isolated from patients with AS in modulating epithelial zonulin levels, bacteria were isolated from ileal AS samples obtained from patients enrolled at the University of Palermo as described by Conte *et al*[10] and incubated with Caco-2 epithelial cells. The modulation of zonulin mRNA was then assessed by RT-PCR. The effect of zonulin on human umbilical vein endothelial cells and PBMCs was evaluated as previously described.[10] For more information, see supplemental methods.

Human leukocyte antigen (HLA)-B27 TG rats

HLA-B*2705 transgenic (TG) rats of line 33-3 (B27-TG) on a Fischer background (F344/NTac-Tg [HLA-B*2705, β2M]) (Taconic, Hudson, New York, USA) were backcrossed with Piebald Virol Glaxo (PVG) rats (PVG/OlaHsd) (Harlan, UK) for a minimum of 10 generations before their use in experiments as previously described.[11] For more information, see online supplementary methods.

Statistical analysis

The non-parametrical Mann-Whitney test was used to calculate the statistical significance between groups. Spearman's rank correlation was used to calculate the correlation between different variables in AS. p Values <0.05 were considered statistically significant.

RESULTS

Assessment of intestinal gut inflammation in AS

IL-8 was overexpressed in patients with AS with chronic inflammation (see online supplementary figure S1C,D) compared with those with acute inflammation (see online supplementary figure S1B,D) and without inflammation (see online supplementary figure S1D) and controls (see online supplementary figure S1A, D). In patients with AS, the number of IL-8 positive cells was correlated with the degree of intestinal inflammation (see online supplementary figure S1D).

Adherent and invasive bacteria are present in the gut of patients with AS

Adherent and invading rod-shaped bacteria were observed in 35 out of 50 patients with AS (25/33 of the Palermo cohort and 10/17 of the Ghent cohort) independent of the presence of acute or chronic inflammation. Among these patients, only four showing acute inflammation and four showing chronic inflammation were taking sulfasalazine. Of these, two out of four patients with acute inflammation and one out of four with chronic inflammation did not show cultivable bacteria. Bacteria were mainly detected within the epithelium and rarely in the context of lamina propria (figure 1A–C). Absence of adherent and/or invasive bacteria was observed in normal ileum (figure 1D). In particular, invasive bacteria, sometimes aggregated in clusters, were observed in 12 patients with AS of the Palermo's cohort and in 7 patients with AS of the Ghent cohort. The bacterial scores significantly correlated with the percentages of infiltrating inflammatory cells ($r^2=0.57$, $p<0.0001$) (figure 1E). Gram-positive (F-G) and Gram-negative (H-I) bacteria were confirmed to be both adherent and invasive in patients with AS. The presence of invasive bacteria in AS was invariably associated with histological changes characterised by the detachment of basal membranes from the lamina propria, forming vacuoles inside the villi, and oedematous lamina propria with extravasated red blood cells (figure 1K–M and see online supplementary table S3). Isolated oedematous lamina propria, without detachment of basal membranes and/or vasculitis, was observed in the intestine of all patients displaying adherent bacteria (see online supplementary table S3). Identification of the bacteria from culture of ileal samples showed that all the patients with AS of the Palermo's cohort had cultivable bacteria essentially the Gram-negative bacteria *Escherichia coli and* Prevotella spp (figure 1N). Conversely, only 5 out of 20 control samples displayed cultivable bacteria (25%), *E. coli* being the only Gram-negative species found (figure 1N). No culture of ileal samples was performed in ileal samples from the Ghent cohort. Cultures of Prevotella spp and *E. coli* were confirmed by PCR.

We next studied the expression of intestinal tight junction proteins. A significant downregulation of claudin 1 (figure 1O), claudin 4 (figure 1P), occludin (figure 1Q) and zonula occludens 1 (figure 1R) was observed in the gut of patients with AS (expecially in those with chronic gut inflammation) compared with controls. The significant downregulation of the tight junction proteins in AS was confirmed by immunohistochemistry (IHC) demonstrating the reduced expression in AS of occludin (figure 2A,C) and claudin 4 (figure 2D,F) compared with controls (figure 2B,C,E and F).

Zonulin is upregulated in the gut of patients with AS and modulated by ileal bacteria

We next evaluated zonulin expression in the biopsies of all patients with AS and controls. Significant upregulation of zonulin mRNA was observed in the ileal samples of patients with AS, expecially in those with chronic gut inflammation, (figure 2G), inversely correlated with the expression levels of claudin 1 ($r^2=0.28$, $p<0.0001$) (see online supplementary figure S1E), claudin 4 ($r^2=0.324$, $p<0.0001$) (see online supplementary figure S1F), occludin ($r^2=0.654$, $p>0.0001$) (see online supplementary figure S1G) and zonula occludens 1 ($r^2=0.245$, $p<0.001$) (see online supplementary figure S1H). Zonulin has been identified as prehaptoglobin 2, one of the two genetic variants (together with haptoglobin 1) of human

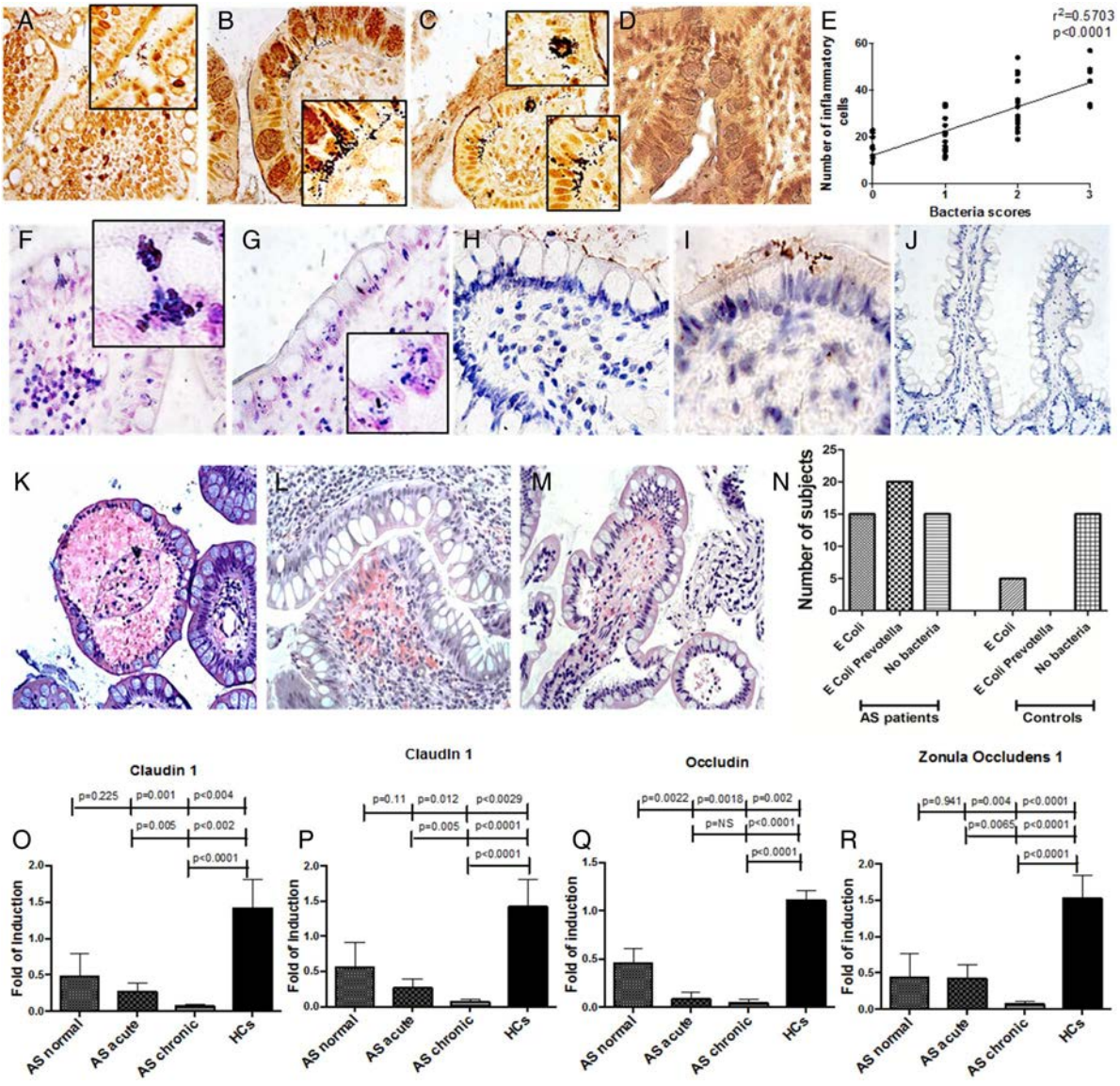

Figure 1 Invasive and adherent bacteria are present in the ileum of patients with ankylosing spondylitis (AS) and are associated with alterations of tight junction proteins. (A–D) Representative microphotographs showing adherent (A) and invasive (B and C) bacteria in AS but not in controls (D). (E) Bacterial scores are directly correlated with the number of infiltrating mononuclear cells. (F–G) Representative images showing Gram staining in patients with AS demonstrating the presence of invading Gram-positive bacteria. (H–J) Representative images showing immunohistochemistry for lipopolysaccharide (LPS) in patients with AS (H and I) and controls (J). (K–M) Histological alterations are associated with the presence of bacteria such as haemorrhages (K and L) and detachment of epithelium from basal membrane (M). (N) Cultures of isolated bacteria displayed mainly *Escherichia coli* and Prevotella spp. (O–R) relative m-RNA levels of claudin1 (O), claudin 4 (P), occludin (Q) and zonula occludens 1 (R) were assessed by quantitative real-time (RT)-PCR in ileal samples obtained from all the patients and all the controls. Data are expressed as mean (SEM). (A–D, F–J): original magnification×250. Insert in (A–C) and (F-G) original magnification×630.

haptoglobins.[3] Since we cannot completely discriminate between pre-HP2 and HP2 by RT-PCR,[12] overexpression of zonulin was also confirmed by immunohistochemistry in frozen ileal samples obtained from patients with AS by using a specific antizonulin antibody (figure 2H–J). Analysis of tissue distribution of zonulin demonstrated its expression among epithelial cells and infiltrating mononuclear cells (figure 2H,I). Interestingly, the number of zonulin$^+$ cells correlated with the number of IL-8$^+$ cells (figure 2K). We next evaluated in vitro the role of isolated ileal bacteria from patients with AS in modulating zonulin expression. As shown in figure 2L, co-culture of Caco-2 cells with bacteria isolated from ileal biopsies of five patients with AS of the Palermo's cohort induced significant upregulation of zonulin.

Impairment of the GVB occurs in patients with AS

In order to evaluate whether increased intestinal permeability was paralleled by impairment of the GVB,[2] RT-PCR for junctional adhesion molecule-A (JAM-A), a vascular tight junctions protein, vascular endothelial (VE)-cadherin, a vascular adherens junctions protein, and PV1, a marker of endothelial cells permeability, was performed. VE-cadherin and JAM-A (figure 3A,B), were significantly downregulated in the inflamed ileum of patients with AS together with a significant upregulation of PV1 expecially in those patients with chronic gut inflammation compared with controls (figure 3C). To confirm the alteration of GVB, confocal microscopy analysis of occludin and CD31 (a specific endothelial cell marker) expression and of CD31/glial fibrillary acidic protein (GFAP)/PV1 was performed next in ileal

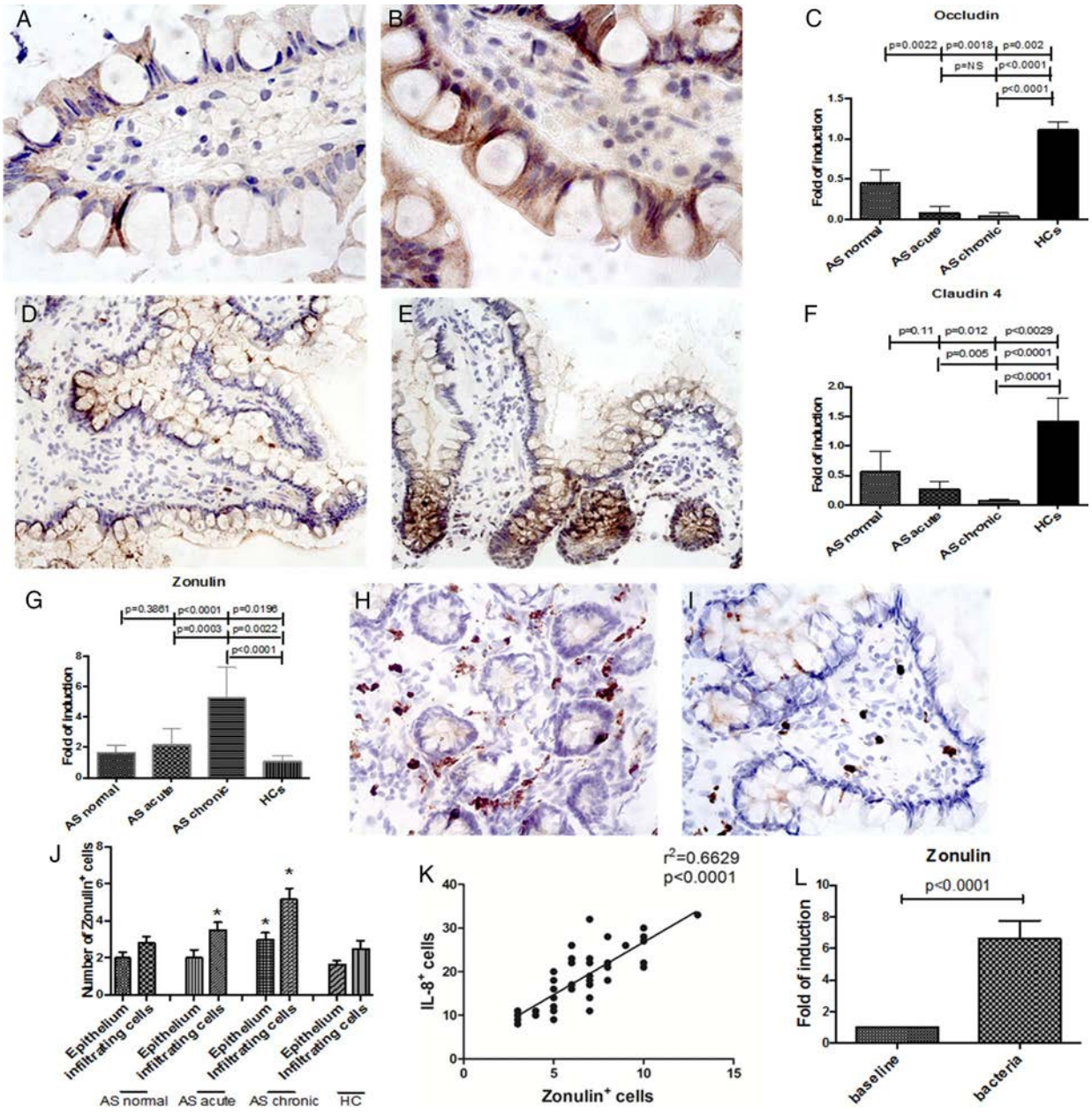

Figure 2 Occludin, claudin 4 and zonulin 1 tissue expression is altered on patients with ankylosing spondylitis (AS) and modulated by intestinal bacteria. (A and B) representative imaging showing occludin expression in the gut of patients with AS (A) and controls (B). (C) Higher numbers of occludin positive cells were observed in healthy controls compared with AS. (D and E) Representative imaging showing claudin 4 expression in the gut of patients with AS (D) and controls (E). (F) Higher numbers of claudin 4 positive cells were observed in healthy controls compared with AS. (G) relative m-RNA levels of zonulin 1 were assessed by real-time (RT)-PCR in the ileal samples obtained from all the patients with AS and HCs. (H and I) Representative imaging showing zonulin 1 expression in the gut of patients with AS (H) and controls (I). (J) Quantification of zonulin 1 positive cells was performed in the ileal biopsies of all the patients and the controls showing higher numbers of zonulin 1 positive cells in patients with AS. (K) The number of zonulin positive cells was significantly and directly correlated with the number of IL-8 positive cells. (L) Caco-2 cells were incubated with bacteria isolated from ileal biopsies obtained from five patients with AS and the modulation of zonulin expression assessed by RT-PCR. Data are expressed as mean (SEM) of five independent experiments. (A and B) Original magnification×630. (D and E) Original magnification×250. (H and I) Original magnification×400. Data are expressed as mean (SEM).

samples from patients with AS and controls. As shown in figure 3D–F, endothelial occludin expression in healthy controls (HC) showed a continuous staining of the junctional protein that surrounded cell borders. In comparison, endothelial cells from patients with AS exhibited the disappearance of the classic occludin continuous staining, showing a jagged and broken vascular distribution (figure 3G–I). Analysis of GVB showed a higher expression of PV1 in AS (figure 3N) compared with HC (figure 3J) and confirmed the disorganised staining for CD31 (figure 3O) and GFAP (figure 3P).

Zonulin alters the expression of endothelial tight junctions

We next evaluated in vitro whether zonulin may influence the expression of endothelial tight junction proteins. As shown in figure 4, zonulin induced a significant downregulation of occludin (figure 4A) and VE-cadherin (figure 4B). Corresponding

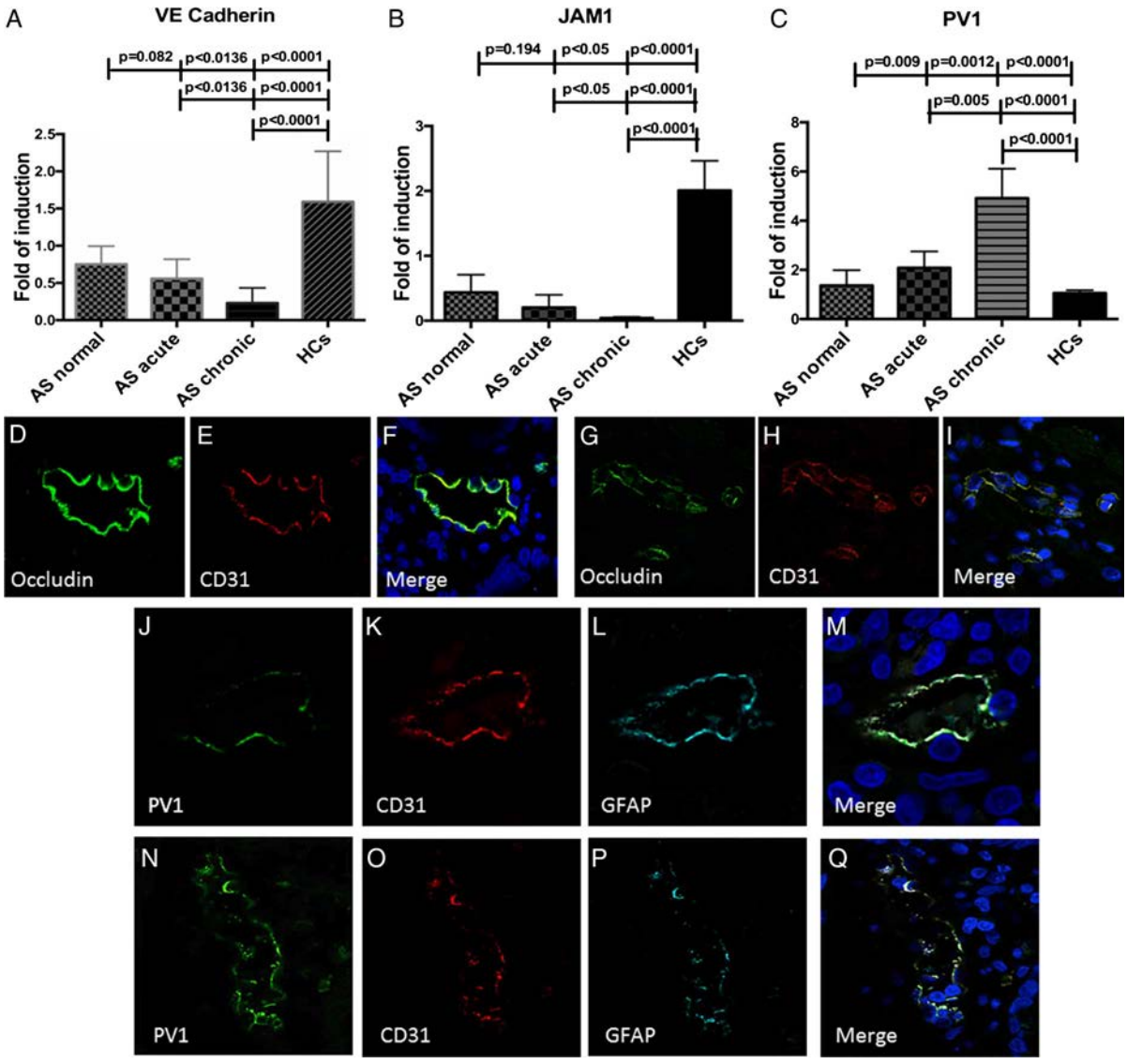

Figure 3 Gut vascular barrier (GVB) in patients with ankylosing spondylitis (AS). (A–C) relative m-RNA levels of VE-cadherin (A), junctional adhesion molecule (JAM)-1 (B) and PV1 (C) were assessed by RT-PCR in AS and HC ileal samples. (D–F) and (G–I) Representative confocal microscopy images showing CD31 and occludin co-localisation in HCs (D–F) and AS (G–I). (J–M and N–Q): Representative confocal microscopy images showing PV1, CD31 and GFAP co-localisation in HCs (J–M) and AS (N–Q). (D–Q): Original magnification×400. Data are expressed as mean (SEM).

with the alteration of the GVB, increased serum zonulin levels (figure 4C) were observed in AS. To establish whether serum zonulin levels are correlated with intestinal permeability, lactulose (LA)/mannitol (MA) urine ratio was determined in 20 patients with AS and 20 controls, all enrolled at the University of Palermo. An increased intestinal permeability, significantly correlated with serum zonulin, was present in patients with AS (LA/MA 0.052±0.002, r^2=0.7236, p=0.01777) (figure 4D) but not in healthy controls (LA/MA 0.021±0.0011; r^2=0.1858, p>0.05) (data not shown). Zonulin has a CD163 binding motif identical to that present in mature haptoglobin 2[10 13] In order to assess the potential functional relevance of zonulin interaction with CD163, isolated PBMCs from patients with AS and controls were incubated with recombinant zonulin. As shown in figure 4E,F,G and H, incubation with zonulin induced a significant expansion of c-MAF$^+$CD163$^+$ cells identified as M2 polarised macrophages[14] in AS (figure 4E,F) but not in controls (figure 4G,H).

Increased serum levels of iFABP, LPS and LPS-BP are found in patients with AS

Since the alterations of epithelial and endothelial permeability, we next evaluated the serum levels of LPS, LPS-BP and iFABP in all the patients with AS and controls. As shown in figure 5, significantly increased levels of LPS (figure 5A), LPS-BP (figure 5B) and iFABP (figure 5C) were observed in patients with AS. Since it has been demonstrated that the presence of high LPS concentration downregulates the expression of CD14,[15] we examined by flow cytometry, the expression of CD14 in circulating monocytes and the effects of LPS and soluble CD14 stimulation on IL-23 production. A significant reduction of CD14$^+$ monocytes (figure 5D–F) and of HLADR$^+$ monocytes (figure 5G,H) was observed only in AS monocytes. Since the soluble form of CD14 (sCD14) has been demonstrated to enable CD14$^-$ cells to respond to LPS,[16] we next evaluated whether sCD14 might rescue AS CD14$^-$ cells from their anergic state. Among AS CD14$^+$ cells, stimulation with LPS, but not with sCD14,

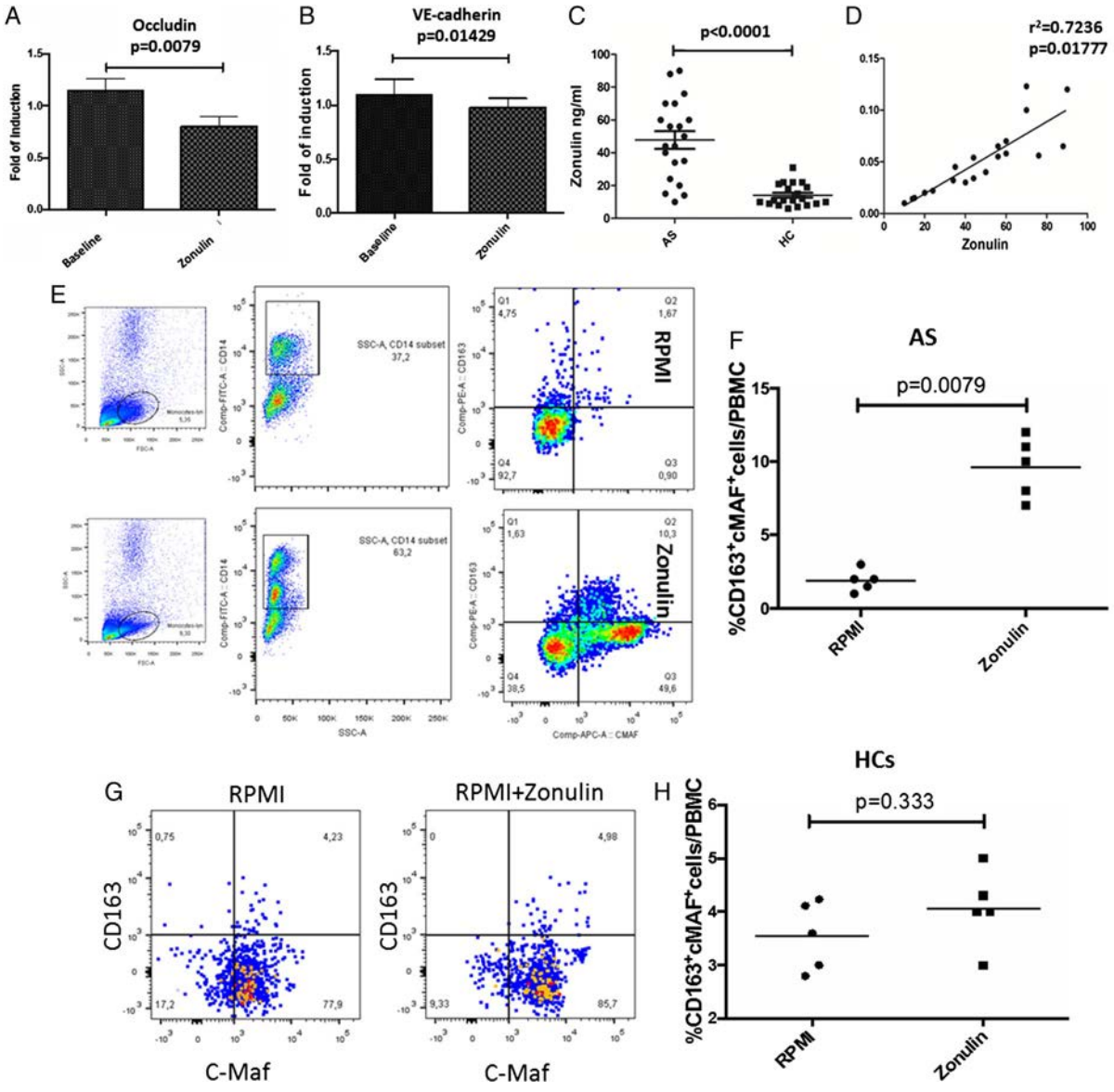

Figure 4 Serum levels of zonulin in patients with ankylosing spondylitis (AS) and in vitro effects of zonulin on human umbilical vein endothelial cells (HUVECs) and peripheral monocytes. (A and B) MRNA expression of occludin (A) and VE-cadherin (B) was assessed in HUVEC cells treated or not with recombinant human zonulin by RT-PCR. Significant downregulation of Occludin and VE-cadherin was observed in HUVEC after incubation with zonulin. (C and D) Serum levels of zonulin were evaluated in 20 patients with AS and 20 controls (C) and correlated with LA/MA ratio (D). (E–H) Peripheral blood mononuclear cells (PBMCs) obtained from five patients with AS (E) and five controls (G) were incubated with recombinant zonulin and the percentage of $CD163^{+}$c-MAF^{+} cells evaluated by flow cytometry; percentages of AS (F) and controls (H) $CD163^{+}$c-MAF^{+} cells before and after zonulin stimulation. (A–B) Data are expressed as mean (SEM). (C, D, F and H): Data are expressed as individual data points.

modified the expression of IL-23 that was not further modified by the combination of LPS and sCD14 (figure 5I–K). Conversely, only the combination of sCD14 and LPS strongly upregulated the production of IL-23 in AS $CD14^{-}$ monocytes (figure 5I–K).

Alteration of epithelial tight junctions occurs in HLA-B27 rats and is restored by antibiotic treatment

In human HLA-B27 and β2-microglobulin TG rats (B27-TG), ileitis develops spontaneously.[17] In order to study whether alteration of tight junctions is present in the ileal samples of these rats, ileal samples from five HLA-B27 TG and five wild type (WT) rats were evaluated. HA-B27 rats displayed ileal inflammation characterised by IL-23 increased expression (figure 6B), occludin downregulation (figure 6F) and the presence of adherent bacteria (figure 6J). Antibiotic treatment caused a significant amelioration of signs of intestinal inflammation as previously described,[18] the reduction of IL-23 expression (figure 6C, D), the normalisation of occludin expression (figure 6G,H) and the disappearance of adherent bacteria (figure 6K,L).

DISCUSSION

In this study we demonstrate that adherent and invading bacteria are present in the ileum of patients with AS and are associated with the alteration of the epithelial barrier and the GVB. The presence of leaky epithelium and endothelium in AS ileum is accompanied by the translocation of zonulin and bacterial products into the bloodstream possibly inducing the modulation of the innate immune system in AS.

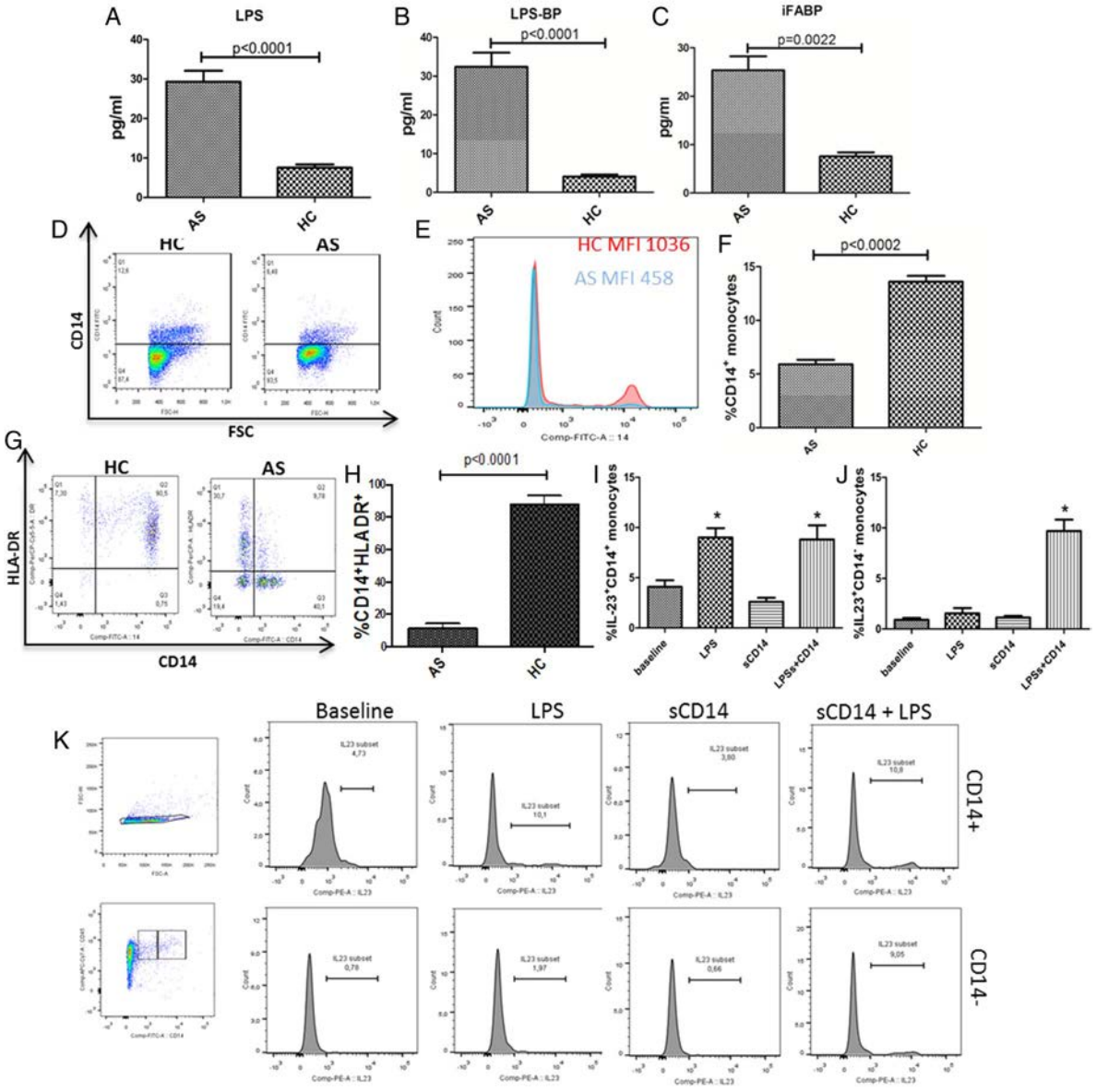

Figure 5 Intestinal bacterial products translocate into ankylosing spondylitis (AS) bloodstream and modulate monocyte behaviour. (A–C) Serum levels of lipopolysaccharide (LPS) (A), LPS-binding protein (BP) (B) and intestinal fatty acid-BP (iFABP) (C) are increased in the sera obtained from patients with AS compared with controls. (D–F) Percentages of $CD14^+$ cells is reduced in peripheral blood mononuclear cells (PBMCs) from patients with AS. (D) Representative dot plot showing the percentage of $CD14^+$ cells gated on CD45 region among PBMCs in patients with AS and controls, (E) representative histogram showing CD14 MFI in patients with AS and HCs. (F) percentages of $CD14^+$ cells in patients with AS and controls. (G and H) Percentage of HLA-antigen D Related $(DR)^+$ cells is reduced in PBMCs from patients with AS. (G) Representative dot plot showing the percentage of $HLA\text{-}DR^+$ cells gated on the monocytes region among PBMCs in patients with AS and controls, (H) percentages of $CD14^+$ cells in patients with AS and controls. (I–K) Effects of monocyte stimulation with LPS alone, sCD14 alone or $sCD14^+LPS$ on $CD14^+$ (H) and $CD14^-$ monocytes. Combination of LPS^+sCD14 increased IL-23 production only in $CD14^-$ cells (I and J). (K) Representative dot plots showing the gating strategy and the percentage of IL-23 expressing cells. Results are showed as mean (SEM).

The intestinal microbiota plays a critical role in modulating the immune response of the gut.[19] The potential role of intestinal bacteria in the pathogenesis of gut inflammation in patients with Spondyloarthritis (SpA) has been highlighted by the identification of dysbiosis in different SpA subsets, including patients with AS.[5 20 21] However, the question of how dysbiosis can influence local and systemic immune responses in AS has not yet been explored.

In this study we confirm and expand our previous results[5] by demonstrating that Gram-negative bacteria, essentially *E. Coli and* Prevotella spp, and Gram-positive bacteria are present in AS ileal samples, displaying both adherent and invasive behaviour. Interestingly, the presence of invasive bacteria was associated with specific histological alterations mainly characterised by the detachment of basal membrane from the lamina propria, leading to the formation of vacuoles inside the villi and haemorrhagic extravasation. These histological findings seem to be directly attributable to the presence of bacteria since similar histological alterations have previously been reported in mice infected with enteropathogenic *E. coli*.[22]

In the presence of pathogenic or non-pathogenic enteric bacteria, mammalian small intestines activate the zonulin pathway[3]

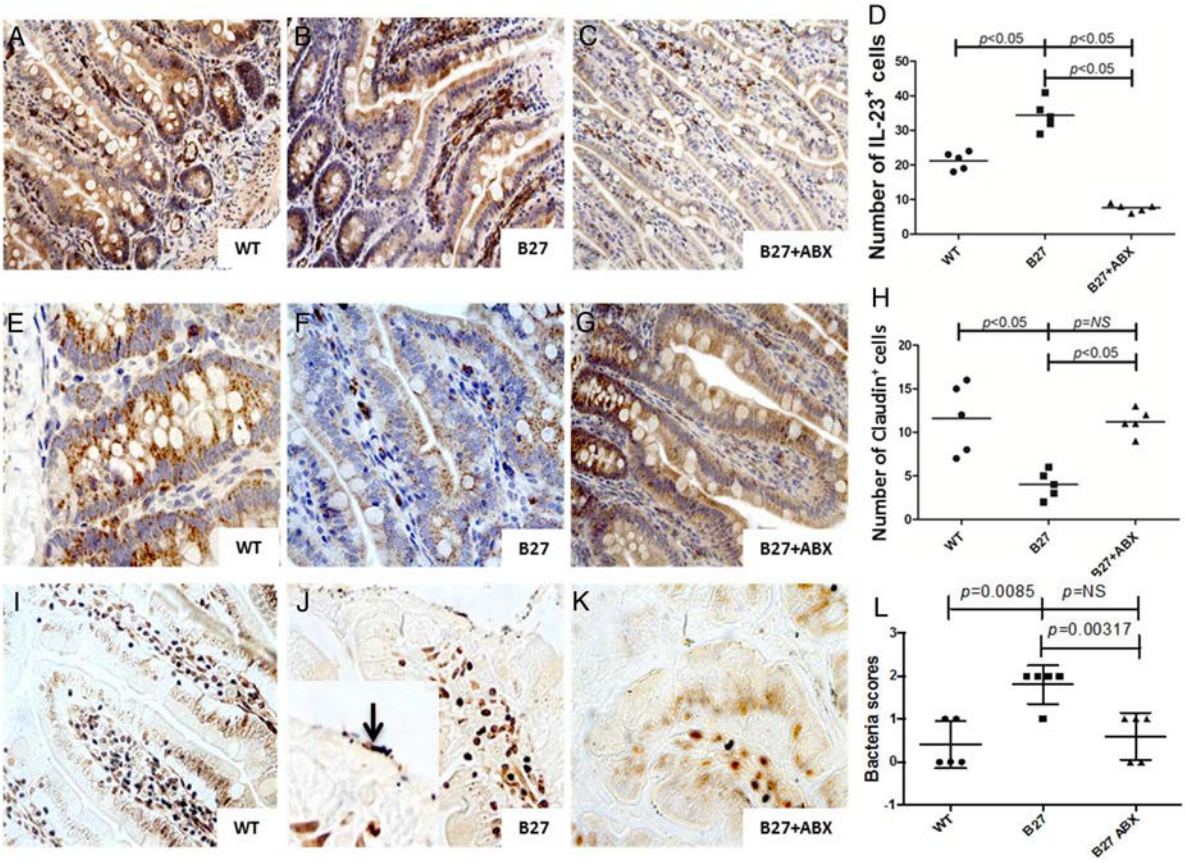

Figure 6 Ileal inflammation and dysbiosis in HLAB27 transgenic rats is modified by antibiotic treatment. (A–C) Representative images showing IL-23 staining in ileal samples obtaining from wild type (WT) rats (A), HLA-B27 transgenic (TG) rats (B) and HLA-B27 TG rats after antibiotic treatment (C). (D) Semiquantitative evaluation of IL-23+ cells. (E–F) Representative images showing IL-23 staining in ileal samples obtained from WT rats (E), HLA-B27 TG rats (F) and HLA-B27 TG rats after antibiotic treatment (G). (H) Semiquantitative evaluation of IL-23+ cells. (I–K) representative images showing Warthin starry staining for identifying bacteria in ileal samples obtained from WT rats (I), HLA-B27 TG rats (J) and HLA-B27 TG rats after antibiotic treatment (K). Higher numbers of adhering and sometimes invading bacteria were observed in HLA-B27 rats (J and insert in J). (L) Semiquantitative evaluation of bacteria in rats ileal samples. (A–C, E–G, I–K) original magnification ×250; J insert: original magnification ×630. Data are expressed as individual data points.

that is involved in the regulation of the permeability of epithelial tight junctions.[4] In our study, tissue levels of zonulin were significantly upregulated in AS ileal samples and accompanied by IL-8 overexpression and a profound reduced expression of tight junction proteins by epithelial cells. These alterations were dependent on the degree of intestinal inflammation, associated with both adherent and invasive bacteria and apparently related to a reduced expression by epithelial cells. We, however, cannot exclude that loss of epithelial cells may also contribute to the reduced tight junction protein expression. Serum zonulin increase was also observed in patients with AS with more pronounced gut inflammation, accompanied by an increased intestinal permeability evaluated by LA/MA urine ratio. Interestingly, isolated bacteria from AS ileal biopsies significantly upregulated zonulin expression in cultured epithelial cells, apparently indicating a specific effect of AS-associated bacteria. It is unclear whether these alterations are the cause or the consequence of intestinal dysbiosis. However, here we demonstrated that alterations of tight junctions, also present in HLA-B27 TG rats, are restored after antibiotic treatments and that antibiotics therapy reduced epithelium-adherent bacteria, suggesting that intestinal dysbiosis might be responsible for the impairment of the epithelial barrier. The reduced number of adherent intestinal bacteria we observed is consistent with previous studies demonstrating that antibiotic treatment reduces mucosal adherent bacteria in mice.[18]

Together with the gut epithelial barrier, a GVB has been recently demonstrated in mice and humans, that acts by preventing the entry into the bloodstream of microbiota-derived products.[2] The GVB shows adherens junctions and tight junctions that seem to be modulated or downregulated, as demonstrated in our in vitro experiments, by zonulin. Increased zonulin expression was in fact accompanied by a significant downregulation of endothelial tight junction proteins, such as occludin, and vascular adherens proteins such as VE-cadherin and by the upregulation of PV1, a marker of increased endothelial permeability.[23] The presence of a 'leaky endothelium' was also confirmed by confocal microscopy experiments showing disorganised staining for CD31, occludin and GFAP and by the demonstration of increased serum levels of zonulin and bacterial products such as LPS, iFABP and LPS-BP in serum of patients with AS. Overall, our results point to a zonulin-dependent epithelial and endothelial loss of barrier function. The fact that gene expression analysis cannot distinguish between pre-HP2 (alias zonulin) and Hp2 and that antibodies used for the IHC experiments may not be specific enough to exclusively detect zonulin and not mature HP2 may raise the possibility that HP2 rather than zonulin is upregulated. However, the decreased expression of tight junction protein and, most importantly, the direct correlation between zonulin and LA/MA point clearly to the involvement of

zonulin and not the mature HP2 that has never been reported to have an effect on barrier function.

Zonulin has a CD163 binding motif identical to that present in mature haptoglobin 2[10] that has been shown to bind the haptoglobin receptor CD163.[13] Therefore, it is conceivable that zonulin binds to CD163 as well as to haptoglobin. The potential functional relevance of this binding in the regulation of monocytes' behaviour, however, has been not previously studied. Here we demonstrated that zonulin induces a significant in vitro expansion of $CD163^+$c-MAF^+ monocytes, compatible with the M2 phenotype, and that these cells were expanded in the peripheral blood of patients with AS. Macrophages play essential activities in homoeostasis maintenance during different organism conditions and may be polarised according to various stimuli into distinct populations. M2 macrophages are macrophages essentially involved in the pathogenesis of asthma, fibrosis, atopic dermatitis, cancer and granuloma formation.[24] Furthermore, an increased frequency of $CD163^+$M2 monocytes, producing IL-23, has been previously demonstrated to be expanded in the peripheral blood and inflamed gut and synovial tissues of patients with AS.[25 26] The in vitro stimulation of AS PBMCs with recombinant zonulin, was also accompanied by a significant expansion of c-MAF^+CD163^+ M2 cells. We also observed the zonulin-dependent expansion of c-MAF^+CD163^- cells. Beyond its role in modulating macrophage differentiation, c-MAF is also involved in the differentiation of T helper cells[27 28] and we cannot exclude that zonulin might also induce the expansion of c-MAF^+ T cells.

We also studied the functional relevance of the increased circulating levels of bacterial products in AS. In the gut, the presence of high LPS concentrations downregulates the monocyte expression of CD14, the receptor involved in the binding of the LPS/LPS-BP complex.[15] Increased LPS levels in AS, were accompanied by the downregulation of CD14 on the surface of monocytes together with the reduced expression of HLA-antigen D Related (DR). $CD14^-HLADR^-$ monocytes have been demonstrated to be functionally anergic[29] and this anergic phenotype was rescued, at least in part, by the co-incubation of these cells with LPS+sCD14 leading to an increased expression of IL-23.

In conclusion, in this study we provide the first evidence that adherent and invasive bacteria are present in the inflamed gut of patients with AS and that these bacteria, through the release of zonulin, may induce a leaky gut epithelial and endothelial barrier, leading to the translocation of intestinal-derived proteins into the bloodstream, ultimately inducing systemic immune alterations that might participate in AS pathogenesis.

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Contributors FC, GG, AR, RA, MML, DE, AF and GT: study design. FC, GG, AR, RA, MML, SM, LS, HC, PDB, TS, AG: acquisition of data, and analysis and interpretation of data. FC, GG, DE, GT: manuscript preparation. FC, GG, PDB, AR: statistical analysis. AF, DE, GT: overall study supervision.

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EXTENDED REPORT

Inhibition of phosphodiesterase 4 (PDE4) reduces dermal fibrosis by interfering with the release of interleukin-6 from M2 macrophages

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ABSTRACT

Objectives To investigate the disease-modifying effects of phosphodiesterase 4 (PDE4) inhibition in preclinical models of systemic sclerosis (SSc).

Methods We studied the effects of PDE4 inhibition in a prevention and a treatment model of bleomycin-induced skin fibrosis, in the topoisomerase mouse model as well as in a model of sclerodermatous chronic graft-versus-host disease. To better understand the mode of action of PDE4 blockade in preclinical models of SSc, we investigated fibrosis-relevant mediators in fibroblasts and macrophages from healthy individuals and patients suffering from diffuse-cutaneous SSc on blockade of PDE4.

Results Specific inhibition of PDE4 by rolipram and apremilast had potent antifibrotic effects in bleomycin-induced skin fibrosis models, in the topoisomerase I mouse model and in murine sclerodermatous chronic graft-versus-host disease. Fibroblasts were not the direct targets of the antifibrotic effects of PDE4 blockade. Reduced leucocyte infiltration in lesional skin on PDE4 blockade suggested an immune-mediated mechanism. Further analysis revealed that PDE4 inhibition decreased the differentiation of M2 macrophages and the release of several profibrotic cytokines, resulting in reduced fibroblast activation and collagen release. Within these profibrotic mediators, interleukin-6 appeared to play a central role.

Conclusions PDE4 inhibition reduces inflammatory cell activity and the release of profibrotic cytokines from M2 macrophages, leading to decreased fibroblast activation and collagen release. Importantly, apremilast is already approved for the treatment of psoriasis and psoriatic arthritis. Therefore, PDE4 inhibitors might be further developed as potential antifibrotic therapies for patients with SSc. Our findings suggest that particularly patients with inflammation-driven fibrosis might benefit from PDE4 blockade.

INTRODUCTION

Fibrosis is a defining characteristic of systemic sclerosis (SSc) as well as a major cause of morbidity and mortality among patients. On a molecular level, fibrosis results from the accumulation of excessive amounts of extracellular matrix proteins released by chronically activated fibroblasts.[1 2]

Particularly in early phases of SSc, leucocytic infiltrates with macrophages, T cells and B cells are a common feature in affected organs. These inflammatory infiltrates are important sources of profibrotic mediators: the release of interleukin-6 (IL-6), transforming growth factor-β (TGF-β) and other profibrotic mediators initiates profibrotic processes through pathological activation of fibroblasts.[1 3] In a subset of patients, these inflammatory processes persist and further drive the progression of fibrosis.[4 5]

Monocytes and macrophages are among the most abundant cell types in leucocytic infiltrates in SSc. Sclerotic skin of patients with early SSc contains an increased number of $CD14^+$ monocytes/macrophages,[6] and the ratio of $CD68^+$ macrophages to T cells is high in sclerotic skin.[7] Higashi-Kuwata *et al* showed an increasing number of cells expressing CD204, a marker for alternatively activated M2 macrophages, in localised scleroderma paralleling the severity of inflammation.[8–10] Although macrophage polarisation has not directly been investigated in fibrotic SSc tissue yet, high levels of IL-4 and IL-13 might favour M2 differentiation.[8] Of note, research on other fibrotic diseases indicates that M2 macrophages may propel fibrotic processes by releasing profibrotic mediators.[11] Molecular profiling of skin biopsies from the FASSCINATE trial has recently suggested that this profibrotic role of M2 macrophages might also hold true in SSc. Gene expression analysis revealed a M2 signature in fibrotic SSc skin, which was downregulated by the IL-6 receptor blocker tocilizumab.[12 13]

Cyclic adenosine monosphosphate (cAMP) is an ubiquitous second messenger molecule that orchestrates physiological responses, such as apoptosis, lipid metabolism and inflammation.[14–19] Its homeostasis is controlled by phosphodiesterases, a superfamily of enzymes that catalyse the breakdown of cAMP to monomeric AMP, thereby inactivating the molecule.[20] The cAMP-specific phosphodiesterase (PDE) isoenzyme PDE4 is almost exclusively expressed within inflammatory cells.[21 22] PDE4 inhibition has well-established disease-modifying activity in specific inflammatory diseases, including psoriasis,[23] psoriatic arthritis[24 25] and Behçet's disease.[26] In the present study, we evaluated PDE4 inhibition as a novel therapeutic approach in treating fibrosis in SSc. We observed that PDE4 blockade ameliorated experimental fibrosis in different models through downregulating the release of profibrotic mediators from M2 macrophages.

MATERIALS AND METHODS

A detailed description of the methods is provided in the online supplementary file.

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Mice and therapeutics

C57/Bl6 and BALB/c mice were purchased from Janvier (Le Genest Saint Isle, France), B10.D2 mice from Jackson Laboratories (Bar Harbor, Maine, USA). (*R*,*S*)-Rolipram (LC Laboratories, Woburn, Massachusetts, USA) was dissolved in dimethyl sulfoxide and further diluted in phosphate buffer saline for intraperitoneal application twice daily. Apremilast was diluted in 1% methycellulose and was applied orally twice daily.

Bleomycin-induced dermal fibrosis

Skin fibrosis was induced in C57Bl/6 mice aged 6 weeks by subcutaneous injections of bleomycin as described previously.[27–33] After 4 (preventive model) or 6 weeks (therapeutic model) of bleomcyin challenge, mice were sacrificed and the injected skin processed for further analysis.

Topoisomerase I mouse model

Skin fibrosis was induced in C57Bl/6 mice aged 6 weeks by subcutaneous injections of 250 U/mL recombinant DNA topoisomerase I as described previously.[34] Controls were injected with 0.9% NaCl. After 8 weeks of topoisomerase I challenge, mice were sacrificed and the injected skin processed for further analysis.

Sclerodermatous, chronic graft-versus-host disease

The B10.D2→Balb/c [H-2(d)] minor histocompatibility antigen-mismatched model was performed as described previously.[35–40]

Analysis of murine skin

Skin thickness, α-smooth muscle actin (SMA) counts, histomorphometry of fibrotic tissue and inflammatory infiltrates were analysed as described.[27–33]

Immunofluorescence for F4/80 and arginase and IL-6

The following primary antibodies were used: F4/80 (AdD Serotec, UK), cMAF (Abgent, USA), arginase (Santa Cruz, Germany), IL-6 (Abcam, UK), IgG (Beckton Dickinson, USA).

Human fibroblasts and macrophages

Fibroblasts and peripheral blood were isolated from healthy donors and lesional skin of patients with diffuse-cutaneous SSc. Fibroblasts were cultured as described.[27 28 33] All healthy individuals and patients with SSc provided written informed consent as approved by the institutional ethics committees.

Quantification of collagen protein

The amount of soluble collagen in cell culture supernatants was quantified using the SirCol collagen assay (Biocolor, Belfast, Northern Ireland).

Cell viability and cytotoxicity assays

Cell viability was quantified using the Cell Counting Kit 8 (Dojindo Molecular Technologies, Maryland, USA).[41]

IL-6 ELISA

IL-6 was determined in the supernatants from the human macrophage experiments with the human IL-6 DuoSet ELISA (R&D Systems, Minneapolis, Minnesota, USA).

Quantitative real-time PCR

Gene expression was quantified by SYBR green real-time PCR on a StepOne System quantitative PCR System (Thermo Fischer Scientific, Waltham, Massachusetts, USA).

Statistical analysis

All data are presented as median with IQR. Differences between the groups were tested for their statistical significance by two-tailed Mann-Whitney U non-parametric test using GraphPad Prism (V.5.03). p Values of <0.05 were considered to be statistically significant.

RESULTS

Inhibition of PDE4 prevents bleomycin-induced dermal fibrosis

We first investigated the effects of PDE4 blockade in bleomycin-induced skin fibrosis. When we treated bleomycin-challenged mice with the PDE4 inhibitor rolipram, a lead compound for the clinically available apremilast, we observed dose-dependent antifibrotic effects as assessed by the reduction of dermal thickening, fibrotic tissue and myofibroblast counts (figure 1A, B). In the group of mice receiving rolipram 5.0 mg/kg twice daily, we found decreases in skin thickening by 64%, in fibrotic tissue by 50% and in myofibroblast counts by 70% (figure 1A, B). Apart from these potent antifibrotic effects, we observed a strong decline in leucocytic infiltrates on PDE4 blockade. Animals receiving 5.0 mg/kg rolipram twice daily showed a reduction of infiltrating leucocytes by 49% (figure 1C). PDE4 inhibition was well tolerated throughout all experiments, as indicated by constant body weight, normal texture of the fur and normal activity.

The antifibrotic effects of PDE4 inhibition are not mediated by direct effects on fibroblasts

Since fibroblasts have been shown to express PDE4,[42] we investigated if PDE4 blockade had direct effects on fibroblasts. We observed that rolipram did not inhibit fibroblast proliferation in healthy and SSc fibroblasts until cytotoxicity occurred at high doses of 1000 μM, which by far exceeds clinical relevant concentrations[43 44] (see online supplementary figure S1). Moreover, PDE4 inhibition left stress fibre formation of healthy and SSc fibroblasts unaffected (figure 2A). Consistently, PDE4 inhibition did neither alter closure time of artificial scratches nor the migration rates of healthy and SSc fibroblasts (figure 2E, F). Finally, we did not detect any inhibitory effects of PDE4 blockade either on *COL1A1 and PAI-1* gene transcription (figure 2B, C) or on collagen release (figure 2D) in resting and TGF-β-stimulated fibroblasts. We therefore hypothesised that the antifibrotic effects of PDE4 inhibition might be leucocyte-dependent, which was supported by decreased leucocyte infiltration in the bleomycin model on PDE blockade.

Inhibition of PDE4 reduces the release of profibrotic cytokines from alternatively activated macrophages

M2 macrophages are a central source of profibrotic mediators. We hence hypothesised that the antifibrotic effects of PDE4 inhibition in bleomycin-induced skin fibrosis might have resulted from a reduced release of profibrotic cytokines from macrophages. We therefore isolated peripheral blood monocytes from healthy volunteers and patients with diffuse-cutaneous SSc and differentiated them into M1 and M2 macrophages. Increased *iNOS* mRNA levels confirmed the differentiation into the M1 phenotype, increased *ARGINASE* mRNA into the M2 phenotype. PDE4 blockade by rolipram inhibited the differentiation of monocytes into M2 macrophages (figure 3A, B), while differentiation into the M1 phenotype remained unaffected (see online supplementary figure S3A, B). In addition, mRNA levels of the profibrotic cytokines *IL-6, IL-13, TGF-β1 and TGF-β2*

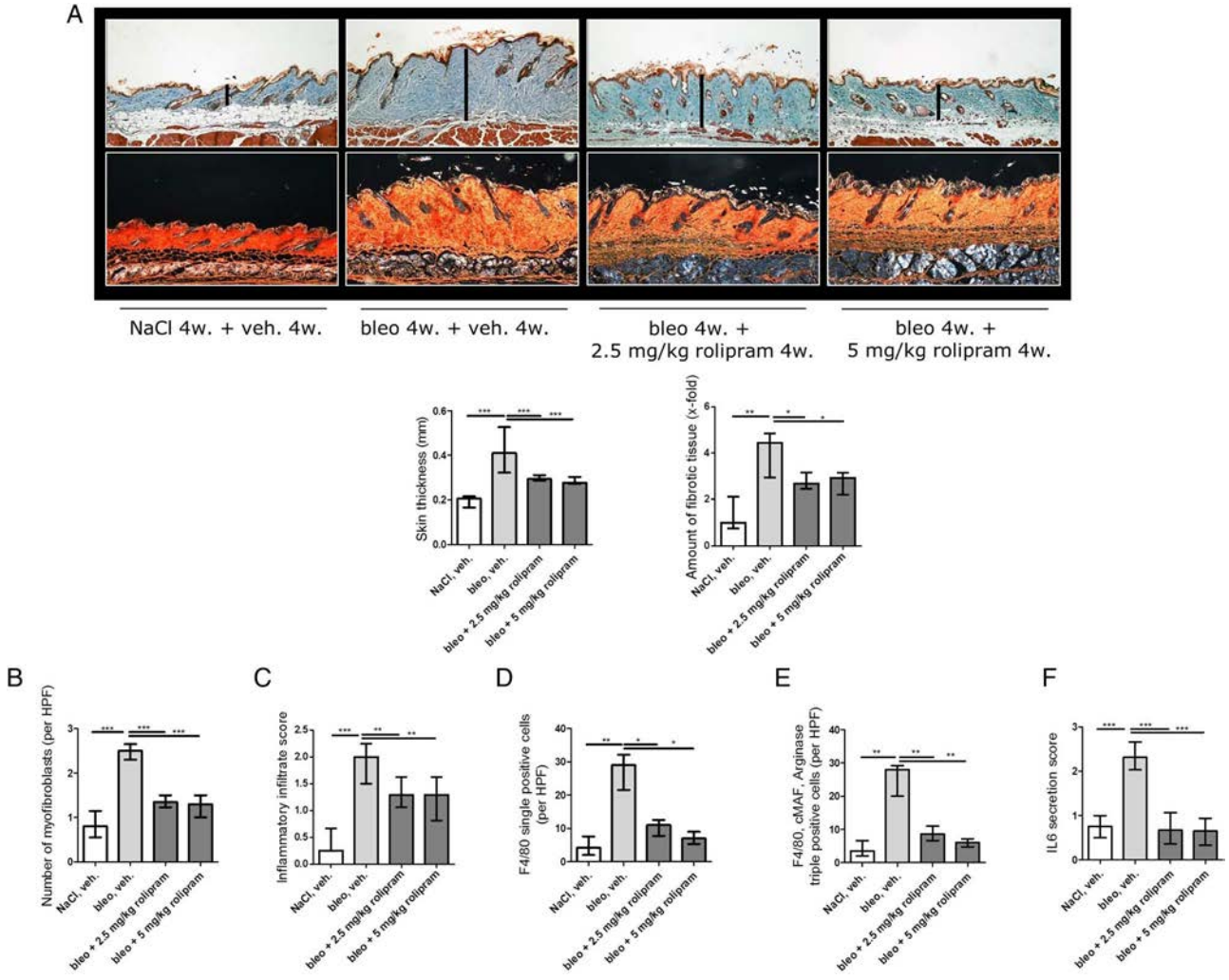

Figure 1 Inhibition of phosphodiesterase 4 (PDE4) by rolipram inhibits the development of bleomycin-induced skin fibrosis. (A) Representative images of Masson's trichrome with blue staining for collagens (upper pictures) and sirius red with orange staining for collagens (lower pictures). Pictures are shown in 100-fold magnification. Skin thickening as determined by Masson's trichrome stainings. Fibrotic tissue as assessed by histomorphometric measurements. (B) α-Smooth muscle actin-positive myofibroblasts. (C) Inflammatory infiltrates as determined in H&E stainings. (D) F4/80 single positive macrophages. (E) F4/80, cMAF and arginase triple positive macrophages. (F) Tissue interleukin-6 (IL-6) levels as assessed by immunofluorescence staining. (A–F) Animal groups consisted of N≥7 mice each. Statistical description: * for 0.01<p<0.05, ** for 0.001<p<0.01, *** for p<0.0001. bleo, bleomycin-treated mice; HPF, highpower field; veh, vehicle-treated mice; w, weeks.

mRNA as well as the secretion of IL-6 were reduced on treatment of M2 macrophages with rolipram (figure 3C–F). Along with our hypothesis that PDE4 blockade particularly affects the M2 macrophages, rolipram treatment did not show significant effects on the expression of *IL-6*, *IL-10*, *IL-13* and *tumour necrosis factor (TNF)-α* in M1 macrophages from healthy individuals and patients with SSc (see online supplementary figure S3C,D). Release of IL-6 protein was not affected by PDE4 inhibition in M1 macrophages (see online supplementary figure S3E, F).

We also assessed the effects of rolipram on already differentiated M2 macrophages. Again, mRNA levels of the profibrotic cytokines *IL-6*, *IL-13*, *TGF-β1* and *TGF-β2* as well as protein levels of IL-6 were reduced significantly, suggesting that PDE4 blockade could prevent and reverse the profibrotic cytokine milieu generated by M2 macrophages (see online supplementary figure S4).

To exclude that inhibition of M2 polarisation is due to off-target effects of rolipram, we knocked down PDE4B, the major PDE4 isoform in macrophages by small interfering RNA (siRNA). Consistent with the findings observed with rolipram, siRNA-mediated silencing of PDE4B inhibited alternative activation and M2 polarisation of macrophages. Consistently, knockdown of PDE4B also reduced the mRNA levels of profibrotic mediators such as IL-6, IL-13, TGF-β1 and TGF-β2 (see online supplementary figure S5).

We next investigated whether the inhibitory effects of PDE4 blockade on M2 macrophages were also relevant in vivo. Indeed, we observed a dose-dependent reduction of F4/80 single positive monocytes and F4/80/cMAF/arginase triple positive M2 macrophages in skin sections of bleomycin-challenged mice (figure 1D, E). Furthermore, tissue IL-6 levels were also reduced (figure 1F and see online supplementary figure S2), indicating that PDE4 inhibition blocks the release of profibrotic cytokines from M2 macrophages both in vitro and in vivo.

Inhibition of PDE4 ameliorates established skin fibrosis

So far, we have demonstrated that PDE4 blockade prevents bleomycin-induced fibrosis by interfering with the release of IL-6 and potentially other mediators from M2 macrophages.

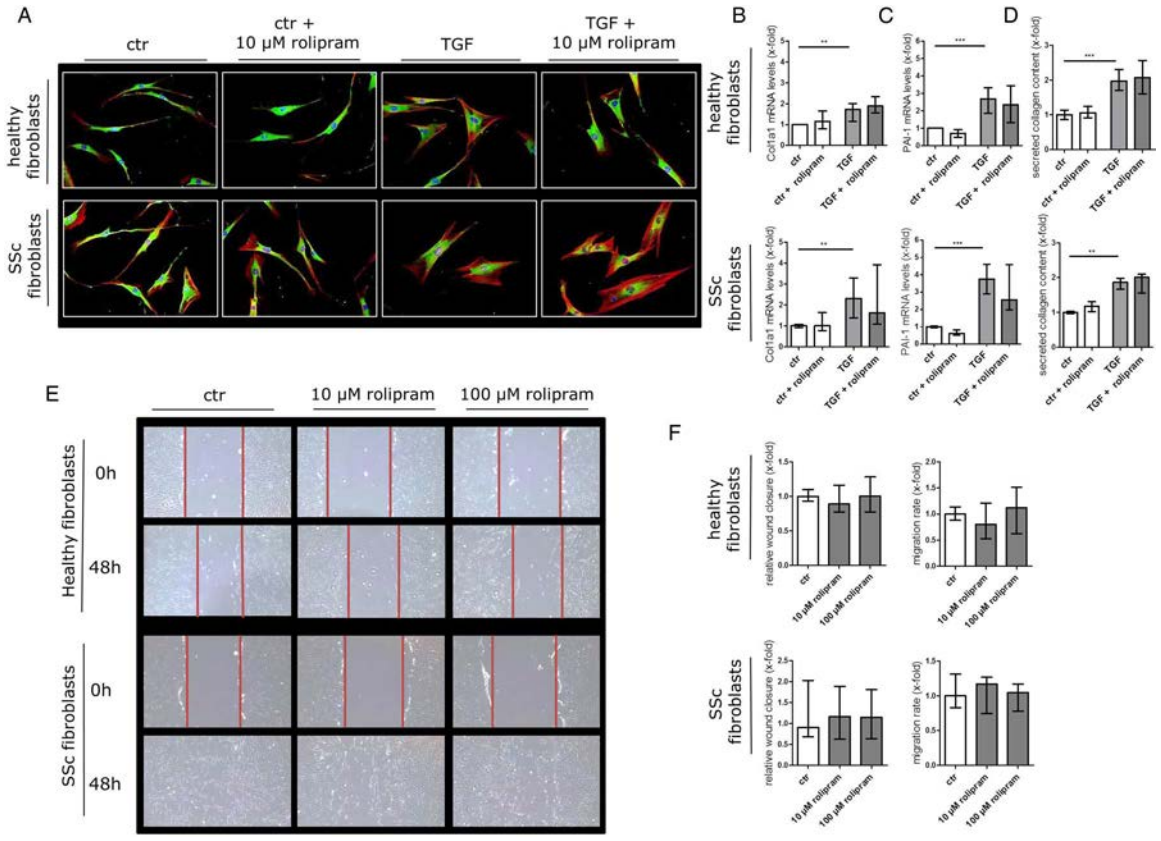

Figure 2 Phosphodiesterase 4 (PDE4) inhibition has no direct effects on fibroblasts. (A) Stress fibre formation in fibroblasts from healthy individuals (upper pictures) and patients with systemic sclerosis (SSc) (lower pictures) as assessed by phalloidin red staining. Nuclei are stained with 4′,6-diamidin-2-phenylindol. Representative stainings are shown in 200-fold magnification. N=3. (B, C) Messenger RNA levels of transforming growth factor-β (TGF-β) target genes *COL1A1* and *PAI-1* of unstimulated and TGF-β-stimulated dermal fibroblasts from healthy individuals and patients with SSc. (D) Secreted collagen proteins in the supernatant of unstimulated and TGF-β-stimulated dermal fibroblasts from healthy individuals and patients with SSc as assessed by SirCol collagen assay. (E) Representative pictures of scratch assay experiments assessing closure of the scratch after 48 hours. (F) Quantification of scratch closure time and migration rate in dermal fibroblasts from healthy individuals and patients with SSc. (A–F) In all experiments N≥3. Statistical description: * for 0.01<p<0.05, ** for 0.001<p<0.01, *** for p<0.0001. ctr, control.

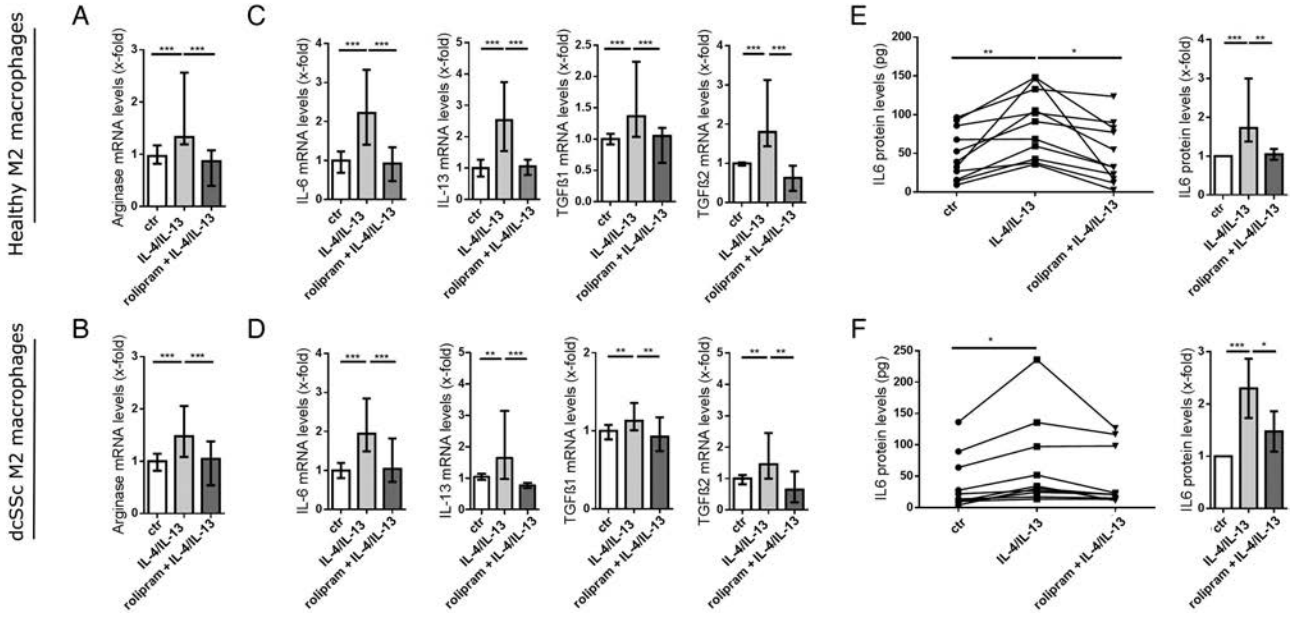

Figure 3 Inhibition of phosphodiesterase 4 (PDE4) interferes with the release of profibrotic cytokines from M2 macrophages. (A–F) M2 macrophages from healthy volunteers and patients with diffuse-cutaneous systemic sclerosis (dcSSc). (A–D) Messenger RNA expression of specific *ARGINASE* as well as interleukin-6 (*IL-6), IL-13,* transforming growth factor *(TGF)-β1 and TGF-β2*. (E, F) IL-6 protein levels in the supernatants. (A–F) In all experiments, N≥10. Statistical description: * for 0.01<p<0.05, ** for 0.001<p<0.01, *** for p<0.0001. ctr, control.

Next, we wondered if PDE4 inhibition might also be effective in pre-established fibrosis. We therefore took advantage of a modified model of bleomycin-induced fibrosis in which PDE4 blockade was initiated once skin fibrosis had already been established. We used the clinically available PDE4 inhibitor apremilast to demonstrate that the antifibrotic effects were applicable to PDE4 inhibitors in general.

When we compared the treatment groups with the first control group (6 weeks of bleomycin challenge) (see online supplementary figure S6), we observed that skin thickness, morphometric fibrosis assessment and myofibroblast numbers were reduced significantly by both doses of apremilast, suggesting that PDE4 blockade effectively prevented chronic progression of fibrosis. Next, we compared both treatment groups with the second control group (3 weeks of bleomycin challenge followed by 3 weeks of NaCl injections). Intriguingly, we observed that apremilast treatment reduced all fibrosis outcome measures below baseline fibrosis levels, indicating that PDE4 blockade induced regression of fibrosis (figure 4A, B).

In addition to the antifibrotic effects, leucocytic infiltrates were reduced on treatment with apremilast (figure 4C). Again, we observed a dose-dependent reduction of both F4/80 single positive monocytes and F4/80/cMAF/arginase triple positive M2 macrophages on treatment with apremilast (figure 4D, E). Furthermore, tissue IL-6 levels were dose-dependently reduced by 58% and 73% after treatment with 5.0 and 25.0 mg/kg apremilast twice daily (figure 4F and see online supplementary figure S7). Together, these experiments indicated that PDE4 inhibition by the clinically approved apremilast prevented progression of chronic fibrosis and even reversed establish fibrosis by reducing M2 differentiation and IL-6 release.

PDE4 blockade inhibits dermal fibrosis in the topoisomerase I mouse model

Since autoantibodies play a central role in initiation and progression of fibrosis, we studied the effects of pharmacological PDE4 blockade in mice immunised with the DNA topoisomerase I. Treatment with rolipram reduced skin thickness by 123%, fibrotic tissue by 75% and α-SMA-positive myofibroblasts by 91% (figure 5A, B). In line with our results from the bleomycin models, PDE4 inhibition by 5.0 mg/kg rolipram reduced the inflammatory infiltrates by 84% (figure 5C). F4/80 single positive monocytes were reduced by 67% and F4/80/cMAF/arginase triple positive M2 macrophages by 63% on treatment with rolipram (figure 5D, E). Furthermore, tissue IL-6 levels were reduced by 137% (figure 5F, see online supplementary figure S8).

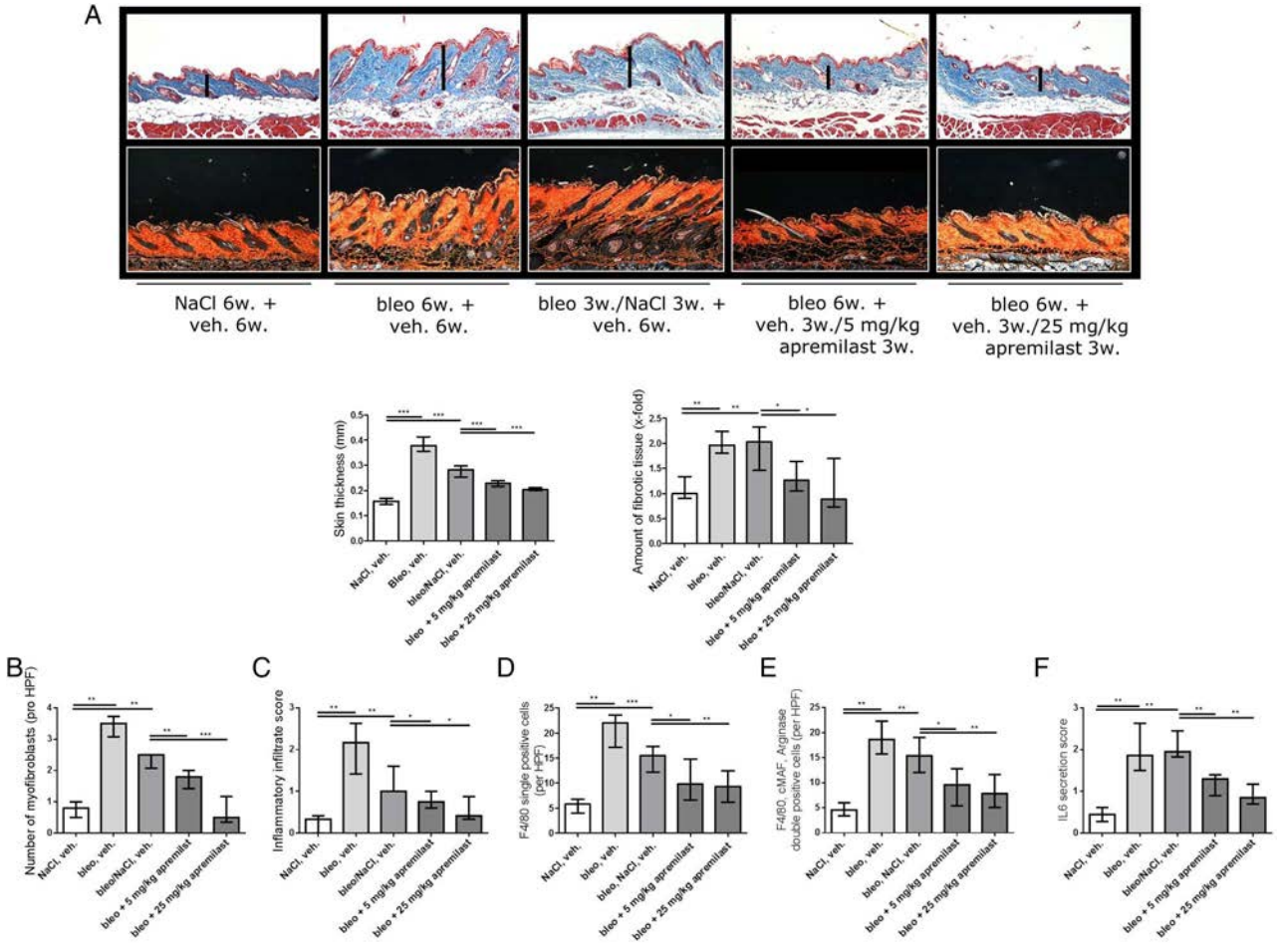

Figure 4 Phosphodiesterase 4 (PDE4) inhibition by apremilast induces regression of pre-established bleomycin-induced skin fibrosis. (A) Representative images of Masson's trichrome-stained sections (upper pictures) and sirius red-stained sections (lower pictures) at 100-fold magnification. Skin thickening as determined by Masson's trichrome stainings. Fibrotic tissue as assessed by histomorphometric measurements. (B) α-Smooth muscle actin-positive myofibroblasts. (C) Inflammatory infiltrates as determined in H&E stainings. (D) F4/80 single positive cells. (E) F4/80, cMAF and arginase triple positive macrophages. (F) Interleukin-6 (IL-6) tissue levels assessed by immunofluorescence staining. (A–F) Animal groups consisted of ≥6 mice each. Statistical description: * for 0.01<p<0.05, ** for 0.001<p<0.01, *** for p<0.0001. bleo, bleomycin-treated mice; veh, vehicle-treated mice; w, weeks.

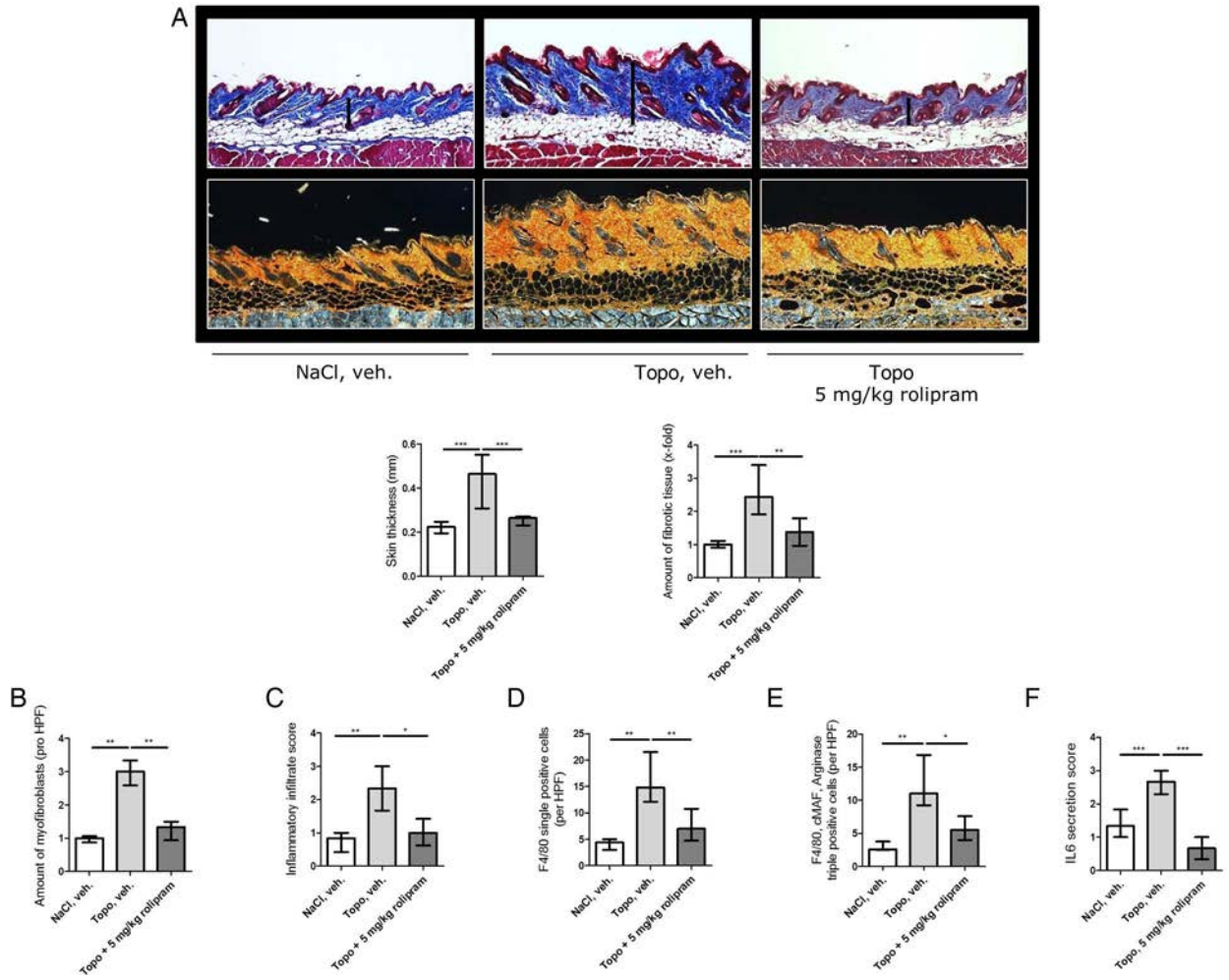

Figure 5 Phosphodiesterase 4 (PDE4) inhibition by rolipram inhibits dermal fibrosis in the topoisomerase I mouse model. (A) Representative images of Masson's trichrome-stained sections (upper pictures) and sirius red-stained sections (lower pictures) at 100-fold magnification. Skin thickening as determined by Masson's trichrome stainings. Fibrotic tissue as assessed by histomorphometric measurements. (B) α-Smooth muscle actin-positive myofibroblasts. (C) Inflammatory infiltrates as determined in H&E stainings. (D) F4/80 single positive cells. (E) F4/80, cMAF and arginase triple positive macrophages. (F) Interleukin-6 (IL-6) tissue levels assessed by immunofluorescence staining. (A–F) Animal groups consisted of ≥7 mice each. Statistical description: * for 0.01<p<0.05, ** for 0.001<p<0.01, *** for p<0.0001. topo, topoisomerase I-treated mice; veh, vehicle-treated mice.

Inhibition of PDE4 reduces dermal fibrosis caused by chronic graft-versus-host reaction

Since SSc is a systemic disease, we finally investigated the efficacy and tolerability of PDE4 blockade in the sclGvHD mouse model. When we treated transplanted animals with the PDE4 inhibitor rolipram, skin thickening, morphometric fibrosis assessment and myofibroblast counts (figure 6A, B) all indicated strong antifibrotic effects in well-tolerated doses. In line with our results from the bleomycin models, rolipram reduced the inflammatory infiltrates (figure 6C). Again, the antifibrotic effects of PDE4 inhibition were, at least in part, the result of reduced release of profibrotic mediators from M2 macrophages as indicated by a potent decreases in the numbers of M2 macrophages (figure 6D and E) and tissue IL-6 levels in the treatment group (figure 6F and see online supplementary figure S9).

DISCUSSION

Intensive research of the last decades resulted in the clinical development of PDE4 inhibitors for the treatment of psoriasis, psoriatic arthritis and potentially Behçet's disease. The current literature suggests that the disease-modifying effects of PDE4 inhibitors in these diseases mainly result from their anti-inflammatory activity. Since autoimmunity and inflammation drive fibrosis in early stages of SSc and persist in a subset of patients, we initiated the current study to investigate a potential disease-modifying activity of PDE4 inhibition in SSc.

Using the lead compound rolipram and apremilast, the PDE4 inhibitor used in the clinic, we observed potent antifibrotic activity on PDE4 blockade. Although PDE4 is expressed in fibroblasts and although previous studies suggested inhibitory effects of PDE4 blockade on fibroblast contraction and chemotaxis,[45] we did not observe any direct effects of PDE4 inhibition on fibroblast activation, migration and collagen release in clinically relevant doses. These observations were consistent with our hypothesis that PDE4 inhibition might have disease-modifying antifibrotic activity through interaction with immune cell activation in SSc.

Both in leucocytic infiltrates in early SSc and in the skin of bleomycin-challenged mice, macrophages represent one of the most abundant cell populations. Accumulating preclinical evidence suggest that subgroups of macrophages, often referred to as M2 macrophages, are key mediators of physiological wound

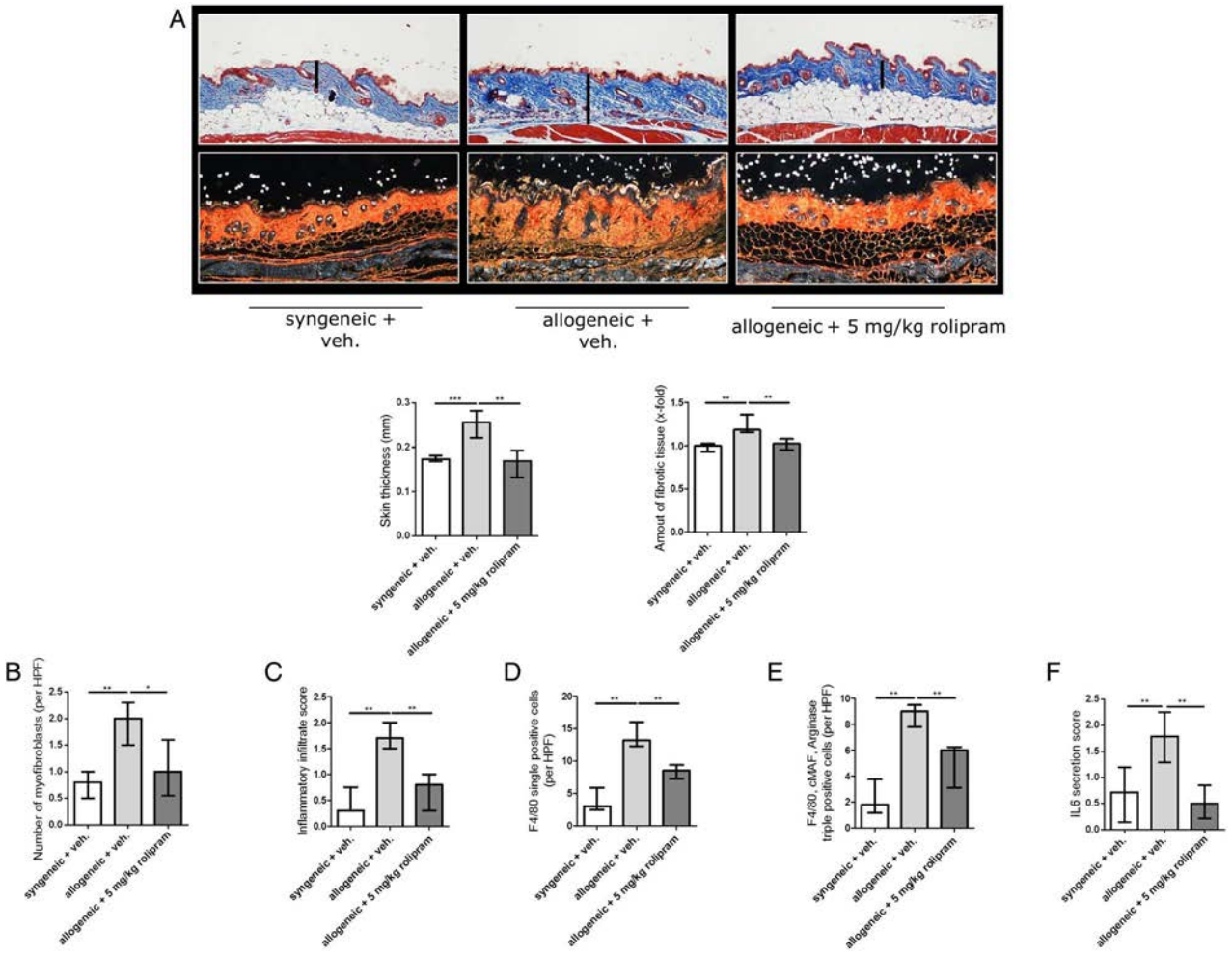

Figure 6 Inhibition of phosphodiesterase 4 (PDE4) ameliorates fibrosis in murine sclerodermatous chronic graft-versus-host disease. (A) Representative images of Massons's trichrome (upper images) and sirius red-stained (lower images) sections at 100-fold magnification. Skin thickening as determined by Masson's trichrome stainings. Fibrotic tissue assessed by histomorphometric measurements. (B) α-Smooth muscle actin-positive myofibroblasts. (C) Inflammatory infiltrates as determined in H&E stainings. (D) F4/80 single positive cells. (E) F4/80, cMAF and arginase triple positive macrophages. (F) Tissue interleukin-6 (IL-6) levels assessed by immunofluorescence staining. (A–F) The groups consisted of ≥6 mice each. Statistical description: * for 0.01<p<0.05, ** for 0.001<p<0.01, *** for p<0.0001. Veh, vehicle-treated mice.

healing and pathological fibrosis.[11] These preclinical findings are corroborated by data from the FASSCINATE trial.[12] Gene expression analysis revealed a M2-macrophage signature in SSc skin, which was responsive to treatment with tocilizumab. We observed that PDE4 blockade was effective in inhibiting the differentiation of monocytes/macrophages into a M2 phenotype and in reducing the release of profibrotic mediators, including IL-6. Since PDE4 inhibition blocked the expression of several fibrosis relevant mediators in M2 macrophages, we hypothesise that its antifibrotic efficacy may exceed the antifibrotic effects of selective IL-6 inhibition in patients with SSc.

Our study provides first evidence that PDE4 blockade has a profound antifibrotic activity. Gobejishvili *et al*[46] and Udalov *et al*[47] investigated PDE4 blockade in models of cholestatic liver disease and chronic lung injury, respectively, supporting the idea that PDE4 might play a general role in chronic organ damage. In detail, cholestatic liver injury induced by bile duct ligation was accompanied by increased PDE expression. Treatment with rolipram reduced inflammatory and profibrotic cytokine expression. Udalov *et al* used the PDE4 inhibitor cilomilast to demonstrate reduced lung injury and fibrosis after a single bleomycin challenge. Similar to our results in the skin, treatment with cilomilast reduced the number of macrophages in bronchoalveolar lavage (BAL) fluid, while neutrophils remained unchanged. By contrast to our results, the authors observed decreased *TNF-α* mRNA but increased *IL-6* mRNA BAL levels. This might reflect the early disease stage after injury during which these investigations were performed. At this early stage, inflammation is still dominated by M1 macrophages, whereas M2 macrophages as a predominant source of profibrotic mediators accumulate during later stages of bleomycin-induced injury.[11]

Since the 'standard' bleomycin model is used to investigate prevention of fibrosis, it was crucial to study the effects of PDE4 inhibition in a modified model, in which treatment is initiated, once fibrosis has already been established. PDE4 blockade was effective in preventing progression of chronic fibrosis and reversing established fibrosis. These observations were accompanied by increased counts of M2 macrophages in established fibrosis, which were normalised by PDE blockade. We believe that this finding might indicate that M2 macrophages may contribute to the persistence of fibrotic disease in patients with SSc. Targeting M2 macrophages might therefore re-establish physiological tissue homeostasis and allow reversal of fibrosis.

In addition to its antifibrotic effects in bleomycin-induced fibrosis, we observed that PDE4 blockade was also effective in treating preclinical sclGvHD, a model to mimic systemic fibrosis as seen in patients with diffuse-cutaneous SSc. Although the sclGvHD model was long thought to be T cell-driven, accumulating evidence suggests a central role of M2 macrophages chronic graft-versus-host disease,[48] which is in line with our observations. In addition to bleomycin-induced fibrosis and murine sclGvHD, PDE4 inhibition also demonstrated potent antifibrotic effects in topoisomerase-induced fibrosis. Histological analyses indicate that early phases of SSc are characterised by inflammatory infiltrates,[3] and genetic profiling studies highlight that SSc may evolve in distinct disease subtypes, including inflammatory subtypes.[49] In this context, our current study highlights potent antifibrotic effects of PDE4 inhibition in SSc models reflecting exactly these early stages and inflammatory subtypes. By contrast to its effects in other rheumatic conditions, PDE4 inhibitors act primarily through modulating the release of profibrotic mediators from macrophages expressing a M2 phenotype. Since accumulating evidence suggests that M2 macrophages may play a more general role in several subtypes of fibrosis, including inflammatory and fibroproliferative disease types, our findings might prompt additional experimental studies to investigate a potential role of PDE4 inhibitors in inflammation-independent, fibroproliferative diseases.

The PDE4 inhibitor apremilast is already approved for the treatment of psoriasis and psoriatic arthritis. Apart from minor gastrointestinal side effects during the initiation of therapy, apremilast is very well tolerated and does per se not require routine laboratory testing compared with other disease-modifying agents. Our results suggest that apremilast, as well as other PDE4 inhibitors, might be tested and further developed for the treatment of patients with SSc at early stages or with persistent inflammatory disease.

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EXTENDED REPORT

Knee and hip intra-articular adipose tissues (IAATs) compared with autologous subcutaneous adipose tissue: a specific phenotype for a central player in osteoarthritis

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ABSTRACT

Objectives Compared with subcutaneous adipose tissue (SCAT), infrapatellar fat pad (IFP), the main knee intra-articular adipose tissue (IAAT), has an inflammatory phenotype in patients with osteoarthritis (OA). We phenotyped suprapatellar fat pad (SPFP) and hip acetabular fat pad (AFP), two other IAATs, to determinate the unique signature of IAATs compared with SCAT.

Methods IFP, SPFP, AFP and autologous SCAT were obtained from patients with OA during total knee (n=38) or hip replacement (n=5). Fibrosis and adipocyte area were analysed by histology and vascularisation, leucocyte and mast cell infiltration were analysed by immunohistochemistry for von Willebrand factor, leucocytes and tryptase, respectively. Secretion of interleukin (IL)-6, IL-8 and prostaglandin E_2 (PGE_2) was assessed by ELISA. The mRNA expression of adipocyte-associated genes (ATGL, LPL, PPAR-γ, FABP4 and CD36) and developmental genes (SFRP2, HoxC9 and EN1) was determined. The inflammatory response of isolated fibroblast-like synoviocytes (FLS) to autologous IFP and SPFP conditioned media was examined.

Results Fibrosis, vascularisation and leucocyte and mast cell infiltration were greater in IAATs than SCAT, and levels of IL-6, IL-8 and PGE_2 were greater in all IAATs than SCAT. IFP and SPFP induced a similar inflammatory response to FLS. Adipocyte area was smaller in IAATs than SCAT. Adipocyte-associated and developmental genes showed a similar gene expression pattern in all IAATs, different from SCAT.

Conclusions IFP but also SPFP and AFP (gathered under the term 'IAAT') may play a deleterious role in OA by affecting joint homeostasis because of their inflammatory phenotype and their close interaction with synovium in the same functional unit.

INTRODUCTION

Osteoarthritis (OA) is a whole-joint disease mainly characterised by progressive cartilage disappearance, subchondral bone remodelling and synovitis, which all act in concert for OA progression. In this context, many studies have focused on cartilage/subchondral bone crosstalk. Cartilage and subchondral bone are indeed now considered a unique functional unit.[1 2] Although synovitis was thought to mainly result from cartilage breakdown, several data suggest that it could be also involved in early stages of OA even before cartilage damage.[3 4] Moreover, we and others have shown that knee OA synovitis may also depend on the release of inflammatory factors by the infrapatellar fat pad (IFP), located at the posterior surface of synovium.[5 6]

IFP is one intra-articular adipose tissue (IAAT), which has received much attention for several years. IFP from patients with knee OA releases many inflammatory factors in higher amounts compared with autologous subcutaneous adipose tissue (SCAT).[6–8] IFP volume[9] and release of tumour necrosis factor α by IFP[8] are both positively associated with the body mass index (BMI) of human patients with OA. Similarly, in mice fed a high-fat diet, weight gain and IFP volume were correlated with development of OA.[10] Positive associations were also found between adipocyte area and vascular infiltrates of IFP.[10] Consequently, IFP may have a paracrine function in other joint tissues in OA, especially the adjacent synovium.[5 6]

In the best of our knowledge, only IFP has been studied in OA, although anatomically, other IAATs are indeed present in the joints. The suprapatellar fat pad (SPFP) is composed of the quadriceps fat pad and the pre-femoral fat pad, which are located above the patella and behind the suprapatellar bursa, respectively (see online supplementary figure S1). The posterior fat pad is in close contact with the posterior articular capsule behind the menisci.[11] Whether IFP has unique properties among IAATs is currently unknown. In addition, IAATs are not restricted to the knee. For instance, the coxofemoral joint contains one IAAT located in acetabular fossa and surrounding the ligamentum teres (acetabular fat pad (AFP)) (see online supplementary figure S1). Nothing is known about AFP in the context of arthritis. Whether IAATs from different joints share similar properties is unknown.

We investigated whether all IAATs acquire an inflammatory phenotype in OA like IFP, belonging to the same and unique type of adipose tissue (AT), which thus may act with the adjacent synovium as a unique functional unit. We used histological, molecular and functional characterisation of IFP, SPFP and SCAT from patients with autologous OA. In parallel, OA AFP and autologous SCAT were characterised. Fibrosis, vascular density, inflammatory

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infiltrates and adipocyte size were determined, as was the expression of inflammatory factors and molecules important for adipocyte function. The effect of different knee IAATs on fibroblast-like synoviocyte (FLS) inflammatory response was compared.

MATERIALS AND METHODS

AT and synovium samples

Tissues were harvested from patients with end-stage symptomatic knee (n=38) or hip OA (n=5) undergoing surgery for total knee or hip replacement at Henri Mondor Hospital (Créteil, France). Sequential patients from whom informed consent was obtained were included. Surgeons harvested the IFP with the synovial membrane lining its posterior surface and the SPFP during knee replacement or AFP located in the bottom of acetabular fossa during hip replacement. The SCAT was harvested immediately below the scar. Tissues were stored in Dulbecco's modified Eagle's medium (DMEM) with 1% bovine serum albumin (BSA).

Omental and autologous SCATs were harvested from patients (n=15; female, n=14) operated at Visceral Surgery Department of Ambroise Paré Hospital (Boulogne-Billancourt, France) for gastric banding (n=2), gastric bypass (n=7) or sleeve gastrectomy (n=6). Mean age and mean BMI were 39.7±3.8 (18–62) and 45.6±1.4 (36.4–53.7), respectively. All subjects are part of the BAR-ICAN study (study of obese subjects in bariatric surgery programmes) (ethical committee no. 2014-April-13533).

Generation of AT-conditioned medium

ATs were carefully dissected as described.[6] For the IAAT samples, special care was taken to separate AT from the synovium. The absence of remaining synovium was checked on several samples by histology. Nevertheless, we cannot exclude the marginal presence of remaining synovium in some cases. To generate the AT-conditioned medium, 300 mg AT, minced into small pieces, was incubated in 1 mL of DMEM, 12.5 mM glucose and 1% BSA for 1 hour at 37°C in a humidified atmosphere of 5% CO_2/95% air. The medium was removed and tissues were incubated in 1 mL of the same medium for 3 hour. Thereafter, conditioned media and tissues were collected separately, spun and frozen at −80°C.

Isolation, culture and stimulation of FLS

As described,[6] synovium was digested in 0.75 mg/mL collagenase/dispase and 0.075 mg/mL DNase (both from Roche Diagnostics) in FLS growth medium (RPMI 1640 Glutamax, 100 units/mL penicillin, 0.1 mg/mL streptomycin, 10 mM HEPES, 2 mM sodium pyruvate, 10% fetal calf serum) before seeding cells in culture plates. FLS were cultured at 37°C in a humidified atmosphere of 5% CO_2/95% air.

FLS from passage 3 (n=6 patients) were seeded at 10^5 cells/well in 6-well culture plates. Confluent FLS were left in depletion medium (growth medium without serum) for 24 hours before washing with phosphate-buffered saline (PBS) and stimulation with IFP-conditioned or SPFP-conditioned medium (150 µL) in depletion medium (850 µL). Control FLS were incubated in depletion medium (850 µL) with 150 µL of medium used to generate tissue conditioned media. After 24 hour stimulation, FLS were rinsed twice with PBS and incubated in depletion medium for 24 hours. Conditioned media were kept, spun and stored at −80°C.

Isolation of adipocytes

IFP and SCAT were digested in 1 mg/mL collagenase (Roche Diagnostics) in DMEM 4.5 g/L glucose, 100 units/mL penicillin, 0.1 mg/mL streptomycin, 15 mM HEPES, 0.2% BSA for 1 hour at 37°C. The adipose suspension was then filtered through a 100 µm mesh and centrifuged for 6 min at 150*g*. The upper phase containing adipocytes was separated and washed two times with PBS. After a final centrifugation (6 min at 150*g*), adipocytes were lysed for gene expression analysis.

Total RNA extraction and quantitative RT-PCR

Total RNA was isolated by using the Reliaprep RNA Cell miniprep system (Promega). RNA (250–1000 ng) was reverse transcribed by using the Omniscript RT kit (Qiagen). Gene expression was analysed by quantitative RT-PCR with Roche Diagnostics LightCycler 480 in a 12 µL final volume with specific primers (10 µM) (see online supplementary table S1) and GoTaq PCR Master Mix (Promega). PCR amplification involved a denaturation step (5 min at 95°C) followed by 40 cycles of 10 s at 95°C, 15 s at 60°C and 10 s at 72°C.

For each PCR, cDNA was run in duplicate in parallel with serial dilutions of a cDNA mixture tested for each primer pair to generate a standard linear curve, which was used to estimate the amplification efficiency. The relative mRNA expression for all genes analysed was normalised to that of 18S RNA (used as the internal reference gene) and determined by using the efficiency method with Light Cycler 480 software.

ELISA

ELISA kits were used to determine the concentrations of interleukin (IL)-6, IL-8 (both from Sanquin-PeliKine), prostaglandin E_2 (PGE_2; Cayman Chemical), matrix metalloproteinase 1 (MMP-1; from R&D Systems) in AT and/or FLS-conditioned medium.

Histology and immunohistochemistry study

AT samples were fixed in 3.7% paraformaldehyde, embedded in paraffin and serially sectioned (5 µm). Sections were stained with picrosirius red (Sigma). Immunohistochemistry involved mouse monoclonal antibodies to CD45 (leucocytes, clone 2B11 +PD7/26, Dako) (dilution 1:100), tryptase (mast cells, AA1, Santa Cruz Biotechnology) (dilution 1:100), CD3 (T lymphocytes, clone F7.2.38, Dako) (dilution 1:50), CD20 (B lymphocytes, clone L26, Dako) (dilution 1:100) and CD68 (macrophages, clone PG-M1, Dako) (dilution 1:100) and von Willebrand factor (vWF) (endothelial cells, clone F8/86, Dako) (dilution 1:500) as primary antibodies. For all antibodies except vWF, the R.T.U Vectastain kit (Vector) was used for detection followed by counterstaining with Mayer's haematoxylin. Immunofluorescent detection of vWF involved horseradish peroxidase conjugated secondary rabbit anti-mouse IgG antibody (Abliance) and the TSA Plus Cyanine 3 System (Perkin Elmer). Irrelevant control antibodies (Dako) were incubated at the same concentration to assess non-specific staining.

Morphometric analysis

Sections stained with picrosirius red were used for fibrosis quantification and adipocyte area determination. Digital images of magnification views (×20) of tissue sections were captured by using an Olympus DP73 camera (Olympus) on an Olympus BX43 microscope. Fibrosis analysis involved histomorphometry with CaloPix software (Chatillon, France) with content colour thresholds. The quantification of total fibrosis was expressed as

the ratio of fibrous tissue area stained with picrosirius red/total tissue surface.[12] For adipocyte mean area determination, two independent observers blindly measured the area of 40 adipocytes located in the centre of the tissue section and values were averaged. Vessel number and vascular density were quantified after vWF immunostaining. The number of vessels was measured in the whole section and normalised to the tissue area. Vascular density was quantified as the proportion of vWF-positive area normalised to tissue area. Infiltration of CD45-positive and tryptase-positive cells within the tissue area was graded as 0, no or sparse positive cells; 1, several positive cells; and 2, numerous positive cells with several clusters.

Statistical analysis

Paired Wilcoxon non-parametric rank test (Statview software, V.4.57, SAS) was used for analysis. Data are presented as mean ±SD. $p<0.05$ was considered statistically significant.

RESULTS

Patient characteristics

We included patients with severe and symptomatic knee (n=38) and hip OA (n=5). Characteristics of each group are in online supplementary table S2. In the knee OA group, the mean age was 73.3±6.5 years and 29 (76.3%) were women. The mean BMI was 29.3±5.0 kg/m^2. In the hip OA group, the mean age was 73.0±3.3 years and three (60.0%) were women. The mean BMI was 27.4±4.4 kg/m^2.

Fibrosis and inflammatory infiltration of OA IAATs

At the time of dissection, all harvested IAATs, whatever their knee or hip origin, had more fibrous tissue than autologous SCAT. This observation was confirmed at histological level after quantification of picrosirius red staining (figure 1). In knee OA, fibrosis in IFP and SPFP accounted for 30.9%±18.6% and 26.9%±9.4% of tissue area, respectively, and was significantly more extended than in SCAT (18.9%±8.5% of tissue area, p=0.028 and 0.028, respectively). This difference was even more pronounced between AFP and corresponding SCAT (69.2%±9.5% and 21.3%±6.0% of tissue area, respectively) (figure 1B,C). Given the small number of patients, no statistical analysis was performed for hip tissue. In IAATs, fibrous tissue accumulated between adipocyte lobules as large fascicles. In some cases, adipocytes and fibres were intermingled (figure 1A, panels a, b). In contrast, the fibrous part of SCAT surrounded adipocyte lobules and few fibres were detected among clusters of adipocytes (figure 1A, panel c). The vascular network also appeared different between IAATs and SCAT. Vessel number to tissue area was significantly increased 2.2-fold between both IFP

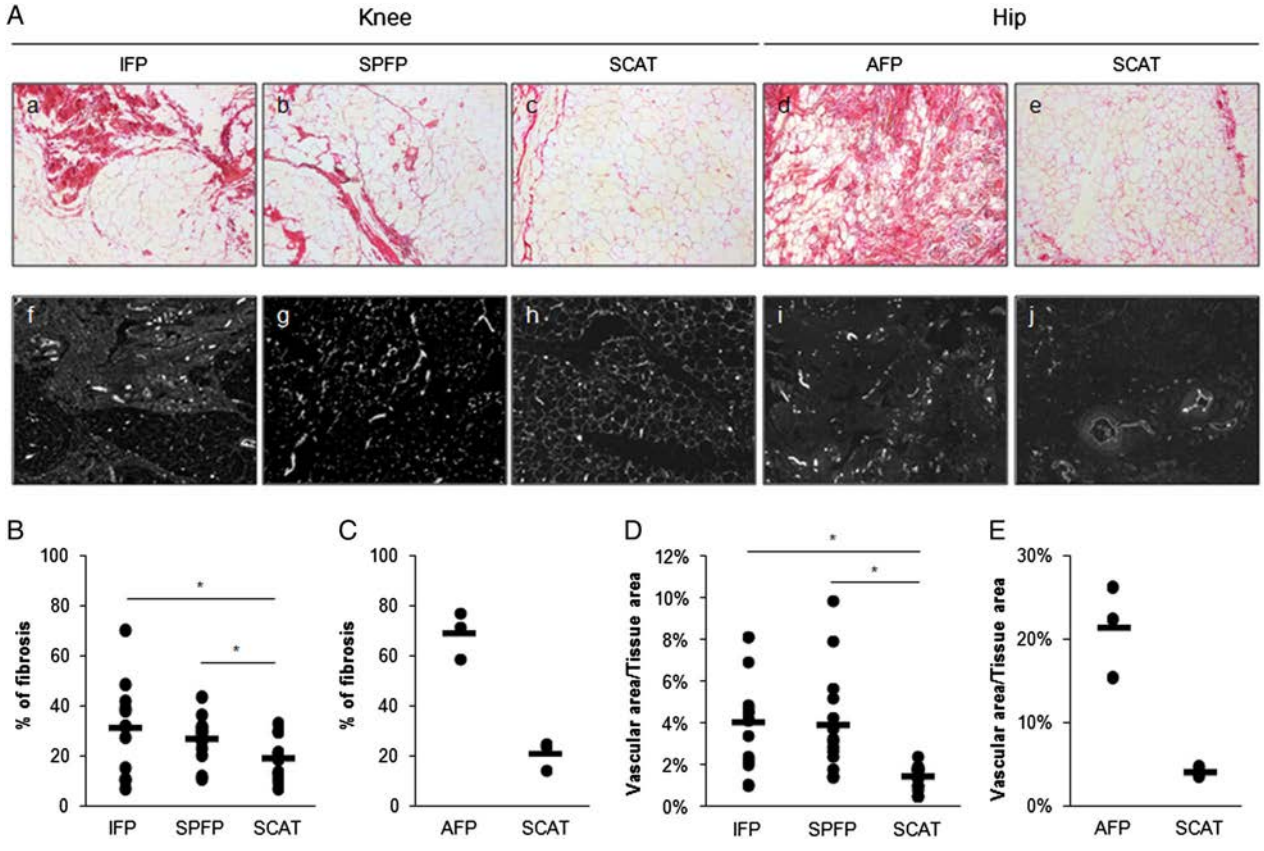

Figure 1 Histological characterisation of fibrosis and vascularisation in intra-articular adipose tissue (IAAT) and subcutaneous adipose tissue (SCAT) from patients with end-stage osteoarthritis. (A) Paraffin sections (5 μm) of infrapatellar fat pad (IFP) (a and f), suprapatellar fat pad (SPFP) (b and g), acetabular fat pad (AFP) (d and i) and SCAT (c, e, h and j) (n=16 for knee and n=3 for hip tissues) were stained with picrosirius red for fibrosis determination (a–e) and for von Willebrand factor (f–j) for vessel quantification. Digital images of magnification views (×4) of tissue sections were captured. In IAATs, fibrous tissue (stained in red) accumulated between adipocyte lobules as large fascicles. In some cases, adipocytes and fibres were intermingled. In contrast, the fibrous part of SCAT surrounded adipocyte lobules and few fibres were detected among clusters of adipocytes (c and e). Quantification of the proportion of fibrosis and ratio of vessel-to-tissue area in knee (n=16) (B and C) and hip tissues (n=3) (D and E). Knee IAATs have significantly greater percentage of fibrosis and vessel area than autologous SCAT. Similar observations are made for AFP compared with hip SCAT. Horizontal bar is mean and dots represent each patient. *p<0.05.

and SPFP and SCAT (data not shown). The vascular area to tissue area was significantly greater in IFP and SPFP than SCAT (p=0.003 and 0.006, respectively) (figure 1D), with no difference between IFP and SPFP. As observed in knee IAATs, a higher vessel number and vascular area to tissue area were quantified in AFP compared with autologous SCAT (figure 1E). The fibrous part of ATs contained more vessels, which were also larger than within adipocyte lobules. Tissue fibrosis and vascularisation were associated neither with the obese/non-obese status of the patients nor with gender, with the exception of a lower vascular area, in females only (see online supplementary table S3). In addition to fibrosis and vascularisation, CD45-positive and tryptase-positive cell infiltration was greater in knee and hip IAATs than autologous SCAT (figure 2). Leucocytes and mast cells preferentially accumulated in perivascular areas in fibrous parts of ATs than in adipocyte lobules. Inflammatory infiltrates consisted mainly of macrophages. Some T lymphocytes and B lymphocytes could also be observed in IAATs (see online supplementary figure S2).

Common inflammatory secretion pattern of IAATs

As we previously reported,[6 7] IFP from patients with OA produced significantly more IL-6, IL-8 and PGE_2 than autologous SCAT (figure 3A–C). Similarly, SPFP also secreted higher amounts of these inflammatory mediators compared with SCAT. The release was 6.2-fold, 6.7-fold and 189.1-fold higher by SPFP than SCAT for IL-6 (p<0.0001), IL-8 (p=0.002) and PGE_2 (p<0.0001), respectively. No significant difference was found between IFP and SPFP. AFP also secreted significantly more inflammatory factors than autologous SCAT (figure 3D–E). No relationship was found between obesity and any of the inflammatory factors. We only found a lower release of PGE_2 by SCAT in females only (see online supplementary table S3).

As a consequence of the inflammatory pattern of IAAT secretion products, both IFP-conditioned and SPFP-conditioned media induced an inflammatory and prodegradative response to autologous FLS (figure 4). The production of IL-6 and MMP-1 was indeed strongly stimulated by IFP (8.6-fold, p=0.028, and 3.7-fold, p=0.028, respectively) and SPFP-conditioned media (15.6-fold, p=0.046, and 3.8-fold, p=0.043, respectively) compared with unstimulated control cells.

Specific phenotype of IAAT-derived adipocytes

Morphometric analysis of adipocytes revealed a smaller size of adipocytes within IAATs than SCAT. IFP and SPFP adipocyte mean area was 0.6-fold (p=0.002) and 0.7-fold smaller (p=0.007), respectively, than that from autologous SCAT (figure 5). IFP and SPFP did not differ in adipocyte mean area. As observed in knee IAATs, the mean surface of AFP-derived adipocytes was 0.7-fold smaller than that from autologous SCAT (figure 5). No relationship was found between obesity or gender with the adipocyte area of knee IAATs and SCAT (see online supplementary table S3). This result suggests that adipocytes present within IAATs and SCAT have distinct phenotypes.

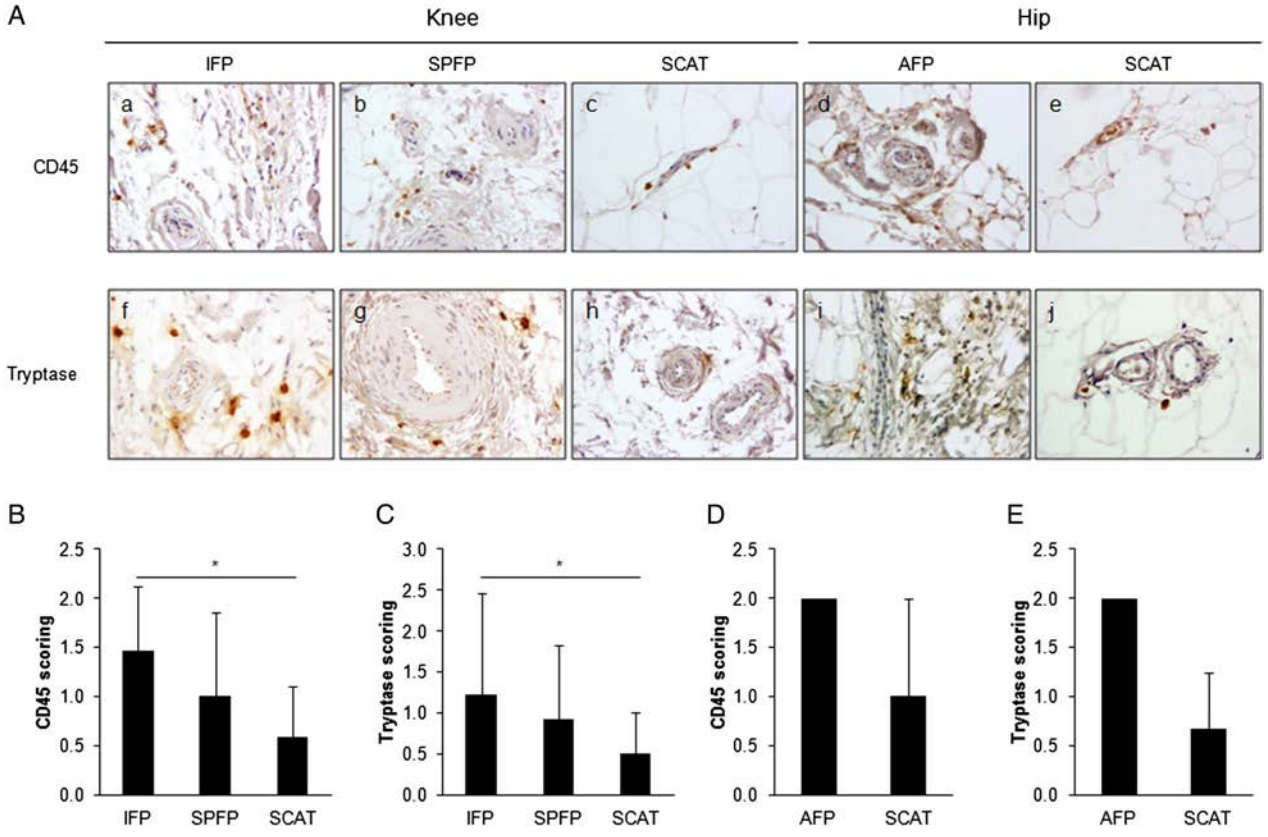

Figure 2 Histological characterisation of leucocyte infiltration in intra-articular adipose tissue (IAAT) and subcutaneous adipose tissue (SCAT) from patients with end-stage osteoarthritis. (A) Paraffin sections (5 μm) of infrapatellar fat pad (IFP) (a and f), suprapatellar fat pad (SPFP) (b and g), acetabular fat pad (AFP) (d and i) and SCAT (c, e, h and j) (n=13 for knee and n=3 for hip tissues) were immunostained for CD45 leucocytes (a–e) and tryptase, which targets mast cells (f–j). CD45-positive and tryptase-positive cells were mainly observed in the fibrous part and in perivascular areas of the adipose tissue. Original magnification ×20. (B–E) Quantification of the leucocyte (B and D) and mast cell infiltrates (C and E) of knee (B and C) and hip IAATs and SCAT (D and E). More leucocytes and mast cells are present in IAATs than SCAT. Data are mean±SD. *p<0.05.

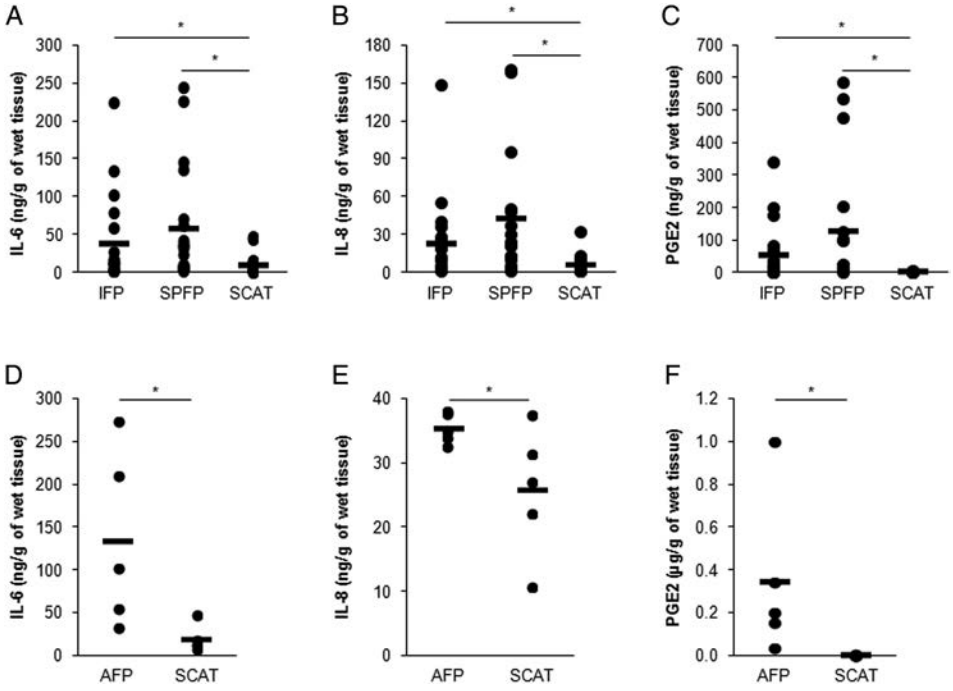

Figure 3 Differential release of inflammatory factors by knee and hip intra-articular adipose tissues (IAATs) and subcutaneous adipose tissue (SCAT) from patients with end-stage osteoarthritis (OA). Secretion of interleukin (IL)-6 (A and D), IL-8 (B and E) and prostaglandin E_2 (PGE_2) (C and F) by knee (A–C) and hip IAATs (D–F) and autologous SCAT in conditioned media. Measurements of 19 and 5 patients with knee and hip OA, respectively. Horizontal bar is mean and dots represent each patient. *p<0.05. AFP, acetabular fat pad; IFP, infrapatellar fat pad; SPFP, suprapatellar fat pad.

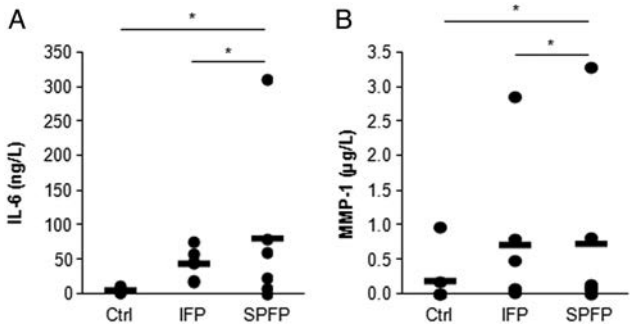

Figure 4 Inflammatory response of fibroblast-like synoviocytes (FLS) by stimulation by autologous knee intra-articular adipose tissues. FLS from six patients with osteoarthritis were treated or not with conditioned media from autologous infrapatellar fat pad (IFP) and suprapatellar fat pad (SPFP). Secretion of interleukin (IL)-6 (A) and matrix metalloproteinase 1 (MMP-1) (B) in FLS-conditioned media was determined by ELISA. Horizontal bar is mean and dots represent each patient. *p<0.05.

EN1, SFRP2, HoxC9 and Wt1 are genes that are differentially expressed by SCAT and intra-abdominal AT and their isolated adipocytes.[13–15] (see online supplementary figure S3) Here, IAATs and SCAT also differentially expressed EN1 mRNA, with an expression strongly decreased in all IAATs compared with SCAT (IFP: 0.3-fold, p=0.006; SPFP: 0.2-fold, p=0.006; and AFP: 0.3-fold, p=0.046) (figure 6H). Similarly, the mRNA level of HoxC9 was lower in SPFP and AFP than autologous SCAT (SPFP: 0.6-fold, p=0.025; and AFP: 0.3-fold, p=0.043). IFP and SCAT did not differ in HoxC9 expression and all ATs showed a similar mRNA expression of SFRP2 (figure 6). Wt1 was not expressed by IAATs.

PPAR-γ, CD36, FABP4, LPL and ATGL are involved in adipogenesis, intracellular fatty acid transfer and trafficking, lipogenesis and lipolysis. They were also differentially expressed in IAATs and SCAT (figure 6). The mRNA expression of CD36, FABP4, LPL and ATGL was significantly decreased in IFP, SPFP and AFP compared with the autologous SCAT. PPAR-γ mRNA expression was lower in SPFP and AFP than SCAT. In contrast, PPAR-γ mRNA expression was similar in IFP and SCAT. Interestingly, some differences were observed between IFP and SPFP. The mRNA levels of PPAR-γ (p=0.002) and CD36 (p=0.007) were lower in SPFP than autologous IFP.

To exclude that differences in gene expression between IAATs and SCAT could be due to the accumulation of fibrosis within IAATs and thus to a lower proportion of adipocytes within the whole AT, we analysed the expression of ATGL, LPL and CD36 in isolated adipocytes from IFP and SCAT (figure 6I). As observed in whole AT, the mRNA expression of ATGL, LPL and CD36 was lower in IFP-derived than SCAT-derived adipocytes.

DISCUSSION

In recent years, IFP has received much attention for its possible involvement in OA. IFP volume or surface is modified in OA and could be associated with structural damage and pain.[16] IFPs from early-stage and end-stage OA display a different gene expression pattern.[17] OA IFP also shows an inflammatory phenotype characterised by a higher expression and secretion of inflammatory factors than autologous SCAT.[6 7] Consistently, IFP stimulates an inflammatory response to FLS,[5 6] which suggests that the functional interaction of IFP with synovium may be a mechanism of inducing OA synovitis. Of note, several IAATs are present within the knee and IAATs are not restricted to the knee. In this study, we wondered whether IFP is unique among IAATs or whether all IAATs share common properties. Interestingly, all IAATs feature a similar histological pattern: increased fibrosis, vascularisation and leucocyte infiltration compared with autologous SCAT. They express and secrete a higher level of inflammatory factors (IL-6, IL-8 and PGE_2). All these features were independent of gender or BMI of patients. Like IFP, SPFP induces an inflammatory response to FLS. All IAATs and isolated adipocytes express a lower level of genes associated with adipocyte function. The size of adipocytes is lower in IAATs than SCAT. These results suggest that all IAATs belong to

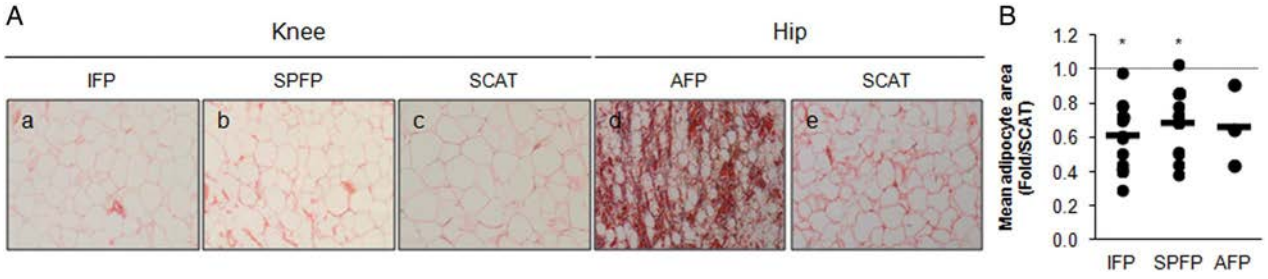

Figure 5 Mean adipocyte size in intra-articular adipose tissues (IAATs) and subcutaneous adipose tissue (SCAT) from patients with end-stage osteoarthritis. (A) Paraffin sections (5 μm) of infrapatellar fat pad (IFP) (a), suprapatellar fat pad (SPFP) (b), acetabular fat pad (AFP) (d) and SCAT (c and e) (n=16 for knee and n=3 for hip tissues) were stained with picrosirius red and adipocyte size was quantified. (B) Quantification of mean adipocyte size of knee and hip IAATs. The mean size of adipocytes from knee IAATs is significantly lower than those of autologous SCAT. A similar observation is made for AFP compared with hip SCAT. Data are expressed as ratio of mean adipocyte size between each IAAT and its corresponding SCAT (set at 1.0, horizontal dotted line). Horizontal bar is mean and dots represent each patient. *p<0.05.

the same type of AT and support that IAATs and their adjacent synovium should be considered a unique functional unit.

Our results show that IAATs differ from SCAT. Although we cannot exclude that the proportion of fibrous and vascular tissue may explain some molecular differences we observed between IAATs and SCAT, the distinct gene expression pattern of isolated adipocytes from IFP and SCAT argues for a peculiar IAAT phenotype. Interestingly, IAATs seem to share similar properties with visceral AT (VAT), the other main type of white ATs. Indeed, the size of adipocytes from omental or perivascular AT is smaller than in SCAT depots,[18 19] as we found for IAATs. Similarly, the level of adipocyte-associated genes is reduced in adipocytes from perivascular and perirenal ATs.[18] SCAT and VATs differentially express developmental genes, including EN1 and HoxC9, whose expression is decreased in intra-abdominal VAT, whereas the expression of SFRP2 is increased (data not shown).[14 15] Our results also show lower levels of both EN1 and HoxC9 in IAATs than SCAT. Similarly, some developmental genes, including EN1, were differentially expressed by perivascular AT and SCAT, with no difference found between SCAT and perirenal AT.[18] This latter observation highlights intrinsic differences between VATs.[15] Nevertheless, Chau *et al*[13] recently showed that VATs from six different depots but not SCAT all express Wt-1. Interestingly, in our study, no expression of Wt-1 was observed in IAATs, whereas it was in omental VAT. This suggests that IAATs may be a specific AT different from SCAT and VAT while sharing several common properties with VATs.

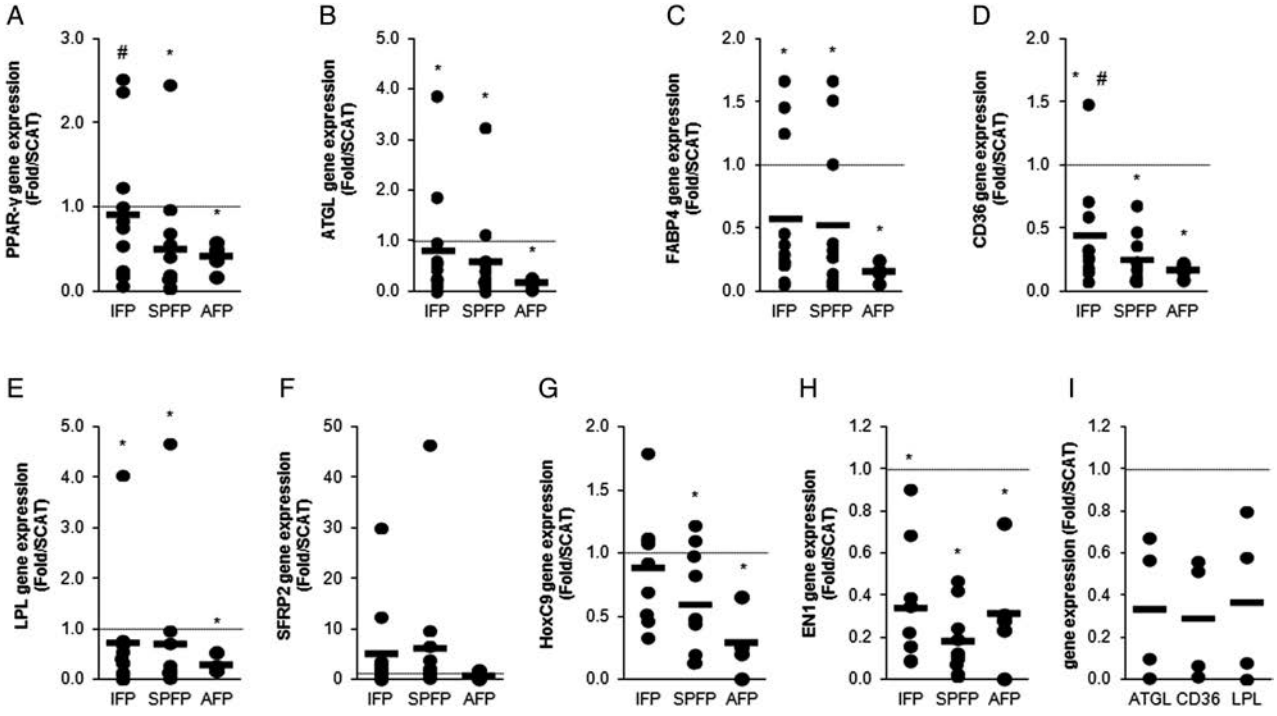

Figure 6 Differential gene expression pattern of knee and hip intra-articular adipose tissues (IAATs) and autologous subcutaneous adipose tissue (SCAT) from patients with end-stage osteoarthritis (OA). (A–H) Relative mRNA expression of PPAR-γ (A), ATGL (B), FABP4 (C), CD36 (D), LPL (E), SFRP2 (F) HoxC9 (G) and EN1 (H) by infrapatellar fat pad (IFP), suprapatellar fat pad (SPFP) and acetabular fat pad (AFP) to their corresponding SCAT from patients with knee (n=12) and hip (n=5) OA. (I) Adipocytes from IFP and SCAT were isolated from four patients before determining mRNA expression of ATGL, CD36 and LPL. Data are fold increase compared with SCAT, whose mRNA expression was set to 1 for each patient (horizontal dotted line). Horizontal bar is mean and dots represent each patient. *p<0.05 vs SCAT and #p<0.05 vs SPFP.

OA IAATs are characterised by a higher fibrotic index, inflammatory infiltrates and vascularisation than autologous SCAT. Increased leucocyte infiltration and mast cell number in OA IFP has been reported.[8] Leucocytes and mast cells preferentially localise within fibrotic areas. Similar observations were reported in omental VAT from obese patients.[12] Inflammation stimulates fibrosis in AT, and macrophages can express extracellular matrix components.[20] Of note, monoiodoacetate injection can induce OA and provoke IFP inflammation and fibrosis.[21 22] IFP area and vascularisation are increased in the murine high-fat diet-induced OA model.[10] In addition to inflammation, AT fibrosis can be induced by mechanical stimulus,[23] which is relevant for knee and hip OA. According to the link between fibrosis, inflammation and mechanical load, we suggest that the peculiar phenotype of IAATs we describe here could depend on intrinsic properties of IAATs. A recent study comparing the histological characteristics of IFP from cadavers without knee OA to autologous knee SCAT and heterologous abdominal SCAT[24] reported that IFP adipocytes were smaller than those from SCAT. However, their results on tissue fibrosis differ from ours. Indeed, interlobular septa of IFP were thinner than those from knee SCAT, whereas no quantitative difference of the intercellular space was observed. We have no explanation for this discrepancy between their study and ours. Nevertheless, we never observed SCAT as fibrous as they showed. The peculiar phenotype of IAATs may also be acquired during the course of OA. Indeed, Gandhi *et al*[17] showed differences in gene expression pattern between IFP from early and late patients with OA.

To conclude, knee and hip OA IAATs share a common phenotype, including a less adipogenic profile but higher fibrotic and inflammatory characteristics than autologous SCAT. IAATs could be considered a subgroup of AT, such as visceral, muscular or perivascular AT. The IAAT impact on joint homeostasis could be related to its inflammatory and metabolic profile and mediated by close interactions with synovium in a same functional unit. IAATs may be new players in OA disease progression.

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EXTENDED REPORT

Transethnic meta-analysis identifies *GSDMA* and *PRDM1* as susceptibility genes to systemic sclerosis

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ABSTRACT

Objectives Systemic sclerosis (SSc) is an autoimmune disease characterised by skin and systemic fibrosis culminating in organ damage. Previous genetic studies including genome-wide association studies (GWAS) have identified 12 susceptibility loci satisfying genome-wide significance. Transethnic meta-analyses have successfully expanded the list of susceptibility genes and deepened biological insights for other autoimmune diseases.

Methods We performed transethnic meta-analysis of GWAS in the Japanese and European populations, followed by a two-staged replication study comprising a total of 4436 cases and 14 751 controls. Associations between significant single nuclear polymorphisms (SNPs) and neighbouring genes were evaluated. Enrichment analysis of H3K4Me3, a representative histone mark for active promoter was conducted with an expanded list of SSc susceptibility genes.

Results We identified two significant SNP in two loci, *GSDMA* and *PRDM1*, both of which are related to immune functions and associated with other autoimmune diseases ($p=1.4\times10^{-10}$ and 6.6×10^{-10}, respectively). *GSDMA* also showed a significant association with limited cutaneous SSc. We also replicated the associations of previously reported loci including a non-GWAS locus, *TNFAIP3*. *PRDM1* encodes BLIMP1, a transcription factor regulating T-cell proliferation and plasma cell differentiation. The top SNP in *GSDMA* was a missense variant and correlated with gene expression of neighbouring genes, and this could explain the association in this locus. We found different human leukocyte antigen (HLA) association patterns between the two populations. Enrichment analysis suggested the importance of CD4-naïve primary T cell.

Conclusions *GSDMA* and *PRDM1* are associated with SSc. These findings provide enhanced insight into the genetic and biological basis of SSc.

INTRODUCTION

Systemic sclerosis (SSc) is an orphan disease with high morbidity and mortality. It is composed of two main subsets, a limited cutaneous form (lcSSc) and a diffuse cutaneous form (dcSSc).[1] SSc is also characterised by production of specific autoantibodies, anticentromere antibody (ACA) and anti-Scl70 antibody. Severe complications in SSc include interstitial lung disease (ILD), digital ulcers (DU), renal crisis and pulmonary hypertension (PH), where fibrosis in tissues and vessel remodelling play fundamental roles.[1] Genetic and environmental elements are associated with the development of SSc.[1 2] While SSc is a heterogeneous disease, it has a significant genetic component.[3] A total of 12 non-*HLA* loci showing significant associations ($p<5.0\times10^{-8}$) were reported for their associations[4–11] (table 1).

In spite of a paradigm shift in the treatment of autoimmune diseases by biological agents,[12] treatment of SSc remains challenging and new molecular targets are still under investigation. Results of previous genome-wide association studies (GWAS) in other autoimmune diseases have successfully identified important pathways as molecular targets, leading to effective treatments.[13–15] Similarly, GWAS on SSc may suggest novel targets for treatment.

To this end, transethnic meta-analysis of GWAS would be a promising way to identify unknown susceptibility genes which were difficult to detect in a single population due to lack of statistical power, different structure of linkage disequilibrium (LD) or different allele frequencies between populations. In fact, transethnic meta-analyses of GWAS for another autoimmune disease, rheumatoid arthritis (RA), have expanded lists of susceptibility genes

Table 1 The results in the current study for the previous GWAS loci and *TNFAIP3*

Previously reported loci					Current GWAS meta-analysis				Japanese GWAS			French GWAS		
SNP	Chr	BP	Neighbouring gene	Risk SNP	Risk SNP	β	SE	p Value	β	SE	p Value	β	SE	p Value
rs3790567	1	67822377	*IL12RB2*	A	A	0.112	0.057	0.050	0.116	0.085	0.17	0.109	0.077	0.16
rs2056626	1	167420425	*CD247*	T	T	0.101	0.059	0.086	−0.048	0.105	0.65	0.170	0.071	0.017
rs7574865	2	191964633	*STAT4*	T	T	0.332	0.054	5.3×10^{-10}	0.375	0.074	3.6×10^{-7}	0.285	0.078	0.00025
rs35677470	3	58183636	*DNASE1L3*	A	A	–	–	–	–	–	–	0.654	0.130	9.4×10^{-7}
rs77583790	3	159694053	*SCHIP1-IL12A*	A	A	–	–	–	–	–	–	0.345	0.285	0.24
rs2233287	5	150440097	*TNIP1*	A	A	–	–	–	–	–	–	0.440	0.107	3.7×10^{-5}
rs9373839	6	106655617	*ATG5*	C	C	–	–	–	–	–	–	0.246	0.083	0.0034
rs2230926	6	138196066	*TNFAIP3*	G	G	0.525	0.102	2.5×10^{-7}	0.352	0.130	0.0066	0.801	0.164	1.9×10^{-6}
rs10488631	7	128594183	*IRF5/TNPO3*	C	C	–	–	–	–	–	–	0.328	0.108	0.0024
rs11642873	16	85991705	*IRF8*	A	A	0.235	0.077	0.0024	0.195	0.132	0.14	0.256	0.096	0.0072
rs2304256	19	10475652	*TYK2*	C	C	0.103	0.054	0.058	0.079	0.074	0.29	0.132	0.080	0.095
rs2305743	19	18193191	*IL12RB1*	G	G	0.104	0.062	0.094	0.040	0.087	0.64	0.171	0.089	0.055
rs137894	22	50467524	*CSK*	C	–	–	–	–	–	–	–	–	–	–

Data not available due to lack of satisfying quality control criteria.
BP, base position; Chr, chromosome; GWAS, genome-wide association studies; SNP, single nuclear polymorphisms.

and led to candidates for target cell types and molecules.[16] However, most of the previous GWAS for SSc are mainly reported from the European population, with only one GWAS from the Asian population using 137 Korean patients.[17] Thus, we performed GWAS for SSc using 716 Japanese cases,[2] and 1797 controls and performed transethnic meta-analysis of GWAS using the previous GWAS from the French population,[5] with comparable numbers of subjects to Japanese GWAS (see online supplementary figure S1).

MATERIALS AND METHODS

Study design

The schematic view of the study design is illustrated in online supplementary figure S1. In brief, after a first analysis of Japanese and French GWAS data, we then performed two replication studies. In the first replication study, we used a Japanese cohort and a European cohort originating from several European countries. In the second replication study, we used Canadian and North American population with European decent. As for markers, we picked up a total of 33 single nucleotide polymorphisms (SNPs) for the first replication study based on the criteria of selection of candidate SNPs. We further selected seven SNPs fulfilling the criteria of selection for the second replication study.

Samples

A total of 1280 cases and 3660 controls in the Japanese population and 3156 cases and 11 091 controls in the European population were recruited. Break down of subjects are shown in online supplementary tables S1 and S2. All case samples fulfilled the American College of Rheumatology classification criteria for SSc.[18] Written informed consent was obtained from all the participants. This study was approved by local ethical committees.

Clinical information

Clinical information regarding subtypes of SSc defined by LeRoy *et al*,[19] but also ILD, PH, renal crisis, DU and possession of ACA, anti-Scl70 antibody and anti-RNA polymerase III antibody were collected. The clinical information was selected based on the importance of SSc outcome and the previous genetic studies identifying specific associations with SSc subtypes or phenotypes. Due to very low prevalence of renal crisis, PH and anti-RNA polymerase III antibody, we did not include these phenotypes for subtype-specific analysis. The availability of clinical information is shown in online supplementary table S1.

Genotyping

The French GWAS data were published previously and the methods are written elsewhere.[5] Genotyping with a competitive allele-specific PCR system for replication in the European samples was performed in the LGC Genomics (Hoddesdon, UK). A part of the replication data in the European samples was obtained by imputation based on GWAS (see online supplementary tables S1 and S2). The Japanese samples in the GWAS and the first replication study were genotyped in Kyoto University and University of Tsukuba, Japan.

Imputation

Imputation and phasing were performed by MaCH software,[20] using the East Asian panel and European panel in the 1000 Genomes Project,[21] as references for Japanese and French populations, respectively. After imputation, we performed quality control (see below). Imputation for the Japanese and French population was performed separately at Kyoto University in Japan and the INSERM UMR 1220 in France, respectively, and only summary statistics for the French imputation data were available due to restriction of data sharing policy of the control samples.

Quality control

We applied different quality control criteria in the two GWAS. The details are shown in online supplementary table S1. Since the current study, especially GWAS, had limited power to find signals in SNPs with low allele frequency (see online supplementary table S3), we filtered SNPs in each data set again after imputation based on allele frequency and used SNPs showing $r^2>0.5$ in the output of MaCH for the subsequent analyses (see online supplementary table S4). Since information of variants in the sex chromosomes was not available in the French GWAS, we focused on variants in the autosomal chromosomes.

Linkage disequilibrium between SNPs

LD structure was evaluated based on the 1000 Genomes data and our genotyping data. Statistical value for LD was calculated by Haploview[22] or PLINK.[23]

Selection of SNPs for the first replication study

For the first replication study, we picked up SNPs whose associations or the associations of other SNPs in the same region were not previously reported, and satisfying the following criteria: (1) whose p values for SSc susceptibility were $<1.0\times10^{-5}$ in the meta-analysis of the two GWAS, (2) whose p values for SSc susceptibility were $<2.0\times10^{-4}$ in the meta-analysis and whose p values were <0.05 in the text-based in silico analysis using Gene Relationships Across Implicated Loci (GRAIL) programme,[24] with use of previously reported genes in SSc as seeds or (3) whose p values for SSc subtypes were $<1.0\times10^{-6}$. When multiple SNPs in the same region ($r^2>0.5$) satisfied the above criteria, we picked up SNPs showing the best p values or SNPs for which probe and primer design for replication studies was not technically difficult.

Selection of SNPs for the second replication study

For the second replication study, we selected SNPs (1) whose p values in the meta-analysis of the GWAS and first replication studies were $<5.0\times10^{-6}$ or (2) whose p values in the GWAS were $<2.0\times10^{-5}$ and whose p values were <0.05 by GRAIL.

Calculation of variance explained by susceptibility SNPs

We evaluated variance explained by new susceptibility SNPs based on liability-scale threshold model. We assumed that there are underlying liability scores following normal distribution and that subjects having a liability score over a predefined threshold to develop SSc. We set prevalence of SSc as 0.05%. OR in the overall study was used as approximation of common relative risk between populations. We performed this estimation separately in each population using control allele frequencies in GWAS.

Associations between clinical manifestation and associated SNPs

After confirming the associations of the two SNPs with SSc, the associations between the two SNPs and SSc subtypes or clinical manifestations including ILD and DU were estimated. We did not perform GWAS for these clinical manifestations due to limited number of subjects who were positive for these manifestations. We further defined two phenotypes, fibrotic and vascular and performed association studies with these two phenotypes. Fibrotic phenotype includes dcSSc or severe lung disease defined by forced volume capacity (FVC) <70% or ILD in combination with FVC <75%. Vascular phenotype is DU, pulmonary arterial hypertension (PAH) or renal crisis.

Amino acid conservation search

We assessed amino acid conservation for the residue of GSDMA altered by rs3894194 across vertebrates in combination with Genomic Evolutionary Rate Profiling (GERP) score by using UCSC Genome Browser. GERP score was calculated for the single amino acid residue in which positive score indicated conservation.

HLA imputation and analyses

HLA alleles and amino acids were imputed by SNP2HLA.[25] We used Asian reference panel and European reference panel for Japanese and French samples, respectively.

Functional annotation and biological insights

HaploReg V.4.0 was used to assess functional annotation of the significant SNPs. The programme of functional enrichment by Trynka *et al*,[15] was used for the enrichment analysis. We looked up the effects of SNPs on gene expression by previous expression quantitative trait loci (eQTL) data.[26 27]

To get biological insight of SSc based on all SSc-associated loci, we also searched for all the SSc-associated loci: (1) missense mutations and functional annotation signals in SNPs in strong LD ($r^2>0.8$) with top SNPs in the loci, (2) the associations with the other diseases by GWAS catalogue using Gene names, (3) cis-eQTL signals based on the largest eQTL study,[26] with p values $<1.0\times10^{-5}$, (4) H3K4me3 signals in CD4-naïve primary T cell with scores more than 0 calculated by the method mentioned above and (5) promoter histone marks in skin tissues based on results of HaploReg V.4.0.

Statistical analysis

Logistic regression model was used for association studies. We used the first three principal components as covariates for the Japanese GWAS since additional PCs did not further improve the results. No inflation of p values was observed in French GWAS. We performed association studies using SSc, dcSSc, lcSSc, anti-Scl70 antibody(+)SSc and ACA(+)SSc as dependent variables. Inverse-variance method assuming fixed effects was applied to integrate the different association studies. Hardy-Weinberg equilibrium was assessed for the SNPs across the studies. SNPs showing association p values $<5.0\times10^{-8}$ in the overall study were regarded as significant. Heterogeneity was evaluated for SNPs showing significant results using Cochran Q test. Interactive effects of two SNPs were evaluated by multiplicative model. Power calculation of the current study was conducted with use of 'Genetics Design' package of R software.

Since the first replication study in the European population was composed of French, Italian and German populations, we put covariates of the three populations as indicator variables. When we excluded imputation data to confirm the results by avoiding batch effects derived from different genotyping methods, German cases were combined with French cohorts because imputation data were used for all German controls.

Since individual imputation data for the French GWAS were not fully available due to restriction of data sharing policy in the control samples, we performed conditional analysis and HLA imputation using all of the case samples and a part of the control samples whose genotyping data were available.

Omnibus test to assess critical amino acid positions in the HLA region was conducted in each population and in the combined set as previously described.[28 29] When we analysed the combined set, the indicator variable of population was added as a covariate.

Statistical analysis was performed by PLINK or R statistical software. LocusZoom[30] was used to draw regional plots.

RESULTS

Japanese GWAS of SSc

We genotyped the Japanese cases and controls with five different Illumina Infinium arrays (see online supplementary table S1). After filtering samples based on quality control criteria, 700 cases and 1797 controls remained (see the Materials and methods section). To maximise the power to find new susceptibility loci, we performed imputation for this dataset with the East Asian panel in the 1000 Genome project[21] as a reference.

We identified rs12612769 in *STAT4* and rs9268636 near *HLA-DRA* showing significant associations ($p=4.7\times10^{-8}$ and 9.6×10^{-10}, respectively, online supplementary figure S2A).

European GWAS for SSc and meta-analysis

Next, we used the previously published French GWAS containing 564 cases and 1776 controls and performed imputation with use of the European population panel in the 1000 Genomes Project European panel as reference (see online supplementary figure S2B). We conducted a transethnic meta-analysis of the two GWAS by the inverse-variance method assuming fixed effects for SNPs satisfying criteria of quality control (see online supplementary table S4). Since no evidence of population structure was obtained (lambda=1.05, figure 1), we did not apply genomic control[31] to correct statistics. As a result, we identified the *STAT4* region showing a significant association ($p=3.0\times10^{-11}$, figure 1 and see online supplementary table S5). The *HLA* locus did not show a significant association ($p\geq1.3\times10^{-7}$, figure 1) in spite of significant associations of the *HLA* locus in both populations (see online supplementary figure S2), suggesting different causative variants between the two populations. In fact, when we conducted HLA imputation using SNP2HLA, the different association patterns of amino acid positions were observed (see online supplementary table S6). The results of the susceptibility loci in the previous studies are shown in table 1. The risk alleles for all of the SNPs in the previous studies were the same in the meta-analysis or the French GWAS, suggesting replication of the previous findings and validity in the current study. In addition, we found an association in the *TNFAIP3* region whose association was previously reported without satisfying genome-wide significance level.[32] All the variants in non-*HLA* region showing p value $<1.0\times10^{-5}$ are shown in online supplementary table S5. We also performed SSc subtype GWAS according to the previous GWAS,[6] namely, lcSSc, dcSSc, ACA(+)SSc and anti-Scl70(+)SSc (see online supplementary figure S3).

Selection of SNPs for the replication studies

We identified 33 SNPs in 33 novel candidates of susceptibility loci (see the Materials and methods section or see online supplementary figure S1). Twenty-seven out of the 33 SNPs were novel candidates of susceptibility loci to SSc. Among the remaining six SNPs, one and two were specific for limited and diffuse types, respectively, and two and one for possession of ACA and anti-Scl70 antibody only, respectively (see online supplementary table S7).

The two-staged replication studies

We recruited 564 cases and 1863 controls in the Japanese population and 1582 cases and 6694 controls in the European population for the first replication study (see online supplementary table S1 and S2). We found that rs3894194 in *GSDMA* showed an association beyond the significance level in the combined population. All the results for the 33 SNPs are shown in online supplementary table S7. We further recruited a total of 1010 cases and 2621 controls in the European population for the second replication study to validate the associations of the seven SNPs showing possible associations (see the Materials and methods section or see online supplementary figure S1). As a result, rs3894194 in *GSDMA* kept its association (overall

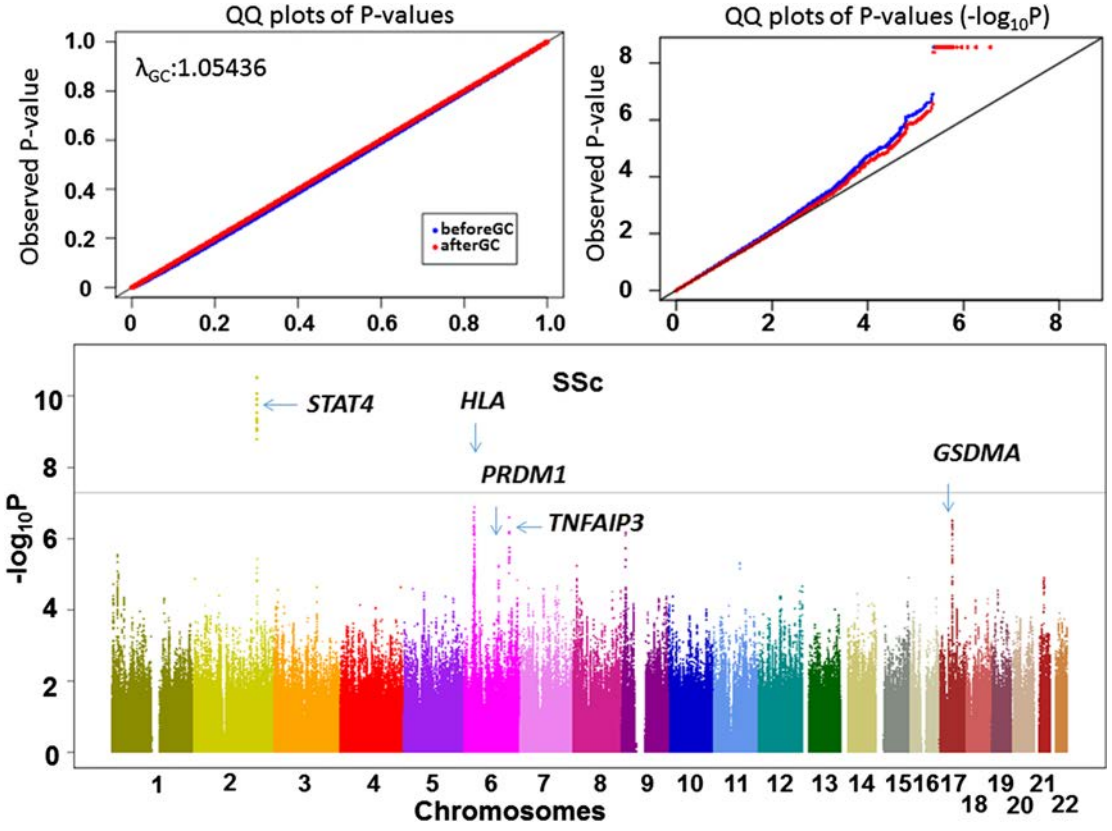

Figure 1 Transethnic meta-analysis of genome-wide association studies (GWAS) revealed multiple susceptibility loci to systemic sclerosis (SSc). The results of the transethnic meta-analysis of GWAS are shown in the Manhattan plot and quantile-quantile (QQ) plot. The newly identified loci and previously reported loci with strong p values are indicated in the Manhattan plot. The horizontal line indicates the genome-wide significance level.

p=1.4×10^{-10}, table 2). rs4134466 in *PRDM1* in chromosome 6 also showed an association beyond the significance level (overall p=6.6×10^{-10}, table 2). The two SNPs did not display deviation from Hardy-Weinberg disequilibrium (p≥0.037) and heterogeneity (p≥0.011) across the studies. When we assessed the liability-scale variance explained by these two SNPs,[33] a total of 0.2% was explained in each population (see the Materials and methods section).

PRDM1 as a novel locus for SSc

rs4134466 is located 20 kbp downstream of *PRDM1*, also known as *BLIMP1*, encoding a transcription factor regulating T-cell proliferation and plasma cell differentiation.[34] The LD block spanning rs4134466 does not contain any other genes (figure 2A). The previous GWAS reported that this region was associated with other inflammatory conditions including RA,[35] systemic lupus erythematosus (SLE)[36] and inflammatory bowel disease (IBD).[37] When we searched for SNPs in the exonic region of *PRDM1* in strong LD with rs4134466, we could not find any coding variants in both Japanese and European populations. While *PRDM1* in chromosome 6 was adjacent to *ATG5*, a previously reported susceptibility gene to SSc,[8] rs4134466 in *PRDM1* was not in strong LD with rs9373839 in *ATG5* showing the strongest susceptibility association in the previous study (r^2<0.15 in our study and the 1000 Genomes Project). In addition, rs9373839 was not polymorphic in the Japanese population. Thus, the association of rs4134466 was not driven by rs9373839. In fact, when we conditioned the association of rs4134466 on rs9373839 using imputation data of French GWAS, the effect size of rs413466 risk allele did not change before and after conditioning (OR 1.102 and 1.105, before and after conditioning, respectively). Since the previous study of SLE GWAS[36] reported that rs65684331 in *PRDM1* is associated with SLE independently from rs2245214 in *ATG5*,[38] SSc seems to have multiple hits in this region as in SLE.

GSDMA as a novel locus for SSc

rs3894194 is a missense mutation of *GSDMA* altering an arginine residue to glutamine (p.R18Q). This amino acid residue is conserved across species with GERP score 3.34 (see online supplementary table S8). Estimation by PolyPhen-2[39] software suggest a benign effect of this variant. The LD block containing SNPs in LD with rs3894194 (r^2>0.8) harboured *LRRC3C* and this region is neighbouring *ORMDL3* and *GSDMB* (figure 2B). This region is a gene-rich region and reported to be associated with various immune-related diseases including RA[16] and IBD.[37 40] However, SNPs located in the LD block tagged by rs3894194 have not been reported to be associated with other diseases. The RA-associated SNP (rs59716545) is in low LD with rs3894194 (r^2=0.25). *GSDMA* is associated with IBD in the previous study and the associated SNPs are in low LD with rs3894194 (rs2872507 or rs12946510, r^2<0.38). This region is also associated with asthma,[41] but the effect of this SNP on asthma is opposite to that on IBD.[41] This opposing effect seems to be true for asthma and SSc (OR of risk allele of this region: 1.26 and 1.18 in asthma and SSc, respectively).

Functional annotation of the two SNPs

Next, we assessed the effects of the two SNPs and the neighbouring SNPs on gene expression and functional annotation. We went through GTEx,[26] and found that rs3894914 in *GSDMA* showed a strong association with expression of *GSDMB* and *ORMDL3*, neighbouring genes to *GSDMA* (p≤2.6×10^{-12}, figure 3A) and whose gene expression strongly

Table 2 The results of the seven SNPs selected for the second replication study

SNP	Ch	BP	Gene	A1/A2	Pop	GWAS meta-analysis			Replication						Overall			
									Replication 1			Replication 2			Japanese+European			
						A2Case	A2Cont	p Value	A2Case	A2Cont	p Value	A2Case	A2Cont	p Value	β	SE	p Value	OR (95%CI)
rs10907300	1	18400980	*IGSF21*	C/A	Japanese	0.61	0.55	0.00025	0.60	0.57	0.038				0.088	0.026	0.00076	1.09 (1.04 to 1.15)
					European	0.39	0.34	0.0031	0.38	0.35	0.018	0.37	0.40	0.012				
rs6714060	2	74210442	*TET3*	C/T	Japanese	0.15	0.19	0.0031	0.15	0.20	0.00041				-0.134	0.037	0.00024	0.87 (0.81 to 0.94)
					European	0.13	0.15	0.016	0.13	0.14	0.34	0.15	0.14	0.74				
rs4134466	6	106577368	*PRDM1*	A/G	Japanese	0.59	0.66	0.0058	0.60	0.66	0.00020				-0.160	0.026	6.6×10^{-10}	0.85 (0.81 to 0.90)
					European	0.57	0.63	0.00030	0.62	0.62	0.35	0.59	0.64	7.2×10^{-5}				
rs12676482	8	42174077	*IKBKB*	G/A	Japanese	0.20	0.15	0.0013	0.19	0.16	0.013				0.221	0.051	1.4×10^{-5}	1.25 (1.13 to 1.38)
					European	0.04	0.03	0.049	0.03	0.03	0.44	0.05	0.04	0.30				
rs2821195	9	11689891	no gene	C/G	Japanese	0.49	0.55	0.00047	0.50	0.51	0.63				-0.093	0.027	0.00048	0.91 (0.87 to 0.96)
					European	0.31	0.36	0.00038	0.32	0.36	0.0062	0.37	0.35	0.080				
rs12357548	10	63803472	*ARID5B*	G/A	Japanese	0.30	0.35	0.00070	0.34	0.35	0.54				-0.073	0.026	0.0053	0.93 (0.88 to 0.98)
					European	0.51	0.55	0.044	0.49	0.53	0.00072	0.51	0.48	0.0065				
rs3894194	17	38121993	*GSDMA*	G/A	Japanese	0.50	0.54	0.012	0.51	0.53	0.28				-0.166	0.026	1.4×10^{-10}	0.85 (0.80 to 0.89)
					European	0.40	0.47	3.7×10^{-6}	0.40	0.46	3.0×10^{-6}	0.42	0.44	0.21				

β and SE are values for A2 allele.
A2Case, frequency of A2 allele in case; A2Cont, frequency of A2 allele in control; BP, base position; Ch, chromosome; Pop, population; GWAS, genome-wide association studies; SNP, single nuclear polymorphisms.

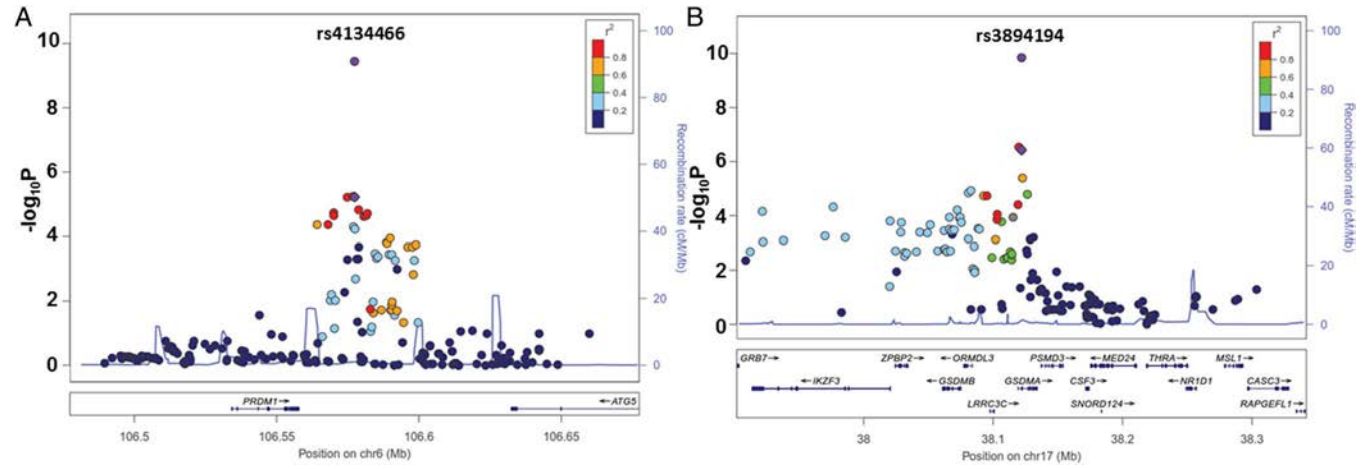

Figure 2 Detailed plot for the two loci found in the current study. The detailed plots in chromosome 6 and 17 are shown for (A and B), respectively. The purple plots indicate the top SNPs in the combined results and GWAS meta-analysis for the upper and lower plots, respectively. The plots are drawn based on the linkage disequilibrium (LD) structure of East Asians by using LocusZoom as a representative.

correlated with each other. The association between gene expression and rs3894194 was also confirmed in the largest eQTL data[27] (see online supplementary table S9). We found that the associations between SSc and SNPs in the *GSDMA* locus correlated well with the associations of the SNPs with gene expressions of *GSDMB* and *ORMDL3* (figure 3B). Thus, the effect of the SNP on gene expression of *GSDMB* and *ORMDL3* in combination with amino acid alteration of the GSDMA protein seems to explain the association of this locus. HaploReg V.4.0[42] revealed that rs3894194 showed enhancer activity and enrichment of histone marks (see online supplementary table S10). While the previous eQTL studies[27] did not show associations between rs4134466 and gene expression, rs4134466 showed DNase hypersensitivity and methylation in various kinds of cells (see online supplementary table S10).

When we assessed interactive effects of the two SNPs on SSc susceptibility, we did not observe a significant effect ($p=0.57$).

Subtype analyses for the two SNPs

When the associations of these two SNPs and the subtypes of SSc were analysed, rs3894194 in *GSDMA* showed a significant association with lcSSc (figure 3C). No other significant associations were observed (see online supplementary figure S4), but this study was underpowered to detect phenotype-specific associations. When we focused on SSc subtypes showing extreme phenotypes of fibrosis and vasculopathy (see the Materials and methods section), we did not find enhanced associations between the two SNPs and the subtypes (data not shown).

Enrichment analysis of histone modification

Next, based on the expanded list of susceptibility genes to SSc, we performed enrichment analysis of H3K4Me3, a representative histone modification mark that was shown to be enriched in autoimmune disease-related variants.[15] We found that the susceptibility SNPs and the neighbouring SNPs in LD with them ($r^2>0.8$) showed suggestive enrichment of H3K4Me3 signal in CD4-naïve primary T cell or CD4 memory T cell (see online supplementary figure S5A). We also found that the suggestive enrichment signal in CD4-naïve primary T cell was mainly brought about by the three SNPs in *GSDMA*, *PRDM1* and *TNFAIP3* found in the current study (see online supplementary figure S5B).

Functional annotation of susceptibility loci

The significant SSc-associated genes including the current results and *TNFAIP3* are summarised in online supplementary figure S6. We combined information of protein alteration, associations with other diseases and functional annotations. The development of promising drug targets by enrichment analysis based on the list may be challenging, but this table would be useful for candidates of future functional analyses and further expansion of SSc-associated loci.

DISCUSSION

This is the largest SSc GWAS from non-European populations and the first transethnic meta-analysis of SSc GWAS. We identified two novel susceptibility loci, namely *GSDMA* and *PRDM1*. Both loci were associated with other autoimmune diseases, consistent with overlapping susceptibility genes among various autoimmune diseases. We also replicated the associations of previously reported GWAS variants and provided evidence of association with *TNFAIP3*. To avoid possible batch effects due to different genotyping methods, we excluded all European subjects in the first replication study whose genotypes were imputed. The associations of the two SNPs remained significant ($p\leq 9.8\times10^{-9}$, data not shown).

We did not find a significant multiplicative interaction between the two SNPs. Since a previous study showed substantial interactive effects limited to the *HLA* loci,[43] it would be interesting to expand SSc cohorts and assess *HLA* interaction.

The enrichment analysis suggested possible involvement of CD4-naïve primary T cells with SSc. However, further expansion of susceptibility loci and convincing evidence of cell-type-specific enrichment are essential. We did not observe suggestive enrichment signal in CD19 primary cells, representing B cells. Interestingly, both SNPs showed evidence of associations of gene-expression including fibroblast or keratinocyte. Since previous loci were associated with gene expression especially in immune-related cells, the current findings would suggest importance of skin-residing cells on SSc pathophysiology. Cell-specific gene expression profile of fibroblast, keratinocyte or other fibrosis-related cell types including endothelial cells in combination with genetic data would be useful to address the importance and involvement of these cells and genes in SSc.

PRDM1, also known as B lymphocyte-induced maturation protein 1, is a transcript factor influencing a broad range of

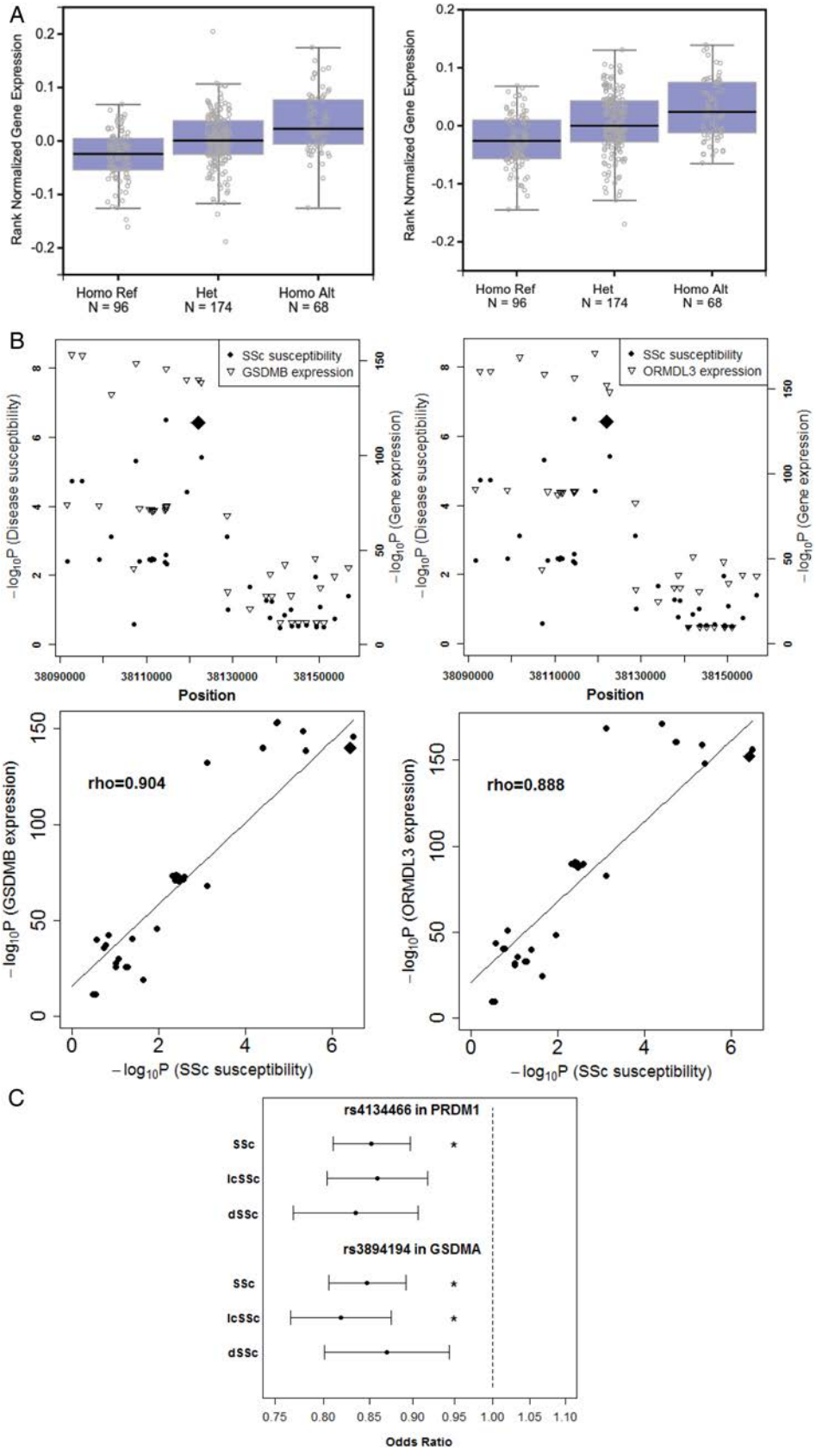

Figure 3 Correlation between the associations of variants in GSDMA region with systemic sclerosis (SSc) susceptibility and gene expression. (A) rs4134466 is associated with gene expression of *GSDMA*-neighbouring genes *GSDMB* (left) and *ORMDL3* (right). The box plots were obtained from GTEx data. (B) The associations between SSc susceptibility and single nuclear polymorphisms (SNPs) in chromosome 17 *GSDMA* locus are plotted together with the associations between the variants and expression of *GSDMB* (left) and *ORMDL3* (right). The gene expression data were obtained from Blood eQTL Browser. The correlation plots are indicated in the lower panels. The black diamonds indicate rs4134466. (C) rs4134466 is associated with limited SSc. The associations between the two SNPs and the two subtypes of SSc are indicated. lcSSc, limited cutaneous SSc; dcSSc, diffuse cutaneous SSc.

genes involved with cell proliferation and the immune system. PRDM1 is critical for epithelial and B cell differentiation,[34] and associated with other autoimmune diseases and haematopoietic malignancies. The association of this locus with SSc suggests a critical role of lymphocytes on SSc susceptibility. In fact, rs4134466 provided the highest score of H3K4me3 in CD4 (+)-naïve primary T cell among the SSc susceptibility variants. The first European replication study might suggest heterogeneity of this allele within the European population. Further expansion of subjects in subpopulations would clarify this point.

rs3894194 is a missense variant of GSDMA protein and associated with neighbouring gene expression. While it is not easy to pinpoint a causative variant, rs3894194 is a promising candidate of a causative SNP. *GSDMA* and *GSDMB* are strongly expressed in the skin and functional annotation revealed that rs3894194 has a regulatory effect of gene expression in various cell types including skin fibroblast. While rs3894194 also provided histone methylation in CD4(+)-naïve primary T cells, this locus may mainly demonstrate its susceptibility effect in the skin. The *GSDMA* locus showed a significant association with limited cutaneous SSc in spite of the reduced number of case subjects. This may suggest that this locus plays a more important role on developing lcSSc than dcSSc. However, since this locus also showed substantial associations with dcSSc, the results were inconclusive.

TNFAIP3 encodes A20 regulating tumour necrosis factor response by inhibiting nuclear factor-κB (NF-κB) activation. A20 also suppresses profibrotic signalling, relevant to SSc pathogenesis.[44] rs2230926 is a missense variant of *TNFAIP3* and associated with other rheumatic diseases.[45] The association of *TNFAIP3* as well as *TNIP1* supports NF-κB involvement with SSc. However, we did not observe significant interactive effect of the two SNPs (data not shown).

Since the two populations substantially contributed to both the associations found in this study, the current findings indicate that transethnic meta-analysis is effective to identify unreported susceptibility loci to SSc, which comprise moderate effect sizes in each population. Furthermore, the current findings, especially rs4134466, would suggest that transethnic meta-analysis is effective by taking advantage of different allele frequencies and LD structure between the populations to discern unreported susceptibility signals from previously reported loci.

HLA and *STAT4* loci showed different association patterns between subtypes of SSc, suggesting genetic heterogeneity in SSc. While the association between *STAT4* and SSc was mainly driven by ACA(+) SSc, intracase analysis did not reveal significant difference in STAT4 between ACA(+) and ACA(–) SSc ($p>0.01$, data not shown). The *HLA* locus showed strong associations with antibody-positive SSc subtypes in spite of the reduced sample numbers even in intracase analyses. The associations of the *HLA* locus were attenuated in overall SSc, and this could be explained by different associations of the *HLA* locus between different SSc subtypes or different antibodies.[46] Our results also suggested different association patterns of the *HLA* locus between Japanese and European populations. It would be feasible to expand SSc to compare the genetic architectures between populations or subtypes.

The different arrays between cases and controls in the Japanese subjects reduced the number of preimputed and postimputed markers. It would be feasible to rescan the control samples using the same arrays as the cases or take advantage of other controls which have used the same arrays to maximise power to find significant signals in future studies.

While it is still challenging to pinpoint a specific cell type contributing to SSc based on genetic findings, most of the susceptibility genes are immune-related and enrichment analysis suggested the importance of immune-related cells. Increasing samples for genetic studies especially from non-European populations would increase SSc susceptibility loci, identify population-specific susceptibility loci, narrow down candidates of causative variants and clarify genetic architecture. Exome,[47] whole-genome or target deep sequencing might also be helpful. Clarification of genetic background of SSc by multiple approaches in combination with functional analyses would lead to the identification of possible therapeutic targets.

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Let's not fool ourselves. In RA, the ACR/EULAR remission criteria are not perfect!

We were interested to read Dr Boer's recent eLetter,[1] in which he outlines the merits of the American College of Rheumatology (ACR) and European League Against Rheumatism (EULAR) 2011 consensus remission criteria in rheumatoid arthritis (RA),[2] and proposes that this definition equates with absence of disease.

The 2011 ACR/EULAR remission criteria do indeed hold several benefits over composite index-based remission definitions such as the disease activity score in 28 joints (DAS28). The ACR/EULAR criteria are widely regarded to be more stringent at defining remission than DAS28-erythrocyte sedimentation rate (ESR) <2.6, supported by a stronger correlation with lower rates of radiographic progression in the ACR/EULAR definition.[2] Furthermore, the ACR/EULAR criteria were developed by consensus agreement among a panel of international RA experts with the express aim of defining remission, whereas DAS28 was developed with the primary intention of measuring disease activity for the purposes of treatment escalation. Although representing a significant international advance in defining remission, it is nevertheless important to acknowledge the several limitations inherent to the ACR/EULAR remission criteria.

First, the ACR/EULAR criteria are based on 28 joint counts that exclude important joint areas; for example, the feet—this shortcoming is described in the original ACR/EULAR criteria publication. Second, the ACR/EULAR Boolean criteria place a strict threshold on patient global assessment of ≤1/10 on a visual analogue scale (VAS) as an absolute requirement for remission. While such a low VAS may be achievable in patients in the controlled clinical trials in which the ACR/EULAR criteria were validated, it is becoming increasingly apparent that patient VAS can be influenced by non-RA factors including osteoarthritis and other medical comorbidities. Indeed, several groups now suggest that the VAS threshold in ACR/EULAR Boolean remission may be overly strict and underdiagnose remission when used in 'real-world' clinical practice.[3–5] In this regard, it is interesting to note that in the original publication of the ACR/EULAR remission criteria, the consensus survey of expert opinion centred on a higher patient VAS threshold of 2.2/10 when all other parameters were consistent with remission.

Third, ACR/EULAR remission criteria neglect measures of synovitis by imaging modalities such as ultrasound (US) and MRI—arguably a more stringent measure of joint inflammation than clinical examination alone. Although ACR/EULAR remission has been shown to correlate with lower levels of US synovitis compared with DAS28,[6] we and other groups have demonstrated that power Doppler synovitis can still be detected in patients who satisfy ACR/EULAR remission criteria, with a prevalence as high as 60%.[7 8]

Fourth, ACR/EULAR remission appears to afford no clear advantage over DAS28-based definitions when applied to the identification of patients in remission who can successfully reduce or even stop their disease-modifying antirheumatic drug (DMARD) therapy. In the Reduction of Therapy in patients with Rheumatoid arthritis in Ongoing remission (RETRO) study, Boolean ACR/EULAR remission at baseline did not predict sustained DMARD-free remission,[9] whereas both autoantibody status and serum cytokine levels provided added value in identifying patients whose disease flared following DMARD withdrawal.[10]

In conclusion, while we acknowledge and support the vital work to reach an international consensus on defining RA remission, this is by no means a fait accompli. ACR/EULAR remission does not always equate with absence of disease and is not necessarily the optimal definition for application in clinical practice, particularly in non-research settings. There is an urgent need for robust and practical biomarkers that can better measure RA remission which, once discovered and validated, could be used to improve future definitions of RA remission.

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Response to: 'Let's not fool ourselves. In RA, the ACR/EULAR remission criteria are not perfect!' by Baker *et al*

Baker *et al*[1] raise many interesting points on the validity and use of the American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) remission criteria that I would like to reflect on.

But first of all I would like to reiterate my main point, one which I think we agree on:

In all clinical trials where remission is one of the outcomes, it should be defined according to the ACR/EULAR criteria.

Baker *et al* list several concerns on these criteria that are well taken, and some that are generic to the way we measure disease activity today.

Indeed, the ACR/EULAR criteria were developed with the express aim to define remission, more specifically 'to develop a definition of remission that is stringent but achievable and could be applied uniformly as an outcome measure in clinical trials'. Although the committee was asked 'to look toward, and make possible a similar definition for use in clinical practice' and in fact presented proposals to this end, the focus was on a definition for use in clinical trials.[2]

Likewise, and contrary to what Baker *et al* claim, the disease activity score (DAS) and DAS in 28 joints (DAS28) were not developed '… with the primary intention of measuring disease activity for the purposes of treatment escalation…'; instead, DAS and DAS28 were developed as composite instruments (indices) of disease activity for use in clinical trials. It is true that in their development, the external criterion for high disease activity was the status of a Dutch clinic patient seen around 1985 in whom therapy was switched for lack of efficacy.[3]

Many of the problems listed in Baker *et al*'s letter are due to the unfortunately widespread, but nevertheless inappropriate use of these indices and definitions to guide clinical practice decisions. This includes the use of 28 joint counts that exclude the feet (OK for trials, but inappropriate in patient care) and the patient global visual analogue scale (VAS) threshold of 1 out of 10 (clearly unachievable for many patients seen in the clinic). The choices made for the ACR/EULAR criteria reflect compromises at several levels to obtain, in the end, a set that performs optimally in the trial setting.

As already noted in the paper publishing the criteria, imaging was purposefully excluded, mainly for reasons of feasibility. Although singly the elements of the criteria can still be compatible with residual disease activity (eg, one swollen and one tender joint), together they result in an optimally specific set, with very limited numbers of 'false positives'. Nevertheless, as Baker *et al* point out, the criteria can still both 'underdiagnose' (as in the case of a VAS >1) and 'overdiagnose' remission (as in the case of a patient with residual disease on imaging). The prognostic relevance of such 'errors' is not clear but likely small on the group level. Another known limitation my group is currently working on is the relative lack of patient-reported input into the criteria.[4–6] Finally, Baker *et al* find fault with the criteria for not performing better than DAS28 <2.6 in the prediction of which patients will flare after treatment discontinuation. I do not think this is a fair argument, because the criteria were never developed with this purpose in mind; also, the argument is built on the unproven assumption that on tapering treatment, patients in remission are less likely to flare than patients in a minimum disease activity state.

In conclusion, I feel the ACR/EULAR criteria are a 'fait accompli' but not forever! They should be put up for revision once sufficient experience in trials has been gained. It may very well be that at that time feasible biomarkers are available to improve their performance or that robust evidence (currently lacking) suggest the thresholds should be altered. In the meantime, they are for the seasoned clinician to 'use wisely', as they were not meant for, let alone optimised for application in clinical practice. There is an urgent need for a disease activity tool that is valid and reliable for use in individual patient care, especially in the range between low disease activity and absence of disease.

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Identifying arthralgia suspicious for progression to rheumatoid arthritis

We read with interest the article by van Steenbergen *et al*[1] in which a definition for arthralgia suspicious for progression to rheumatoid arthritis (RA) was proposed. The authors used a three-phase Delphi exercise to crystallise the concept of clinically suspect arthralgia (CSA), which is inherently subjective, into a core set of definable parameters. We agree that this set of characteristics should provide a useful secondary care framework for identifying homogeneous at-risk populations for future clinical studies. Recent data suggest that rheumatologists can use symptoms and signs to identify which patients with arthralgia referred to them will imminently develop RA.[2 3] In the current cohort, up to 20% of individuals identified as CSA by their rheumatologist developed RA during follow-up, the majority doing so within 6 months.[2] Although a useful signpost for the experienced rheumatologist, it is not yet clear whether CSA can be as effectively identified in primary care. This is important as the vast majority of patients with RA will first present to their general practitioner (GP) when they develop symptoms. In general, GPs have less expertise in assessing arthralgia; in the UK it is estimated that patients with RA visit their GP on average four times before being referred to a specialist for diagnosis.[4] Furthermore, patients are usually only referred once synovitis has developed. Thus for many patients with RA, there is no opportunity for the symptomatic pre-RA phase to be captured in secondary care at all. We would, therefore, argue that including primary care in any strategy to identify at-risk individuals would be optimal.

One such approach is to send individuals with any new musculoskeletal (MSK) complaint in primary care for an anti-cyclic citrullinated peptide (anti-CCP) test. Those who test anti-CCP positive are at high risk of imminent RA, with 45% progressing to clinical arthritis, the majority within 1 year.[5] A key advantage of this approach is that it can be performed by healthcare professionals without any specific rheumatology expertise. It also allows at-risk individuals to be identified when they first access healthcare. Interestingly, symptoms in the hands, shoulders and feet were associated with anti-CCP positivity[5] and the European League Against Rheumatism taskforce also agreed that symptoms and signs in the hands were important in identifying arthralgia that precedes RA.[1] One limitation of this primary care approach is that only anti-CCP positive at-risk individuals will be identified. As it is also important to identify seronegative at-risk individuals, a potential algorithm combining the two approaches in a primary care setting will be a strategy worth investigating in the future.

We agree that the next important step is to develop criteria for imminent RA. As suggested by van Steenbergen *et al*,[1] it is likely that this will need to incorporate clinical, laboratory and imaging parameters to achieve superior predictive accuracy compared with clinical parameters alone. Prediction models that combine clinical and laboratory markers in at-risk cohorts have been published.[6–8] Measurement of T-cell subset dysregulation has recently been shown to add predictive accuracy to clinical symptoms in those at risk of RA.[8] The Leeds prediction model also included ultrasound imaging and identified high-risk individuals with a 62% risk of progression to arthritis.[7] MSK ultrasound is now routinely used alongside clinical markers for real-time decision making in early arthritis clinics. Ultrasound examination in at-risk individuals has also recently been included in a diagnostic algorithm for patients with RA.[9]

Identifying individuals at high risk of imminent RA is now achievable. Incorporating clinical, laboratory and imaging biomarkers into an agreed criteria for imminent RA is an important ambition. This will likely accelerate the identification of homogeneous groups of at-risk individuals necessary for larger observational studies and future interventional trials.

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Appropriate use of the EULAR definition of arthralgia suspicious for progression to rheumatoid arthritis

We thank Mankia *et al*[1] for their interest in the European League Against Rheumatism (EULAR) definition of arthralgia suspicious for progression to rheumatoid arthritis (RA).[2] The authors agree with the taskforce that derivation of criteria for imminent RA is an ambitious next step and that such criteria will probably consist of a combination of clinical, serological and imaging biomarkers.[1] In this light we appreciate the work the authors have done to identify serological and imaging markers that are predictive in patients with anti-citrullinated protein antibody (ACPA)-positive arthralgia.

While studies on blood and imaging markers in arthralgia are relatively frequent, only few studies have addressed the symptoms and signs of the disease stage that may precede clinical arthritis. In addition, the clinical delineation of this preclinical stage, reflected by the intuitive contrast in the description 'clinically suspect arthralgia (CSA)', is difficult: there is not one key symptom. Still, in our experience rheumatologists are capable of identifying patients with arthralgia that may progress to RA based on their expertise and on (intuitive) pattern recognition. In an attempt to strip the term 'suspect arthralgia' of its connotation of subjectivity and to promote the inclusion of homogeneous groups of patients with arthralgia in future studies, the taskforce has agreed on a consensual definition of 'arthralgia at risk for RA'. This definition is deliberately meant to be used in secondary care, for patients in whom imminent RA is considered a more likely explanation for the complaints than another disease, but who not (yet) have clinical arthritis.

Mankia *et al* rebut that in several settings patients with arthralgia are followed in primary care (too long) until clinical arthritis has become manifest. They propose to use the CSA definition as a referral tool in primary care.[1] Referral tools share characteristics of screening tools, such as high sensitivity and lower specificity, that pose huge challenges: Unlike the specialist setting, most patients with musculoskeletal symptoms presenting in primary care will have other more trivial explanations for their complaints than (imminent) RA. Consequently, the prior risk of RA will be low, as will be the predictive value of a positive CSA definition. The impact on specialist care may be significant.

We reiterate that the EULAR definition was not developed for the primary care setting, nor was it designed as a diagnostic test. The EULAR definition of CSA was designed by rheumatologists, with their perception of imminent RA as a reference frame, and was tested in patients from secondary care. Its seven items should be assessed in patients presenting to the rheumatologist in whom the specialist does not find clinical arthritis but imminent RA is still considered a likely diagnosis.[2] General practitioners often find it difficult to detect synovitis and to evaluate if imminent RA is more likely than other, trivial arthralgia's (instead, this uncertainty will often be the reason to refer to secondary care anyway). Therefore, the entry condition cannot be adequately evaluated in a primary care setting. Even so, there is a chance that the seven items will perform better than expected as a reference tool (either when applied in isolation or in combination with an additional test). Still to arrive at accurate referral criteria, these are ideally designed in primary care.

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