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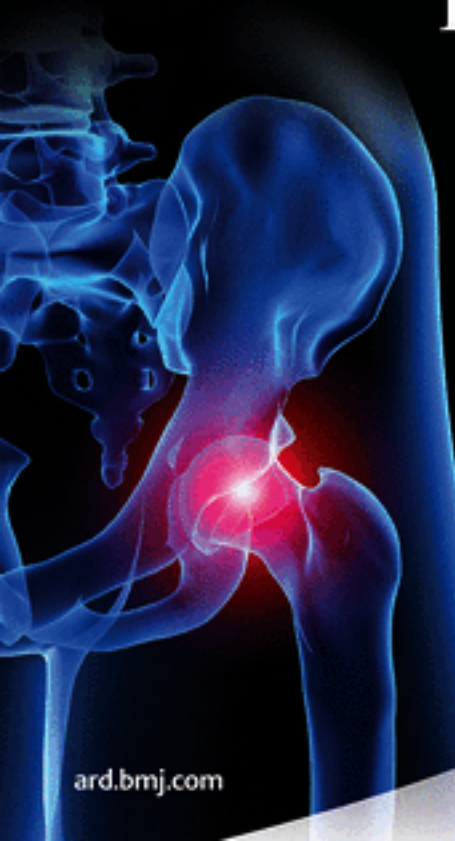

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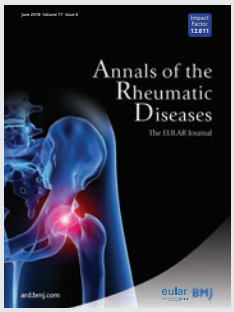

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Greetings from the editor

Josef S Smolen

The June issue of the *Annals of the Rheumatic Diseases* (*ARD*) that you are holding in your hands today constitutes an usual annual breaking point. While the *ARD* volume numbers relate to a full calendar year, January–December, the June issue of each volume marks the first issue for the subscription to *ARD* that participants of the Annual European League Against Rheumatism (EULAR) Congress receive every year in the Congress educational package and that lasts until May of the following year. Long ago, when EULAR and the BMJ Publishing Group agreed on the venture to elect *ARD* as 'The EULAR Journal', EULAR was faced by the fact that it did not have individual members (but rather national societies as official members) and, therefore, no individual membership fees that could include an organisation's official journal, as is the case with many other specialty societies. Therefore, EULAR decided to make *ARD* part of the educational package for all individually registered Congress participants. Since the EULAR Congress is attended by over 14 000 participants every year, this also makes *ARD* one of the most widely circulated journals in the field.

This year's June issue comprises a number of highlights: among many other papers, you will find two EULAR recommendations and a recommendation by an international task force[1–3]; all three will help improve outcomes for patients with various rheumatic diseases. You will also find a viewpoint by Maarten Boers[4] on the optimal design of graphs for scientific data to demonstrating their results in an easily digestible format. ARD wishes to provide you, the readers, with the best science in the field and with publications that attempt to present the data as clearly as possible; here the aspirations of the journal will undoubtedly meet with those of the authors, since both sides wish to present data in the best way to the readers. To this end, we are now also making sure that figures in *ARD* include the actual numeric data within graphs as I promised in my first editorial last year[5]; it has meanwhile become a requirement in the *ARD* submission instructions for clinical trial (and other) data, and you will have already seen the distinct data within the figures or in supplementary material in some of the latest issues as well as this issue.

In my September 2017 editorial, I also announced three new sections: 'Views on News', 'Heroes and Pillars of Rheumatology' and 'Thinking the Unthinkable'. The 'Views on News' section has already successfully started and you will find another piece by David Pisetsky in this issue.[6] The first 'hero' to be covered will be Jacques Forestier by Maxime Dougados, and Gerd Burmester has started to 'think the unthinkable'—these papers will be published very soon. We welcome proposals of topics for these three sections.

This June 2018 issue comprises many articles that passed my desk as the new Editor-in-Chief of *ARD*, but many were also handled by my predecessor Tore Kvien, who led this journal for almost a decade. This gives me a renewed opportunity to thank him deeply for his vision and dedication in bringing this journal to the forefront in the field and for teaching me (and so many others) many valuable lessons—thank you, Tore!

At the end, let me please wish you the joy and satisfaction of discovering new knowledge both at the 2018 EULAR Congress and when reading *ARD*—this issue as well as all of the upcoming ones.

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Chronic widespread pain and increased mortality: biopsychosocial interconnections

Jose A P Da Silva,[1] Rinie Geenen,[2] Johannes W G Jacobs[3]

Chronic widespread pain (CWP) is associated with excess mortality. Robust evidence is provided in *ARD* by a study that included over half a million participants and a meta-analysis.[1] This adds to the (already overwhelming) recognised burden of CWP and imposes an additional urgency in improving our understanding and management of the condition. Reducing excess mortality in patients with chronic pain requires an accurate knowledge of relevant mechanisms and mediators. Previous research and this new study indicate that this excess mortality is, to a large extent, explained by lifestyle factors such as decreased physical activity, increased body mass index, unhealthy diets and smoking. In this editorial, we will offer arguments and evidence emphasising the need to also take account of psychological factors to explain the link between CWP and early death.

BIOPSYCHOSOCIAL MODEL OF CHRONIC PAIN

Decades of research led to the current concept that chronic pain encompasses multiple and mutually interacting biological, psychological and social factors. These include—but are not limited to—nature of pain, peripheral and central pain processing mechanisms, physical disability, sleep disturbance, obesity, smoking, alcoholism and other health risks, psychological resilience and vulnerabilities (emotions, cognitions, behaviour) and social factors (work, support, facilities, financial resources). Relations between all factors of this biopsychosocial model are recognised to be dynamic and reciprocal, with mutually influencing pathways similar to a hanging mobile toy, in which movement of one component may induce change in all others and back. The weight of the distinct factors differs between individuals.

Psychological factors are important players in this context. To start with, they are core determinants of unhealthy lifestyles that turned out to be important in the recent study.[1] For instance, reduced physical activity has been shown to be influenced by fear of movement-related pain (kinesiophobia)[2] and by catastrophising cognitions (ie, rumination, magnification and helplessness).[3] Lifestyle factors including unhealthy diet, excess weight and smoking are also (reciprocally) associated with psychological determinants. Thus, addressing the lifestyle factors contributing to increased mortality would certainly require consideration of the psychological dimensions affecting their adoption and correction. However, the potential roles of psychological dimensions in the link between CWP and mortality are more complex.

PSYCHOLOGICAL VULNERABILITIES PREDICT EXCESS MORTALITY

Depression has been associated with increased all-cause mortality in a large variety of settings, from the general population (relative risk (RR) 1.52[4]) to different disease conditions. Especially in cardiovascular disease, meta-analyses have demonstrated an association between depressive symptoms and the incidence of coronary events (RR 1.81[5]), myocardial infarction (RR 1.32[6]) and stroke (RR 1.45[7]). Psychological stress and depression have also been associated with a higher incidence,[8] faster progression[9] and increased mortality[10] of cancer.

In a hallmark study,[11] it was shown that depression predicted risk of death in older persons. Early death was more likely in major than minor depression, but risk of death was still significantly higher in minor depression, even after correction for age, education level, chronic disease, smoking, obesity and sedentary lifestyle. In a study from the UK, over 68 000 members of the general population aged 35 years and over, at baseline free of cardiovascular disease and cancer, were included and followed up for an average of 8.2 years, accumulating more than 8000 deaths. A dose–response relation between intensity of psychological distress at baseline and mortality was shown, which remained significant after controlling for age, gender, comorbidity and behavioural and socioeconomic factors.[12] Of note, this association was observed across the full range of distress, including people enduring common, subclinical levels of distress, who would not usually require mental health assistance.

Neuroticism has been directly and indirectly associated with a negative impact on a variety of health dimensions,[13] including all-cause mortality.[14 15 16] In the UK Health and Life-Style Survey publication of 2007,[17] 1 SD increase in neuroticism was associated with a 9% HR 1.09 (95% CI 1.03 to 1.16) increased risk of mortality from all causes.

PSYCHOLOGICAL RESILIENCE PROMOTES LONGEVITY

Conversely, a variety of resilience factors has been identified that protect against early death. We will discuss optimism as an example of a cognition, positive affect and social support.

In a pivotal study, optimism was measured in a sample of 7007 college students.[18] Forty years later, 91.1% of the most pessimistic students were still alive as compared with 94.1% of the most optimistic group. Similar results were observed in a recent publication of the Nurses Health Study[19] involving more than 70 000 participants: a higher degree of optimism was associated with a lower mortality risk, which remained significant after adjustment for sociodemographic confounders, health behaviours, health conditions and depression (HR 0.91, 95% CI 0.85 to 0.97). The associations were verified for various causes of death, including cancer, heart disease, stroke, respiratory disease and infection. Data from a cohort of 602 Dutch elderlies, followed for 9 years, corroborated the view that optimism is associated with increased longevity.[20]

Also positive affect has been associated with increased longevity.[21 22] A truly prospective study analysed handwritten biographies of 180 catholic nuns composed when the sisters joined the congregation at a mean age of 22.[23] Emotion words in the biographies were counted and related to survival between ages 75 and 90. The results showed a

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strong positive correlation between the number and variety of positive emotional expressions in the early life biographies and longevity in later life, resulting in a 2.5-fold difference between the lowest and highest quartiles (figure 1). An important aspect of this study is that the nuns shared a very similar lifestyle, thus reducing its potential confounding role in the observations.

Findings of a pioneering study showed that social support predicted 30-month longevity in an elderly community population, even after controlling for age, sex, race, economic status, physical health status, self-care capacity, depressive symptoms, cognitive functioning, stressful life events and smoking.[24] This finding has been consistently replicated in all kinds of groups. An interesting prospective study on mortality showed that providing social support may be even more beneficial than receiving it.[25]

Thus, overall, studies show that psychological vulnerability factors predict mortality but also that psychological resilience predicts longevity. All of these psychological factors are, to a certain extent, amenable to change, but how do they link CWP and mortality?

PSYCHOLOGICAL DIMENSIONS OF CWP

Most accumulated evidence indicates that CWP is associated with a high prevalence of the vulnerability factors and a significant scarcity of the resilience factors described above. CWP has been consistently associated with an increased prevalence of a number of distressful psychological conditions, with emphasis on depression, anxiety and 'overall' negative affect.[26 27 28] The annual prevalence of major depressive disorder among patients with fibromyalgia has been estimated at 22%, which is three times higher than in individuals without fibromyalgia.[29 30] Other studies report even higher prevalence estimates.

Although CWP seems not strictly linked to a *specific* type of personality,[31] some studies indicate that fibromyalgia is associated with higher rates of neuroticism.[32 33 34] We have shown that healthcare professionals are able to distinguish patients with fibromyalgia from healthy controls, based solely on a limited set of items extracted from a personality profile, most being related to neuroticism.[35]

Depression, anxiety and neuroticism have well-established positive correlations with the prevalence and severity of pain, comorbid symptomatology and disability, in patients with CWP and in the general population.[36 37 38] Psychosocial distress and stress have been identified as a predictor of new-onset CWP,[39 40] and neuroticism has been shown to predict joint pain even over 23 years.[41]

Conversely, patients with CWP seem to present lower levels of resilience factors such as positive affect[42 43] and social support.[44 45 46] All of these risk and resilience factors have demonstrated correlations with daily intensity of fibromyalgia symptoms and have been the object of promising intervention studies in this context.

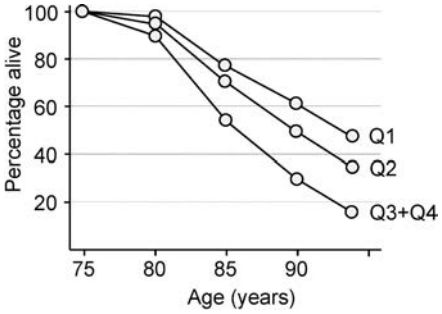

Figure 1 Positive emotion expression at average age 22 and survival after age 75 years. Q1: highest quartile of number of positive emotion sentences; Q2: second highest quartile; Q3+Q4: quartiles 3 and 4 together (because of overlap), reflecting lowest number of sentences with positive emotions. Adapted from Danner *et al*.[23]

CLINICAL IMPLICATIONS

The results presented by Macfarlane *et al* reinforce the evidence that CWP is associated with increased mortality of all causes, underlining the importance of developing our understanding of this condition to improve the care we provide to these patients. The complexity of the potential causative interactions underlying these observations cannot be overexpressed. Our editorial emphasises the importance of also attending to the psychosocial domains of CWP and their multifaceted potential consequences, including the mediation between CWP and increased mortality. This claim neither refutes the importance of lifestyle factors nor does it exclude the participation of yet additional risk factors, such as the adverse effects of non-steroidal anti-inflammatory drugs and other medications commonly used in this clinical context. The socioeconomic context also continues to deserve attention as it has been associated with both the prevalence of CWP[47 48] and with higher overall cardiovascular and cancer mortality.[49] Similar observations apply to sleep deprivation.[50]

We stress the potential importance of psychological domains, as they appear to have key influences in all 'phases' of these complex interactions, from the origin or maintenance of CWP, to the emotional and behavioural response to pain, the adoption of unhealthy lifestyles and the overall risk of death.

This is especially important in view of the efficacy of psychological therapies on CWP and its psychological correlates sleep problems, depression, functional status and catastrophising.[51] A recent population-based intervention study[52] demonstrated that a depression management programme significantly improved the long-term survival among older adults with high levels of medical comorbidity, which supports the view that targeting depression and other psychological correlates of CWP might decrease early mortality.

CONCLUSION

CWP should be seen as a biopsychosocial condition requiring a biopsychosocial understanding and therapeutic approach.

Contributors All authors contributed equally to the text through provision of ideas, writing, discussing and editing.

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Tumour necrosis factor: out of my heart!

Lars Vereecke,[1,2] Dirk Elewaut[1,2]

Increasing evidence points to cardiovascular comorbidities in patients with rheumatic diseases, particularly in rheumatoid arthritis (RA) and spondyloarthritis (SpA).[1] Ischaemic heart disease, valvular involvement including insufficiency and stenosis and arrhythmias can be observed as typical manifestations. They are a major cause of increased morbidity and mortality. While the epidemiological evidence is overwhelming, the underlying mechanisms why chronic inflammatory diseases affect the cardiovascular system are only partially understood. Treatment with anti-tumour necrosis factor (TNF) biologics ameliorates the risk for these comorbidities.[1] However, insights into the underlying mechanisms and information on responsible cell-type linking arthritis and cardiac disease are yet unclear.

In this issue, Ntari *et al* describe spontaneous cardiac pathology in a preclinical model of arthritis driven by chronic human TNF expression. They show that TNF signalling in mesenchymal cells is both necessary and sufficient for the development of heart valve pathology in the presented mouse model.[2]

Human TNF transgenic mice which develop spontaneous arthritis (Tg197 line)[3] were backcrossed to colVI-Cre TNFR1$^{fl/fl}$ versus TNFR1$^{cneo/cneo}$ mutant mice. This approach permits selective mesenchymal-specific genetic ablation versus reactivation of TNF/TNF-receptor 1 (TNFR1), including valve interstitial cells (VICs) of the heart. The same group previously showed that TNF signalling in mesenchymal cells is sufficient to drive chronic gut and joint pathology in an alternative TNF-overexpressing mouse model.[4] In humans, synovial fibroblasts in the joints and intestinal mesenchymal cells in the gut regulate pathological mechanisms during arthritis[5] and inflammatory bowel disease (IBD),[6] which indicates a central role for mesenchymal cells in the aetiology of these diseases.

Under chronic TNF exposure, the authors show development of spontaneous left-sided heart valve pathology with fibrosis and thickening of the aortic and mitral valves. This was linked to activated and hyperproliferating VICs. Valvular stenoses develop which leads to degeneration and left ventricular dysfunction. All these features mimic very well the cardiac abnormalities detected in patients with RA and SpA. Using a number of complementary strategies the investigators pinpoint this to a role of TNFR1 on mesenchymal cells. Mechanistically, VICs ex vivo exhibit an activated phenotype, increased TNF production and enhanced proliferative and migratory features. This mirrors abnormalities found in other stromal cell compartments such as synovial fibroblasts in the joint. RNA sequencing of VICs and synovial fibroblasts from huTNF transgenic mice were compared and were shown to display similar dysregulated pathways, including nuclear factor (NF)-κB signalling and pathways regulating extracellular matrix remodelling and deregulated growth, together indicating an inflammatory and profibrotic phenotype.

One of the intriguing findings of the study is the development of pathological alterations localised in the left side of the heart, particularly the aortic and mitral valvular area while the pulmonary valve as well as blood arteries and vessels appeared unaffected. This is of particular interest given that mesenchymal VICs are equally distributed in both the left and right sides of the heart. The authors suggest that this reflects regions of enhanced biomechanical stress in the heart, in line with the main pump function of left ventricle. This mirrors earlier findings of inflammation at mechanical strain-sensitive entheseal sites in another TNF-driven model of SpA, where biomechanical unloading of the limbs prevents the development of enthesitis.[7] In line with this, the present study suggests sites of enhanced cardiovascular stress to be particularly sensitive to localised tissue remodelling and development of valvular abnormalities. However, unlike in joint tissues, inflammation in aortic and mitral valve appeared to be minimal since no massive immune cell recruitment could be observed. This points to differences of inflammatory cells to enter these heart structures under high velocity or could be due to specific structural features of valve which disfavours immune cell accumulation. Alternatively, it could indicate a rapid clearance of inflammatory cells which is less likely. Despite low immune cell numbers, mesenchymal cells are sufficient to drive inflammatory signalling and orchestrate profound structural remodelling, ultimately leading to fibrosis. This is intriguing as tissue fibrosis is not a typical finding in RA and SpA at synovial sites where accumulation of inflammatory cells and stromal cell hyperplasia seems to dominate along with secondary destruction of articular structures. These findings indicate that synovial fibroblasts and VICs drive TNF-dependent joint and heart pathology, respectively, through site-specific pathological mechanisms. This was also reflected in different expression profiles in VICs versus synovial fibroblasts, which only partially overlapped (40% of genes), especially genes linked to TNF and NF-κB signalling were commonly dysregulated, as well as several chemokines.

While this study suggests that mesenchymal expression of TNFR1 is sufficient to drive TNF-mediated heart disease, the study does not rule out contribution of other cell types including endothelial and myeloid cells to heart pathology. In this regard it is interesting to note that recent studies have highlighted a critical role for dendritic cell subsets as well as macrophages[8–10] in preserving normal cardiac function under ischaemic conditions. As these cells not only produce but also respond to TNF, it is likely that TNFR1 signalling may also be very important on these cell types in the context of TNF-driven cardiovascular disease.[11]

Although the paper convincingly demonstrates the contribution of mesenchymal cells to TNF-driven heart pathology in the presented mouse model, questions remain to what extent arthritis and heart pathology develop independently in response to high hTNF levels, or alternatively, whether these pathologies are mechanistically linked. To address this question, specific synovial fibroblast or VICs TNFR reactivation, instead of general mesenchymal reactivation, in order to evaluate the selective development of arthritis versus cardiac pathology could be very interesting. In this respect, the mechanistic link between

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mesenchymal driven joint and gut pathology in an alternative TNF-driven mouse model is equally intriguing[4] as gut inflammation is a common comorbidity in SpA.[12] Interestingly, also IBD is linked to increased cardiac pathology.[13] Together, these data suggest that intestinal, joint and heart disease can either develop independently in response to chronic inflammation (TNF exposure) or that the aetiology of these diseases is mechanistically closely intertwined.

From a therapeutic perspective, the data illustrate the importance of early detection of cardiovascular morbidity in patients suffering from inflammatory rheumatic diseases and the impact of tight disease control. This underscores the relevance of early intervention and treat-to-target regimens on long-term outcome; not only in joint but also in the cardiovascular system.[14 15] Since human TNF is known to only bind mouse TNFR1, not TNFR2, the data in this paper solidify the crucial role of TNFR1 signalling on the inflammatory features exerted by TNF. In contrast, there is growing evidence that TNFR2 signalling exerts immune regulating properties mainly through its impact on T regulatory cells.[16–19] Thus the interaction of TNF–TNFR1 appears to be the main driver of proinflammatory pathways during the development of arthritis and cardiac abnormalities.[4] Therefore, strategies to neutralise the detrimental effects of TNF through TNFR1-selective antibodies may be interesting alternate strategies of TNF blockade.[20] Overall, the paper by Ntari *et al* shows a crucial contribution of the mesenchymal cells to the development of heart pathology in a TNF-driven mouse arthritis model with many similarities to human disease.

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Effects of immune checkpoint inhibitors on B cells: relationship to immune-related adverse events

Immune checkpoint inhibitors (ICI) are remarkable agents that represent a revolutionary approach to treat cancer. Rather than directly killing tumour cells, ICIs inhibit the regulatory pathways that prevent a T cell response to the tumour. With checkpoints blocked and T cells free to act, the tumour becomes a target for cytotoxic T cells. ICIs interdict key costimulatory systems involved in checkpoint regulation, binding to either cytotoxic T lymphocyte associated antigen 4 (CTLA-4) or the programme cell death 1 (PD-1) molecule or its ligand, PD-1L. These antibodies can be used either alone or in combination.[1]

In view of the effects of ICIs on T cell regulation, this form of therapy is associated with considerable toxicity and a wide variety of immune-related adverse events (irAE). The nature and frequency of these effects vary among agents and include effects on the gastrointestinal tract (colitis), liver, lung (pneumonitis), skin (rash and pruritus) and endocrine glands including hypophysitis. Since CTLA-4 and PD-1 systems involve different lymphocyte populations and act at different sites, the combination shows a broader range of side effects and an increasing frequency.[2]

For the rheumatologist, the use of ICIs raises important questions related to the emergence or exacerbation of rheumatic conditions during their use. While the literature is thus far limited, studies available indicate that ICI therapy can lead to inflammatory arthritis of large and small joints; polymyalgia rheumatica; sicca syndrome; and reactive arthritis among other musculoskeletal conditions.[3–6] When these conditions occur, symptoms can be usually managed by conventional agents, allowing therapy to proceed.[7] Concerns also arise because of the possibility that a person with a pre-existent rheumatic condition may flare once the checkpoints that restrain autoreactivity are blocked.[8] Biomarkers to predict those patients who would either develop a new rheumatic complaint or flare a pre-existing condition would therefore be important in identifying patients at risk, perhaps allowing prophylactic therapy.

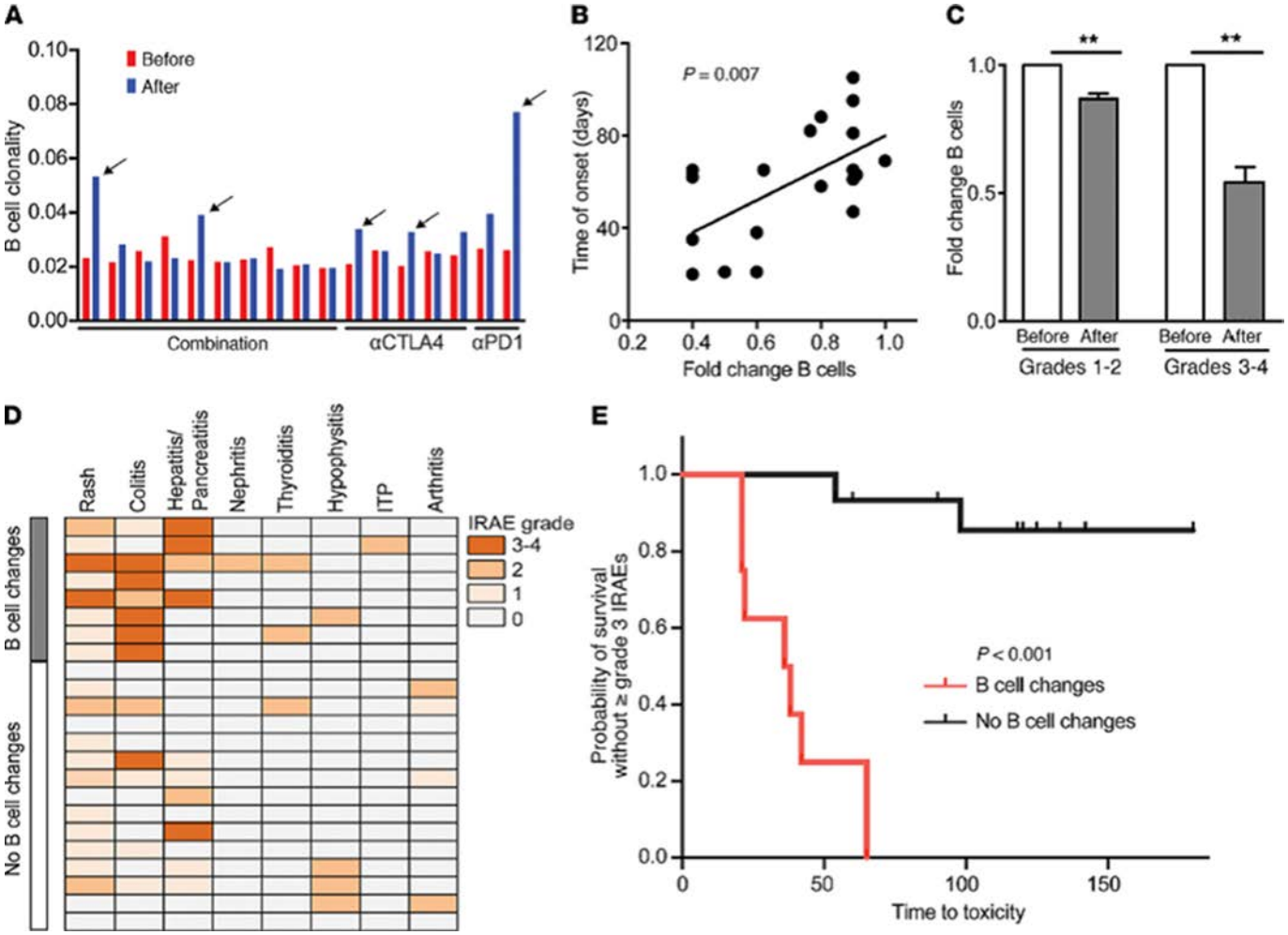

Figure 1 The relationship between the development of irAEs and B cell changes. The figure shows assays of B cell number and phenotype following the administration of immune checkpoint inhibitors (ICI) and the development of irAEs. Panel A shows an index of B cell clonality before and after ICI therapy, with arrows indicating increased clonality in some patients. Panel B shows the relationship between the time of onset of an irAE and the fold change in B cell numbers following combination checkpoint blockade (CCB). Panel C shows the relationship between the magnitude of change of B cell numbers and the grades of irAE. Panel D shows the type and grade of irAEs in patients receiving CCB. Figure E shows a Kaplan-Meier plot of the relationship between the B cell changes and the probability of survival without grade 3 or more irAEs. In this plot, B cell changes are defined as a decrease in B cell numbers (0.7 mean fold change), with a two-fold or greater increase in either $CD21^{lo}$ cells or plasmablasts. Reproduced with permission from the *Journal of Clinical Investigation*.[9]

A recent paper by Das *et al* published in the *Journal of Clinical Investigation* provides perhaps unexpected findings on blood biomarkers that may predict irAE.[9] While checkpoint inhibitors act on T cells, Das *et al* examined B cells obtained from patients with melanoma who were treated with anti-CTLA-4 or anti-PD-1, alone or in combination. Using elegant flow cytometry and other molecular approaches, these studies demonstrated a number of important changes in B cell populations in the blood. These changes include a decline in circulating B cells and an increase in plasmablasts and a population of B cells defined as $CD21^{lo}$. Importantly in the study of Das *et al*, changes in the B cell populations showed a correlation with the frequency and timing of irAEs (figure 1). Furthermore, patients who developed B cell changes early in the onset of therapy had an increased frequency of more severe adverse events.

While checkpoint inhibitors act on T cells, these studies show an important effect on B cells which resemble those observed in CTLA-4 haploinsufficient patients; such patients display abnormal T regulatory cell function, lymphocytic infiltration of organs, autoimmune cytopenias and B cell abnormalities that include an accumulation of $CD21^{lo}$ cells.[10] A variety of studies have suggested that $CD21^{lo}$ cells are a functionally and phenotypically distinct population that represent recent emigrants from germinal centres on the path to differentiation into long-lived plasma cells.[11] Given the important role of autoantibodies in the pathogenesis of diseases such as rheumatoid arthritis and systemic lupus erythematosus, it will be important to look specifically at patients with these autoimmune diseases who are treated with ICIs to characterise any changes in B cells that may occur and their impact on serology. It will also be important to characterise any novel autoantibodies that may emerge during this form of therapy and mediate irAEs.

Immune checkpoint inhibition is an important new therapy to treat advanced malignancy. The utilisation of these agents is also an opportunity to garner unique insights into immune regulation especially as they relate to the mechanisms of tolerance. These mechanisms are beneficial when they restrain autoreactivity and prevent recognition of self-antigens that lead to autoimmunity. In malignancy, these mechanisms are detrimental since they prevent recognition of the neoantigens that arise from genetic or epigenetic disturbances and can decorate tumours. Future studies should hopefully provide new approaches to identify and control irAEs as well as explore new forms of immunotherapy relevant for both autoimmunity and malignancy.

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EULAR recommendations for the health professional's approach to pain management in inflammatory arthritis and osteoarthritis

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ABSTRACT

Pain is the predominant symptom for people with inflammatory arthritis (IA) and osteoarthritis (OA) mandating the development of evidence-based recommendations for the health professional's approach to pain management. A multidisciplinary task force including professionals and patient representatives conducted a systematic literature review of systematic reviews to evaluate evidence regarding effects on pain of multiple treatment modalities. Overarching principles and recommendations regarding assessment and pain treatment were specified on the basis of reviewed evidence and expert opinion. From 2914 review studies initially identified, 186 met inclusion criteria. The task force emphasised the importance for the health professional to adopt a patient-centred framework within a biopsychosocial perspective, to have sufficient knowledge of IA and OA pathogenesis, and to be able to differentiate localised and generalised pain. Treatment is guided by scientific evidence and the assessment of patient needs, preferences and priorities; pain characteristics; previous and ongoing pain treatments; inflammation and joint damage; and psychological and other pain-related factors. Pain treatment options typically include education complemented by physical activity and exercise, orthotics, psychological and social interventions, sleep hygiene education, weight management, pharmacological and joint-specific treatment options, or interdisciplinary pain management. Effects on pain were most uniformly positive for physical activity and exercise interventions, and for psychological interventions. Effects on pain for educational interventions, orthotics, weight management and multidisciplinary treatment were shown for particular disease groups. Underpinned by available systematic reviews and meta-analyses, these recommendations enable health professionals to provide knowledgeable pain-management support for people with IA and OA.

INTRODUCTION

Pain is the predominant symptom in the majority of people with inflammatory arthritis (IA) and osteoarthritis (OA) which both broadly contribute to the global burden of rheumatic and musculoskeletal conditions.[1–5] Knowledgeable pain-management support can reduce pain, increase functioning and well-being, and reduce individual and societal costs.[6] Therefore, practitioners in all healthcare settings should have the knowledge and skills required to help people with IA and OA to better manage their pain. Rheumatology health professionals are ideally placed to provide comprehensive, evidence-based and patient-centred care.[7]

Pain is a complex and multifaceted experience. Besides pathological processes such as inflammation and tissue damage, multiple individual factors influence pain, for example, illness beliefs, mood, avoidance behaviour, obesity, sleep disturbance, and the pattern of rest and activity throughout the day.[8–13] These factors are commonly mentioned in educational materials and are part of the pain-management approach by health professionals in rheumatology.[9 14 15]

Meta-analyses and randomised controlled trials (RCTs) have, for instance, been performed with respect to pharmacological pain treatment in OA,[16 17] aerobic exercise in rheumatoid arthritis (RA) and OA,[18 19] activity pacing in chronic pain,[20] and broad education and self-management approaches in rheumatic diseases.[15 21] Evidence regarding pain-management support in IA and OA ranges from RCTs to expert reports, but as yet the effect of pain-management options on pain have not been studied in a comprehensive way for multiple pain treatment modalities. The aim of this review was to evaluate the existing scientific evidence associated with the benefits of the health professional's approach to pain management for people with IA and OA, and to use this evidence and expert opinion to provide recommendations that enable health professionals to provide knowledgeable pain-management support.

METHODS

The standardised operating procedures for European League Against Rheumatism (EULAR)-endorsed recommendations were followed,[22] including a systematic literature review and definition of the strength of recommendations by a task force of experts. In the current article, the recommendations regarding management options mostly include advice with sufficient data-driven evidence, whereas the overarching principles and recommendations regarding assessment are based on expert opinion because they could not be substantiated with evidence from systematic reviews.

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Task force

The task force that included 18 members (16 from Europe and 2 from the USA) from 12 countries consisted of patient representatives, nurses, physiotherapists, psychologists, rheumatologists, a general practitioner, an occupational therapist, a clinical epidemiologist and a research fellow. The executive committee of the task force consisted of a convener (RG), methodologist (RC) and research fellow (CLO). During the first of two task force meetings, the research questions, scope of the project and pain-management options were defined.

Scope

Definition of the scope and framing of questions addressing management options in systematic reviews helped the task force to achieve focused recommendations. This process was guided by PICO which specifies the patient Population, Interventions, Comparator and Outcomes of interest.[23]

The target users of the recommendations are health professionals in the field of rheumatology including rheumatologists. The target population for these recommendations are patients with OA and patients with the following types of IA: RA, spondyloarthritis (SpA) and psoriatic arthritis (PsA).

It was decided that recommendations should cover core general pain management that virtually any health professional should be able to give including the appraisal of treatment options which require referral to specialised pain treatment. These options requiring referral included in the recommendations should be readily available to most healthcare practitioners. Moreover, it was decided to exclude general pharmacological and joint-specific medical and surgical treatments such as arthroplasty and glucocorticoid injections from the systematic literature review because these are better covered by the existing EULAR task force recommendations for the management of IA and OA.[16 17 24–29]

To restrict the systematic literature review to pain-management strategies, the target outcome of our systematic review was pain. However, consistent with other expert groups,[30 31] the task force unanimously endorsed that, besides pain, physical functioning (eg, activity) and psychological functioning (eg, emotional well-being and participation) are core domains of any management intervention in rheumatic care. This focus on pain as an outcome but also the multiple management options that are reviewed and the broad group of patients to which this study is relevant, differentiates our study from studies with a more general focus on education in IA,[21] or non-pharmacological management of OA.[15]

Systematic literature review

The bibliographic databases Cochrane, Embase, PsycINFo, PubMed, Scopus and Web of Science were searched with the name of one or more of the diseases of interest in the title and the word 'pain' and a word referring to a mode of intervention or care in the title, abstract or keywords (search date: 19 October 2015). For efficiency in answering the broad question of the literature review and to benefit from the work that was done previously, the search was limited to systematic reviews, meta-analyses, (practice) guidelines and recommendations. If no systematic reviews were available, we searched for RCTs. No time or language restrictions were applied in the initial search. Thus, included in the literature search were systematic reviews in one of the selected diseases (RA, SpA, PsA, OA) with pain as an outcome measure. Excluded were studies involving general pharmacological and joint-specific medical and surgical treatment.

All abstracts were independently read and judged on their suitability for inclusion by two reviewers. Results were compared and, in case of discrepancy, discussed until consensus was reached. Excluded were duplicate articles, articles that were withdrawn, those not written in English, animal studies, conference abstracts, articles including (practice) guidelines or recommendations without a systematic review or meta-analysis included, previous versions of reviews and meta-analyses (eg, Cochrane reviews), articles that did not have pain as a reported outcome measure or did not report outcomes for OA, RA, SpA or PsA, articles not reviewing the effect of one or multiple modes of intervention or care, and articles that only reviewed the effects of pharmacological treatments, surgical treatments, complementary medicine, herbs or nutraceuticals. Reference lists of the selected articles were hand-searched for additional relevant systematic reviews and meta-analyses. The detailed search keys and exclusion criteria are shown in online supplementary file 1 .

Evaluating the evidence

The evidence for OA was divided into evidence for OA in general; OA of the knee, hip or knee and hip; OA of the hand/wrist and OA of the foot/ankle. The systematic reviews and meta-analyses commonly included a mean effect size for pain. For every treatment option and per disease subgroup, the effect found by the included articles was recorded and effect categories were distinguished: 'positive effect' (ie, articles (unanimously) state positive effects of the treatment option on pain), 'no effect' (ie, articles state the treatment option has neither positive nor negative effects), 'unclear effect' (ie, articles state both no effects and positive effects) or a combination thereof meaning that articles were divided in their conclusions on the effect of the treatment option (eg, some state unclear effects and others only positive effects). 'Negative effect' could have been a category but none of the included studies stated harmful overall effects of the examined treatment options.

Our systematic review protocol was developed as a review of reviews including systematic reviews and meta-analyses of RCTs. Therefore, the Oxford Centre for evidence-based medicine 'level of evidence' for all recommendations was 1A (from meta-analysis or RCTs) or occasionally 1B (when only one RCT was available).[22] The Grades of Recommendation, Assessment, Development and Evaluation system was used to rate the overall quality of evidence of the reviews and meta-analyses.[32] Two assessors independently graded the quality of the available evidence as high, moderate, low or very low. In case of discrepancy, the quality was discussed until consensus was reached.[33] 'Strength of recommendation' was determined for the recommendations. These scores vary from A ('category of evidence' 1A: meta-analysis of RCTs) to D ('category of evidence' 4 from expert committee reports or opinions and/or clinical experience of respected authorities or extrapolated recommendation from 'category of evidence' 2 or 3 from non-randomised experimental, correlation or descriptive studies).[22]

Developing recommendations

During the second and last task force meeting, the results of the systematic literature were presented and discussed, and the wording of recommendations was started. Treatment recommendations were supported by findings in the systematic literature review. Overarching principles and assessment recommendations were mostly based on expert opinion in the task force. After this meeting, the wording was finished through email, and each task force member indicated the 'level of agreement' on a numerical

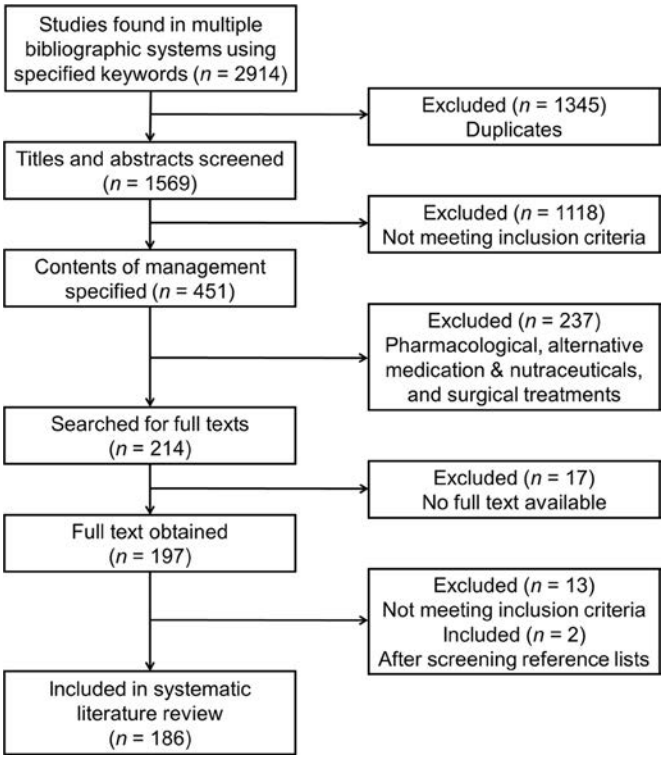

Figure 1 Flow chart of the systematic literature review of systematic reviews.

rating scale ranging from 0 (completely disagree) to 10 (completely agree).

RESULTS

Figure 1 shows a flow chart of the systematic literature review. The 2914 selected titles reduced to 214 after exclusion of duplicates and check of exclusion criteria. Full texts of 17 articles could not be obtained. Two assessors read 197 articles in full-text. Another 13 articles were excluded for being a narrative (non-systematic) review (3x), guideline without systematic review (1x) or duplicate (2x), not having pain as an outcome (4x) or treatment effects on pain as an aim (2x) or involving only results of pharmacological treatment (1x). Two additional meta-analyses were included after scanning the reference list of the selected articles. In total, 186 systematic reviews and meta-analyses were included, and their content was assessed for the following information: population, types of intervention, effects and level of quality. This information is provided in online supplementary files 2, 3 and 4 for all included studies.

Evaluation of effects on pain (systematic literature review)

No studies were found that systematically reviewed effects on pain for PsA. Moreover, it was found that of the included treatment options, sleep interventions and most assistive devices were not evaluated for their effect on pain in systematic reviews. Studies were heterogeneous with respect to intervention and comparator conditions. For example, comparisons of exercise included land-based versus water-based, strengthening versus aerobic, group versus individual, supervised versus home-based, multimodal versus unimodal, progressive versus non-progressive and one or various exercises versus standard care, sham or medication. The reviewed studies, direction of effects and level of quality are shown in tables 1 and 2 for the treatment modalities

Table 1 Overview of systematic reviews of randomised trials (evidence category 1A) regarding education, orthotics, psychological interventions, weight management and multimodal treatment: treatment modality and disease, direction of effect and the quality of the evidence according to GRADE in patients with RA, SpA and OA

Treatment modality Disease Specific treatment modality	Reviews (n)	Direction of effect	GRADE quality of evidence
Education and self-management			
RA	8	o/+	⊕⊕
SpA	1	o	⊕⊕
OA-general	6	o/+	⊕⊕⊕
OA-hand/wrist	1	o	⊕
OA-hip/knee	4	+	⊕⊕⊕
OA-knee	4	+	⊕⊕⊕
Orthotics			
RA			
Orthotic gloves	2	o/+	⊕⊕
Splints	5	o/+	⊕⊕
Insoles	8	o/+	⊕⊕
Orthopaedic shoes	3	+	⊕⊕
Padded hosiery	1	+	⊕
OA-hand/wrist			
Orthotic gloves	1	o	⊕
Splints	8	+	⊕⊕
OA hip			
Insoles	1	+	⊕
OA-knee			
Braces	10	?/+	⊕⊕
Sleeves	1	+	⊕⊕
Elastic bandages	2	+	⊕⊕
Taping	3	?/+	⊕⊕
Orthoses in general	1	+	⊕⊕
Insoles	15	?/+	⊕⊕
Orthopaedic shoes	1	+	⊕⊕
Cane	1	+	⊕⊕
Psychological interventions			
RA			
Cognitive–behavioural therapy	7	+	⊕⊕⊕
Biofeedback	1	+	⊕⊕
SpA			
Cognitive–behavioural therapy	1	o	⊕
OA-general			
Cognitive–behavioural therapy	1	+	⊕⊕⊕
Psychosocial and coping interventions	1	+	⊕⊕⊕
Relaxation techniques	1	+	⊕
OA-hip/knee			
Relaxation techniques	1	+	⊕
OA-knee			
Biofeedback	1	o	⊕
Weight management			
RA	2	+	⊕⊕
SpA	1	+	⊕
OA-hip/knee	2	+	⊕⊕⊕
OA-knee	10	o/+	⊕⊕⊕
Multimodal treatment			
RA			
Comprehensive occupational therapy	1	o	⊕⊕
OA-hand/wrist			
Multidisciplinary therapy	1	o	⊕⊕

Continued

Table 1 Continued

Treatment modality Disease Specific treatment modality	Reviews (n)	Direction of effect	GRADE quality of evidence
OA knee			
Comprehensive physical therapy	1	o	⊕⊕⊕

Diseases without a review were excluded from the table.
Direction of effect:+, positive; o, no; –, negative; ?, unclear (effect equivocal), or a combination thereof meaning that different reviews reached different conclusions about the effect of the treatment.
References are shown in online supplementary file 2.
GRADE, ⊕⊕⊕⊕, high; ⊕⊕⊕, moderate; ⊕⊕, low; ⊕, very low.
GRADE, Grades of Recommendation, Assessment, Development and Evaluation; OA, osteoarthritis; RA, rheumatoid arthritis; SpA, spondyloarthritis.

Table 2 Overview of systematic reviews of randomised trials (evidence category 1A) regarding 'physical activity and exercise': treatment modality and disease, direction of effect and the quality of the evidence according to GRADE in patients with RA, SpA and OA

Treatment modality Disease	Reviews (n)	Direction of effect	GRADE quality of evidence
General exercise			
RA	5	o/+	⊕⊕
SpA	6	+	⊕⊕
OA-general	6	+	⊕⊕⊕
OA-hand/wrist	4	o/+	⊕⊕
OA-hip/knee	11	+	⊕⊕⊕
OA-hip	11	o/+	⊕⊕
OA-knee	18	+	⊕⊕⊕
OA-foot/ankle	2	+	⊕⊕
Aerobic exercise			
RA	3	o/+	⊕⊕
OA-general	3	+	⊕⊕⊕
OA-hip/knee	2	o/+	⊕⊕
OA-hip	1	o	⊕
OA-knee	9	+	⊕⊕⊕
Strength and resistance			
RA	2	o/+	⊕⊕
OA-general	3	+	⊕⊕⊕
OA-hand/wrist	2	o/+	⊕⊕
OA-hip/knee	4	+	⊕⊕⊕
OA-hip	3	+	⊕⊕
OA-knee	14	+	⊕⊕⊕
Tai chi, yoga, qigong, whole body vibration			
RA	3	?/+	⊕
OA-general	6	o/+	⊕ to ⊕⊕
OA-hand/wrist	3	+	⊕
OA-hip/knee	1	o/+	⊕⊕
OA-knee	12	o/+	⊕ to ⊕⊕

Diseases without a review were excluded from the table.
Direction of effect: +, positive; o, no; –, negative; ?, unclear (effect equivocal) or a combination thereof meaning that different reviews reached different conclusions about the effect of the treatment.
References are shown in online supplementary file 3.
GRADE: ⊕⊕⊕⊕, high; ⊕⊕⊕, moderate; ⊕⊕, low; ⊕ to ⊕⊕, very low to low; ⊕, very low. (The combined ⊕ to ⊕⊕ grade is due to difference in quality between studies of different modalities.)
GRADE, Grades of Recommendation, Assessment, Development and Evaluation; OA, osteoarthritis; RA, rheumatoid arthritis; SpA, spondyloarthritis.

Table 3 Review of reviews (evidence category 1A) regarding miscellaneous therapies: treatment modality and disease, direction of effect and the quality of the evidence according to GRADE in patients with RA, SpA and OA

Treatment modality Disease	Reviews (n)	Direction of effect	GRADE quality of evidence
Acupuncture			
RA	5	o/+	⊕⊕
OA-general	4	o/+	⊕⊕
OA-hand/wrist	3	o/+	⊕
OA-hip/knee	2	+	⊕⊕
OA-hip	1	o	⊕⊕
OA-knee	16	+	⊕⊕⊕
Balneotherapy and massage			
RA	3	o/+	⊕⊕
SpA	2	o/+	⊕
OA-general	5	o/+	⊕ to ⊕⊕
OA-hand/wrist	3	+	⊕
OA-hip/knee	2	o/+	⊕
OA-knee	8	+	⊕⊕
Thermotherapy			
RA	4	o/+	⊕⊕
OA-general	1	o	⊕
OA-hand/wrist	3	o/+	⊕⊕
OA-knee	4	o/+	⊕⊕
Ultrasound, radiotherapy and diathermy			
RA	3	+	⊕⊕
OA-general	2	o/+	⊕⊕
OA-hip/knee	2	?/o	⊕⊕
OA-knee	12	o/+	⊕⊕ to ⊕⊕⊕
Electromagnetic therapy			
RA	2	o/+	⊕⊕
OA-general	1	+	⊕⊕⊕
OA-hip/knee	3	+	⊕⊕
OA-knee	18	?/+	⊕⊕ to ⊕⊕⊕
Laser therapy			
RA	3	+	⊕⊕
OA-general	2	o/+	⊕⊕
OA-hand/wrist	4	o	⊕⊕
OA-knee	7	o/+	⊕⊕⊕
Magnet therapy			
OA-general	1	o/+	⊕⊕
OA-hand/wrist	2	o/+	⊕⊕
OA-knee	2	o	⊕⊕
Manual therapy/joint mobilisation			
OA-hand/wrist	4	+	⊕⊕⊕
OA-hip/knee	1	o/+	⊕⊕
OA-hip	1	+	⊕⊕
OA-knee	1	+	⊕⊕
Diverse			
OA-general (healing, qigong, chiropractic)	1	o/+	⊕
OA-hand/wrist (leeches, copper bracelets)	2	o/?	⊕

Diseases without a review were excluded for the table.
Direction of effect: +, positive; o, no; –, negative; ?, unclear (effect equivocal) or a combination thereof meaning that different reviews reached different conclusions about the effect of the treatment or that the direction of effects differed between treatment modalities such as between transcutaneous electrical nerve stimulation and pulsed electromagnetic field therapy which are both included in 'electromagnetic therapy'.
References are shown in online supplementary file 4.
GRADE: ⊕⊕⊕⊕, high; ⊕⊕⊕, moderate; ⊕⊕ to ⊕⊕⊕, low to moderate; ⊕⊕, low; ⊕ to ⊕⊕, very low to low; ⊕ very low. (The combined grades are due to difference in quality between studies of different modalities such as balneotherapy vs massage studies.)
GRADE, Grades of Recommendation, Assessment, Development and Evaluation; OA, osteoarthritis; RA, rheumatoid arthritis; SpA, spondyloarthritis.

that were included in the recommendations and in table 3 for miscellaneous treatment modalities.

Education and self-management

The reviewed review studies on education and self-management programmes generally concluded that available studies showed 'positive effects' (n=8) or showed both 'no effects' and 'positive effects' (n=14), but for SpA and OA of the hand/wrist, single meta-analyses observed 'no effect' on pain (table 1).

Physical activity and exercise

Of all treatment options, the effects of physical exercise have been studied most extensively (table 2). For general exercise, aerobic exercise, and strength and resistance training, the quality of studies was low to moderate and effects on pain were mostly 'positive' in IA and OA, with some reviews observing 'no effects'. For tai chi, yoga, qigong and whole body vibration, the quality of studies was low to very low, and it was unclear whether there were positive effects on pain. Table 2 can be used as a guide for choosing an appropriate intervention; for instance, strength and resistance training is more relevant and more extensively studied for OA of the knee than for other conditions. Reviews do not answer the question whether high-intensity exercise is as safe as low-intensity exercise which is an ongoing issue of debate.[34 35]

Orthotics

In mostly low quality studies, 'positive effects' of orthotics on pain have been consistently observed for orthopaedic shoes in RA and OA of the knee, splints in OA of the hand, and sleeves and elastic bandages in OA of the knee and less consistently for other orthotics (table 1). Except for use of a cane,[16] no systematic reviews evaluated daily living aids such as a tin-opener or assistive devices using pain as an outcome. Although several orthotics can be recommended based on positive effects on pain, there is not enough evidence to give recommendations regarding design or materials.

Psychological and social interventions

In very low to moderate quality studies, effects of psychological interventions (eg, cognitive–behavioural therapy (CBT), mindfulness-based interventions, stress management training) on pain as summarised in reviews were 'positive' with the exception of reviews showing 'no effect' on pain of CBT in SpA and biofeedback in OA (table 1).

Weight management

In very low to moderate quality studies, effects of weight management have been frequently reviewed for OA of the knee; 'no effects' and 'positive effects' were observed. For RA and SpA, 'positive effects' were observed in three reviews (table 1).

Sleep interventions

It has been proposed that sleep disturbance should be systematically assessed and managed in patients with IA and OA.[13 36–38] Our systematic review did not extract systematic reviews that evaluated effects of sleep interventions on pain in OA or IA, but randomised trials examined the effects of CBT for insomnia in OA. CBT was observed to improve sleep and pain in one study.[39] In another study, both CBT and a placebo condition resulted in improved sleep and comparable reductions of pain over 6 months, but the CBT group had significantly greater reductions in wake after sleep onset which predicted subsequent decreases in clinical pain.[40] Outside the field of rheumatic diseases, meta-analyses support the effectiveness of behavioural, including self-help, interventions on sleep outcomes.[41 42] Face-to-face treatments of at least four sessions seem to be more effective than self-help interventions.[43] In meta-analyses, small but significant effects of sleep interventions on pain have been observed in people with varied chronic medical conditions.[44 45]

Pharmacological treatment

Pharmacological treatment is a core ingredient of pain management in IA and OA. It includes analgesics (eg, paracetamol, codeine and other opiate-like drugs); oral or topical non-steroidal anti-inflammatory drugs (NSAIDs); intra-articular injections, for example, with glucocorticoids; and occasionally also agents for neuropathic pain. The evidence for pharmacological pain treatment was not part of the current review but has been evaluated by other task forces.[16 17 24–29 46–50] In brief, previous task forces recommended paracetamol as first-line treatment, with topical agents such as topical NSAIDs and capsaicin also recommended for specific joints, for patients with OA,[17 26 29] and consideration of intra-articular injections for specific joints in OA and IA.[17 24–26 28 29] Existing EULAR recommendations should be consulted regarding the safe use of NSAIDs.[17 26 29 46]

Miscellaneous therapies

Table 3 shows an overview of less commonly available therapies in rheumatological clinical practice that were therefore not included in the recommendations. If a patient shows interest in one of these therapies, and skilled professionals are available to administer them, then this overview can be used as a quick guide to the appropriate meta-analyses. These therapies have especially been studied in OA of the knee with positive effects on pain of acupuncture and 'balneotherapy and massage' and less clear effects of 'electrical' therapies.

Psoriatic arthritis

Pain is as high or higher in PsA than in RA,[51] and patients have an educational need to manage their pain.[52] Nevertheless, none of the extracted studies reviewed the health professional's approach in PsA for effects on pain. Given the lack of specific knowledge, the health professional may use pain treatment options in RA to guide pain treatment in PsA.

Overarching principles (expert opinion)

The task force defined overarching principles based on expert opinion (box 1).

First, patient-centred care was considered important. Care that is respectful of and responsive to individual patient preferences, needs and values, and ensures that patient values guide clinical decisions,[53] may improve adherence and persistence with treatment.[21 54–57] Validation of the patient's pain experience is considered a prerequisite for trust, communication and engagement in treatment.

Second, a biopsychosocial model of pain was recommended. Relationships between all factors of this model are recognised to be interactive and reciprocal with mutually influencing pathways similar to a hanging mobile toy in which movement of one part causes movement of other parts. The importance of the distinct factors differs between individuals.

Third, in order to achieve pain control, it is crucial to treat disease activity and to prevent tissue damage. To meet that aim, the health professional should have basic knowledge of IA and OA. Common treatment goals in rheumatic diseases are to optimise control of inflammation, decrease disease activity, improve

Box 1 Overarching principles

- The assessment and treatment process should be guided by a patient-centred framework.
- The health professional should understand that (any type of) pain encompasses multiple and mutually interacting biological, psychological and social factors that include but are not limited to pain severity, peripheral (inflammation and joint damage) and central neurophysiological processes, physical (dis)ability, resilience and vulnerabilities (emotions, cognitions, behaviour, lifestyle), social factors (work, support, facilities, economic), sleep quality, obesity and other health risks (eg, smoking, alcoholism).
- The health professional should have basic knowledge of the pathology, treatment and sequelae of inflammatory arthritis and osteoarthritis.
- The health professional should be able to differentiate between localised and generalised pain and should know that these types of pain may coexist.

function and well-being, and to reduce pain and other disease-related symptoms.[15–17 21 24–29]

Fourth, the ability to differentiate between types of pain helps the health professional to direct the optimal pain-management strategy. Pain localised in a specific region of the body might be due to peripheral nociceptive input such as inflammation or damage. Generalised pain is more often non-specific with regard to pathological findings and can be due to a dysfunction in the regulation of pain pathways. Pain is commonly regarded as generalised or widespread when pain is present in both sides of the body, above and below the waist and in axial body regions. In IA, generalised pain may remain despite good inflammatory control. Such pain requires comprehensive pain-management strategies.[58 59]

Not all health professionals may currently have the required knowledge and skills to apply these principles. For those health professionals who have identified that they require further education in this area, the overarching principles can be used to direct their learning. This may involve work-based supervision with another health professional or undertaking an educational course that addresses some or all of these principles.

Recommendations (systematic literature review and expert opinion)

Table 4 shows the recommendations. Proper assessment is a prerequisite for proper pain treatment. Recommendations 1 (assessment) and 2 (personalised treatment plan) were based on expert opinion in the task force. Treatment recommendations 3–10 were based on the systematic literature review summarising effects on pain. Specific considerations regarding application of these treatment options (indicated with an asterisk in table 4) were based on expert opinion of the task force.

Assessment

The extent of assessment depends on many factors such as available time. A first step in ensuring that pain management is patient-centred is to invite patients disclosing the impact of pain on their daily functioning, to assess their ideas and concerns regarding the cause of their pain and the perceived control over pain episodes, and to take account of their expectations and preferences for treatment. It is deemed important to establish the patient's functional and valued life goals, that is, what it is that they cannot currently do as well as they would wish to. Research shows that individuals differ widely in terms of management needs.[21 54 60 61]

Second, assess pain severity using a numerical or visual analogue pain rating scale,[62] and the onset, duration, location and spread (pain manikin), quality, interference, triggers and progression of pain. Furthermore, appraise the type of pain (localised or generalised) and whether referral is needed to a pain specialist to evaluate the type of pain, current treatment or current medication (safe use, interactions with other medication, side effects). Generalised pain can be recognised in a clinical interview and by the use of a pain manikin such as the Michigan body map.[63] Use validated questionnaires to assess the potential presence of neuropathic pain.[62 64–66]

Third, assess ongoing pharmacological and non-pharmacological treatments, previous treatments tried and the effects and side effects of these treatments, patient beliefs about the ability to control and overcome pain and its consequences, and willingness of the patient to engage in additional treatment if deemed necessary.

Fourth, assess current inflammation and joint damage as sources of pain following the most recent recommendations.[67 68] In case of poorly controlled inflammation, optimise disease control or refer to a rheumatologist for treatment according to recommendations.[25 27 48] In case of localised (nociceptive) pain relating to OA, consider (to refer to) joint-specific treatments in line with recommendations.[16 69]

Fifth, assess pain-related biological, psychological and social factors that might need attention, specifically:

- The nature and extent of disability: physical activity, mobility, activities of daily living, social participation, general physical fitness (aerobic capacity, muscle strength, endurance), pain-related fear and avoidance of activities, balance of activities and rest (pacing).
- Beliefs and emotions about pain and pain-related disability: the psychological response to pain and psychological vulnerability factors, psychological distress, psychiatric comorbidity and cognitions such as catastrophising (rumination, magnification and helplessness),[70] fear of movement-related pain,[71] catastrophising and pain self-efficacy.[72]
- Social factors related to pain and its consequences: the way family members and other significant others react to patient's pain or pain-related disability; work; family and friends; economic problems; housing.
- Sleep problems: the quantity and quality of sleep, including whether the patient feels refreshed on waking and sleep hygiene habits such as regular exercise during the day, stress management, noise, sleep timing and avoidance of caffeine, nicotine, alcohol and daytime napping.[73]
- Presence of obesity.[74]
- Other factors that might influence pain or pain management, such as dependence on tobacco, alcohol or drugs.[75]

Treatment

Tables 1–3 offer an overview of the number of the reviewed reviews and meta-analyses, the observed effects of specific treatments on pain and the quality of evidence of the studies.

Through shared decision-making, treatment is guided by the expressed needs of the patient, the health professional's assessment and evidence-based treatment options. A stepped-care approach is recommended including education and self-management support in step 1 (recommendation 3), one or more treatment options by a specialist if indicated in step 2 (recommendations 4–9) or multidisciplinary treatment in step 3 (recommendation 10).

Table 4 EULAR recommendations for the health professionals' approach to pain management in inflammatory arthritis and osteoarthritis

	Level of evidence	Strength of recommendation	Level of agreement task force: mean (SD)
1. Assessment by the health professional should include the following aspects (the assessment is brief or extensive depending on factors such as available time, whether it is a first or regular consultation, and the needs of the patient):	4	D	9.3 (0.8)
Patient's needs, preferences and priorities regarding pain management and important activities, values and goals in daily life.			
Patient's pain characteristics including severity, type, spread and quality.			
Previous and ongoing pain treatments and the perceived efficacy.			
Current inflammation and joint damage as sources of pain, and whether these are adequately treated.			
Pain-related factors that might need attention: (a) the nature and extent of pain-related disability, (b) beliefs and emotions about pain and pain-related disability, (c) social influences related to pain and its consequences, (d) sleep problems and (e) obesity.			
2. The patient should receive a personalised management plan with the aim of reducing pain and pain-related distress and improving pain-related function and participation in daily life. This plan is guided by shared decision-making, the expressed needs of the patient, the health professional's assessment and evidence-based treatment options. A stepped-care approach may include, in step 1, education and self-management support (recommendation 3); in step 2, one or more treatment options by a specialist if indicated (recommendations 4 to 9); or, in step 3, multidisciplinary treatment (recommendation 10).	4	D	9.0 (0.8)
3. The patient should receive education. * All patients have easy access to (1) educational materials (such as brochures or links to online resources with encouragement to stay active, sleep hygiene guidelines and so on), (2) psychoeducation by the health professional and (3) online or face-to-face self-management interventions.	1A	A	9.7 (0.6)
4. If indicated, the patient should receive physical activity and exercise. * The health professional and patient appraise whether advice to stay active, supervised physical exercise or multidisciplinary treatment is needed. * If the patient is not able to initiate physical activity and exercises without help, then consider the possibility for referral to a physiotherapist for individually tailored graded physical exercise or strength training. * If psychosocial factors such as fear of movement [71 80] or catastrophising cognitions [70] underlie a disabled, sedentary lifestyle, then consider a multidisciplinary intervention including cognitive – behavioural therapy.	1A	A	9.8 (0.8)
5. If indicated, the patient should receive orthotics. * If a patient has pain during activities of daily living which impedes functioning, orthotics (such as splints, braces, gloves, sleeves, insoles and shoes), daily living aids (such as a tin opener), an assistive device (such as a cane or rollator) or ergonomic adaptation (at home, workplace) can be offered. If the patients wants to use this assistive support, then consider referral to the occupational therapist, who can proceed with several actions: offer education about appropriate ways to use joints and ergonomic principles, appraise the need for the use of an orthotic or assistive device, give advice about how to acquire it, fit the customised aid to the patient , offer training in the use of it, refer to the appropriate specialist who will do this, eg, orthopaedic shoemaker.	1A	A	8.6 (0.9)
6. If indicated, the patient should receive psychological or social interventions. * If there are indications that social variables or psychological factors interfere with effective pain management and functional status, then consider (depending on the severity) providing basic social and psychological management support or referral to a psychologist, social worker, self-management support programme, CBT or multidisciplinary treatment. * If psychopathology (eg, depression and anxiety) is present, discuss treatment options with the patient and the patient 's primary care physician.	1A	A	9.5 (0.6)
7. If indicated, the patient should receive sleep interventions. * If sleep disturbance is reported, inquire about causes (eg, pain, persistent worrying, poor sleep habits) and offer basic education about good sleep hygiene practices. * If sleep remains (severely) disturbed, refer to a therapist or programme aimed at restoring sleep, or to a specialised sleep clinic.	1B	B	8.4 (1.1)
8. If indicated, the patient should receive weight management. * If the patient is obese, explain to the patient that obesity can contribute to pain and disability. Discuss accessible weight management options with the patient or signpost appropriate specialised weight management support; for example, dietitian , psychologist, community lifestyle services or bariatric clinic/surgery.	1A	A	9.1 (1.0)
9. If indicated, the patient should receive pharmacological and joint-specific pain treatment according to recent recommendations. * Ask about the patient 's existing use of prescribed and over - the - counter pain relief including homeopathic remedies and consider if the frequency of use is safe (not over dosing) and appropriately regular. Ask or refer for further specialist or medical advice if there are concerns or if additional pharmacological treatment may be indicated.	See refs 16 17 24–29		9.5 (0.8)
10. If indicated, the patient should receive multidisciplinary treatment. * If more than one treatment options are indicated, for example, to treat psychological distress in combination with a sedentary lifestyle, and if monotherapy failed, consider a multidisciplinary intervention.	4	D	8.8 (1.1)

'Level of evidence' and 'Strength of recommendation' for treatment modalities refer to specific diseases in which uniform positive effects on pain (excluding studies with 'very low' quality of evidence) were observed (tables 1 and 2). Overarching principles and recommendations regarding assessment are based on expert opinion.
Level of evidence: 1A, from meta-analysis of randomised controlled trials; 1B, from at least one randomised controlled trial; 2A, from at least one controlled study without randomisation; 2B, from at least one other type of quasi-experimental study; 3, from descriptive studies, such as comparative studies, correlation studies or case–control studies; 4, from expert committee reports or opinions and/or clinical experience of respected authorities.
Strength of recommendations is a combination of the information from the systematic literature review and expert opinion: A, category I evidence; B, category II evidence or extrapolated recommendations from category I evidence; C, category III evidence or extrapolated recommendation from category I or II evidence; D, category IV evidence or extrapolated recommendation from category II or III evidence.[22]
Level of agreement by the task force on a scale from 0 to 10.
*Specific considerations regarding application of recommendations that are indicated with an asterisk are based on expert opinion of the task force.
CBT, cognitive–behavioural therapy; EULAR, European League Against Rheumatism.

The choice for a specific intervention is not only determined by effects on pain but also by effects on functioning, social participation and well-being. Moreover, evidence for effects of specific pain treatments differ for specific diseases. The 'strength of recommendation' for recommendations 3–10 (table 4) holds for specific diseases in which uniform positive effects on pain (excluding studies with 'very low' quality of evidence) were observed.

Box 2 Research agenda

- To examine omissions in knowledge such as effects of treatment options on pain in psoriatic arthritis, the effects of sleep interventions on pain in inflammatory arthritis (IA) and osteoarthritis (OA), and pain as an outcome measure in studies of assistive devices such as a cane or rollator in more diseases than OA of the knee alone.
- To examine in meta-analyses the effects of multidisciplinary treatment on pain.
- To improve the methodological quality of treatment outcome studies.
- To conduct an analysis examining effect sizes for more specific treatment modalities that are now merged into comprehensive treatment packages.
- To examine moderators of treatment effects (eg, in which patient subgroups each specific treatment option causes a reduction of pain).
- To examine mediators of outcome, that is, how pain treatments work in IA and OA.
- To examine the effects on pain of minimal interventions such as advice during a consultation, use of brochures and e-health psychoeducation.
- To examine whether combined pharmacological and non-pharmacological pain management is more effective than monotherapy.
- To contribute to personalised medicine by analysing customised pain treatments; for example, using replicated single-case experimental designs with idiosyncratic outcome measures.
- To examine in which way healthcare could best be organised to be able to provide the best possible and knowledgeable pain-management support for people with IA and OA.

- Education had a uniform positive effect on pain in OA (hip/knee, knee).
- Physical activity and exercise showed uniform positive effects on pain for general exercise in SpA and OA (general, hip/knee, knee, foot/ankle), aerobic exercise in OA (general, knee), and strength and resistance training in OA (general, hip/knee, hip, knee).
- Orthotics showed small but consistent positive effects on pain for orthopaedic shoes in RA and OA of the knee, splints in OA of the hand and knee orthoses (especially sleeves, elastic bandages) in OA of the knee.
- Psychological and social interventions showed a uniform positive effect on pain for CBT in RA and OA (general), psychosocial and coping interventions in OA (general), biofeedback in RA and relaxation interventions in OA (general, hip/knee).
- There was no meta-analysis that evaluated effects of sleep interventions on pain in IA or OA but small effects of sleep interventions on pain were observed in meta-analyses in people with varied chronic medical conditions.
- Weight management showed a uniform positive effect on pain in RA, SpA and OA of the hip/knee.
- Multidisciplinary treatment is cautiously recommended considering the absence of studies examining the added effect on pain of multidisciplinary treatment to monodisciplinary therapies and considering that meta-analyses on multimodal treatment did not observe effects on pain.

DISCUSSION

Results and conclusions derived from 186 systematic reviews and meta-analyses were reviewed to identify the evidence associated with the benefits of the health professional's approach to pain management for people with IA and OA. Effects on pain were most uniformly positive for physical activity and exercise interventions, and for psychological interventions. Effects on pain for educational interventions, orthotics, weight management and multidisciplinary treatment were shown for particular disease groups. Recommendations for patient-centred pain management were guided by this scientific evidence and by clinical expert opinion.

The task force unanimously endorsed that in rheumatic care, besides pain severity, physical functioning and psychological functioning are major outcomes of any management intervention by health professionals, in agreement with other expert groups.[30 31] Although pain was selected as the target outcome, our systematic literature review also included interventions that were not aimed at alleviating pain but, for instance, at increasing muscle strength, physical activity or emotional functioning. Nevertheless, our study showed that many evaluations of treatment options—especially physical activity and psychological interventions—showed a reduction of pain in most patient groups. This included treatment options in which pain reduction was not the primary goal.

Box 2 presents questions for the future research agenda. Our systematic review showed that there is ample evidence for specific pain treatment options in specific groups. Nevertheless, there are several omissions in our knowledge with respect to effects of pain management on pain, especially in PsA, sleep interventions, assistive devices and multidisciplinary treatment. Moreover, it is inherent to the multifaceted nature of pain, the heterogeneity of the group of patients with IA and OA, and specific needs of individual patients that pain-management options of choice will differ among patients. From a clinical point of view, a multimodal approach will likely result in the best outcome, but from a scientific point of view, it would be more fruitful to learn whether a single treatment option is able to bring about change in pain and other outcomes. Thus, a main challenge in future research is to examine in which patient subgroups each specific treatment option causes a reduction of pain. Moreover, most studies pertain to systematic interventions in groups, but the most frequent clinical intervention is patient-customised education and advice given during a consultation, handing a brochure or offering information through the internet. The effects of these minimal interventions should be investigated as well.

As pain is the predominant symptom and burden in IA and OA,[1–5] clinical training of rheumatology health professionals in pain management is essential. To ensure patient-centred pain management, health professionals need knowledge, confidence, communication skills and skills to support patients to translate intentions into action plans, which should be part of educational programmes.[76–78] It has been demonstrated that training of professionals helps to improve pain management of OA.[79] Health professionals can use the handout shown in figure 2 as a guide in their work, while the more detailed findings and recommendations can be used to fine-tune treatment. Further, knowledge and skills indicated in the overarching principles and recommendations should be used when reviewing pain curricula in higher education and in postgraduate clinical education.[76] Within EULAR, the current recommendations can be included in the 'EULAR online course for health professionals' which comprises specific diseases such as RA and OA as well as broader modules:

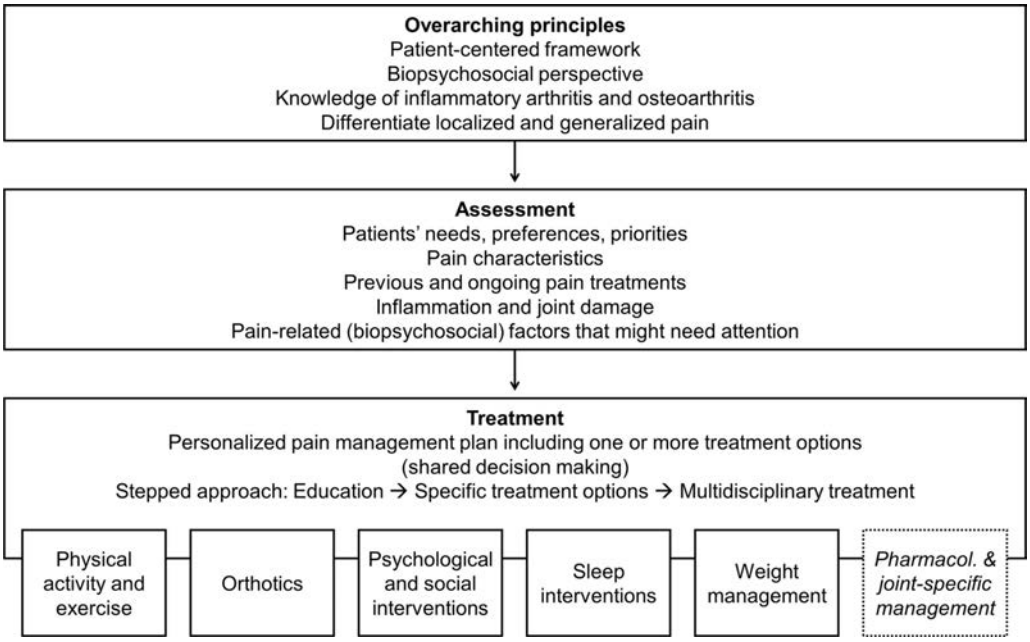

Figure 2 Handout with guide to pain management in inflammatory arthritis and osteoarthritis.

https://www.eular.org/edu_online_course_hpr.cfm. Finally, these recommendations will be disseminated through this publication and through a lay summary of the recommendations that will be disseminated among national patient associations.

In conclusion, guided by expert opinion and partly underpinned by a considerable number of systematic reviews and meta-analyses, an expert group developed and launched the first set of recommendations that enable health professionals to provide knowledgeable and evidence-based pain-management support for people with IA and OA.

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2018 update of the EULAR recommendations for the management of Behçet's syndrome

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ABSTRACT

Several new treatment modalities with different mechanisms of action have been studied in patients with Behçet's syndrome (BS). The aim of the current effort was to update the recommendations in the light of these new data under the auspices of the European League Against Rheumatism (EULAR) Standing Committee for Clinical Affairs. A task force was formed that included BS experts from different specialties including internal medicine, rheumatology, ophthalmology, dermatology, neurology, gastroenterology, oral health medicine and vascular surgery, along with a methodologist, a health professional, two patients and two fellows in charge of the systematic literature search. Research questions were determined using a Delphi approach. EULAR standardised operating procedures was used as the framework. Results of the systematic literature review were presented to the task force during a meeting. The former recommendations were modified or new recommendations were formed after thorough discussions followed by voting. The recommendations on the medical management of mucocutaneous, joint, eye, vascular, neurological and gastrointestinal involvement of BS were modified; five overarching principles and a new recommendation about the surgical management of vascular involvement were added. These updated, evidence-based recommendations are intended to help physicians caring for patients with BS. They also attempt to highlight the shortcomings of the available clinical research with the aim of proposing an agenda for further research priorities.

INTRODUCTION

Behçet's syndrome (BS) is a systemic variable vessel vasculitis that involves the skin, mucosa, joints, eyes, arteries, veins, nervous system and the gastrointestinal system. Physicians from several different disciplines are involved in the care of patients with BS. The disease shows geographic differences in its clinical features. Thus a multicentre collaboration of experts from different specialties and from different parts of the world is necessary for the optimisation of the recommendations for managing BS.

The first European League Against Rheumatism (EULAR) Recommendations for the management of Behçet's disease that were published in 2008 has gained a lot of interest and helped physicians from different disciplines in the management of patients with BS.[1] At that time a total of nine recommendations were formed after a literature review, a Delphi exercise and two expert consensus meetings by a task force that included rheumatologists, ophthalmologists, dermatologists, a neurologist and a patient. In five of the nine recommendations, the strength of the recommendation was 'D', indicating that it was based only on expert opinion for the whole or at least a part of the recommendation.

The task force felt that there was a need for updating these recommendations as there had been several related new publications and data with new agents were available. Especially the experience with the use of biological agents in BS has substantially increased during the recent years. There is also more evidence to guide us in the management of gastrointestinal involvement and about other issues such as the use of anticoagulants in BS patients with vascular involvement. One of the shortcomings of the previous recommendations was that it lacked guidance regarding the surgical and interventional treatment options for vascular involvement.

The objective of the current project was to update and improve the EULAR Recommendations for the management of BS in the light of the new studies, in addition to identifying the hitherto uncovered areas for future research. The target population for these recommendations includes all physicians and surgeons who are involved in the treatment of BS.

METHODS

The standard operating procedures for developing EULAR-endorsed recommendations was followed and when applicable the Appraisal of Guidelines, Research and Evaluation instrument was used.[2] A task force was formed including 20 BS experts from seven European countries and Korea, 1 healthcare professional (a nurse), 2 patients with BS, 2 fellows responsible for the systematic literature review who are EMEUNET members and 1 senior methodologist. The experts were from various specialties that are involved in the management of patients with BS including internal medicine, rheumatology, ophthalmology, dermatology, neurology, gastroenterology, oral health medicine and vascular surgery.

An initial Delphi was conducted among the task force members to identify the questions and problem areas which were not covered by

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the previous recommendations and areas that need updating. A total of 52 clinical questions were decided on with input from both physician and patient members of the task force. The questions were amalgamated and formulated into Population, Intervention, Comparison and Outcome questions for the systematic review.[3] A protocol was prepared for the systematic review according to the recommendations given in Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols and registered in International Prospective Register of Systematic Reviews before starting the systematic literature search (registration number CRD42015027033). The systematic literature search was conducted by two fellows independently and disagreements were resolved by the convenor. Systematic reviews for mucocutaneous and joint involvement and for major organ involvement including eye, vascular, nervous system and gastrointestinal system involvement are prepared in detail for publication separately (Ozguler *et al*. Management of Major Organ Involvement of Behçet's Disease: Systematic Literature Review for the Update of the EULAR Recommendations for the Management of Behçet's Syndrome, submitted for publication; Pietro *et al*. Management of Skin, Mucosa and Joint Involvement of Behçet's Syndrome: A Systematic Literature Review for Update of the EULAR Recommendations for the Management of Behçet's Syndrome, submitted for publication). These systematic reviews and the recommendations manuscript form an integral and inseparable sum and should be read as such.

MEDLINE (from 1950), EMBASE (from 1980), The Cochrane Library, including the Cochrane Central Register of Controlled Trials, Cochrane Database of Systematic Reviews, Database of Abstracts of Reviews of Effects and Health Technology Assessments, International Pharmaceutical Abstracts Database and the http://www.ClinicalTrials.gov website were searched using the predefined keywords and keyword combinations. Any randomised controlled trial (RCT), controlled clinical trial whether open label or not comparing an active intervention (alone or in combination) in patients with BS with any other comparator (drug or placebo) were included. If controlled trials were not available for answering a specific research question, uncontrolled evidence from preferably prospective cohort studies or case series was considered. Studies including patients meeting any of the criteria sets for BS or with a given diagnosis of BS as described by the authors were considered eligible. Authors and/or sponsors were contacted when additional data were required.

Results of the systematic reviews for mucocutaneous and joint involvement and for major organ involvement including eye, vascular, nervous system and gastrointestinal system involvement were presented to the task force during a one-and-a-half-day meeting. Following these presentations, thorough discussions led to the formation of draft recommendations. At the end of the meeting, these draft recommendations were discussed again and modified accordingly. Each recommendation was designated with a strength of recommendation from A to D, where A indicates that this is based on category I evidence (data from meta-analysis of RCTs or from at least one RCT), whereas D corresponds to category IV evidence.[2]

This 'Oxford system' was used for designating the level of evidence and strength of recommendation as advised by the standard operating procedures for developing EULAR-endorsed recommendations.[2] Consensus was reached explicitly via voting with the prespecified decision to include only the statements that obtain agreement by at least 70% of the experts. Additionally, the level of agreement from 0 to 10 for each recommendation was determined by a closed vote.

RESULTS

The systematic search of the literature databases yielded 3927 articles. After reviewing the title and abstracts, 395 were selected for full-text evaluation and 11 additional articles were identified through hand search. Finally, 192 studies on the management of mucocutaneous, joint, eye, vascular, nervous system and gastrointestinal system involvement of BS were included (figure 1). The detailed methods and results of the systematic reviews for mucocutaneous and joint involvement and for major organ involvement are submitted separately. Based on the results of these systematic reviews and experts' opinions, 5 overarching principles and 10 recommendations (table 1) were formed.

Overarching principles

- BS is a condition that typically runs a relapsing and remitting course, and the goal of treatment is to promptly suppress inflammatory exacerbations and recurrences to prevent irreversible organ damage.
- A multidisciplinary approach is necessary for optimal care.
- Treatment should be individualised according to age, gender, type and severity of organ involvement and patients' preferences.
- Ocular, vascular, neurological and gastrointestinal involvement may be associated with a poor prognosis.
- Disease manifestations may ameliorate over time in many patients.

The relapsing and remitting nature of BS and the differences in natural course of different types of organ and system involvement, as well as differences in the disease course between men and women, mandate that the treatment should be individualised accordingly. In patients with BS, skin, mucosa and joint involvement can cause impairment of quality of life but do not cause permanent damage whereas untreated eye, vascular, nervous system and gastrointestinal system involvement can cause serious damage and even death. When there is only skin, mucosa and joint involvement, treatment can be tailored according to the patient's need and how much the symptoms impact on their quality of life compared with the risks associated with adverse effects of any medication used. When chronic oral and genital ulceration caused scarring, vigorous treatment is required to prevent oropharyngeal narrowing, and obliterative and deforming genital scarring. On the other hand, when the patient has organ involvement, it is important to rapidly suppress the inflammation and prevent relapses in order to prevent loss of function. Immunosuppressives are usually necessary to accomplish this. The more severe disease course among men with an early age of disease onset prompts more aggressive treatment and increased caution during follow-up in such patients.[4] As the disease manifestations usually abate over time, treatment may be tapered and even stopped during the course of the disease.[5]

Recommendation 1: mucocutaneous involvement

Topical measures such as steroids should be used for the treatment of oral and genital ulcers. Colchicine should be tried first for the prevention of recurrent mucocutaneous lesions especially when the dominant lesion is erythema nodosum or genital ulcer. (Level of evidence: IB; strength of recommendation: A)

Papulopustular or acne-like lesions are treated with topical or systemic measures as used in acne vulgaris. (Level of evidence: IV; strength of recommendation: D)

Leg ulcers in BS might be caused by venous stasis or obliterative vasculitis. Treatment should be planned with the help of

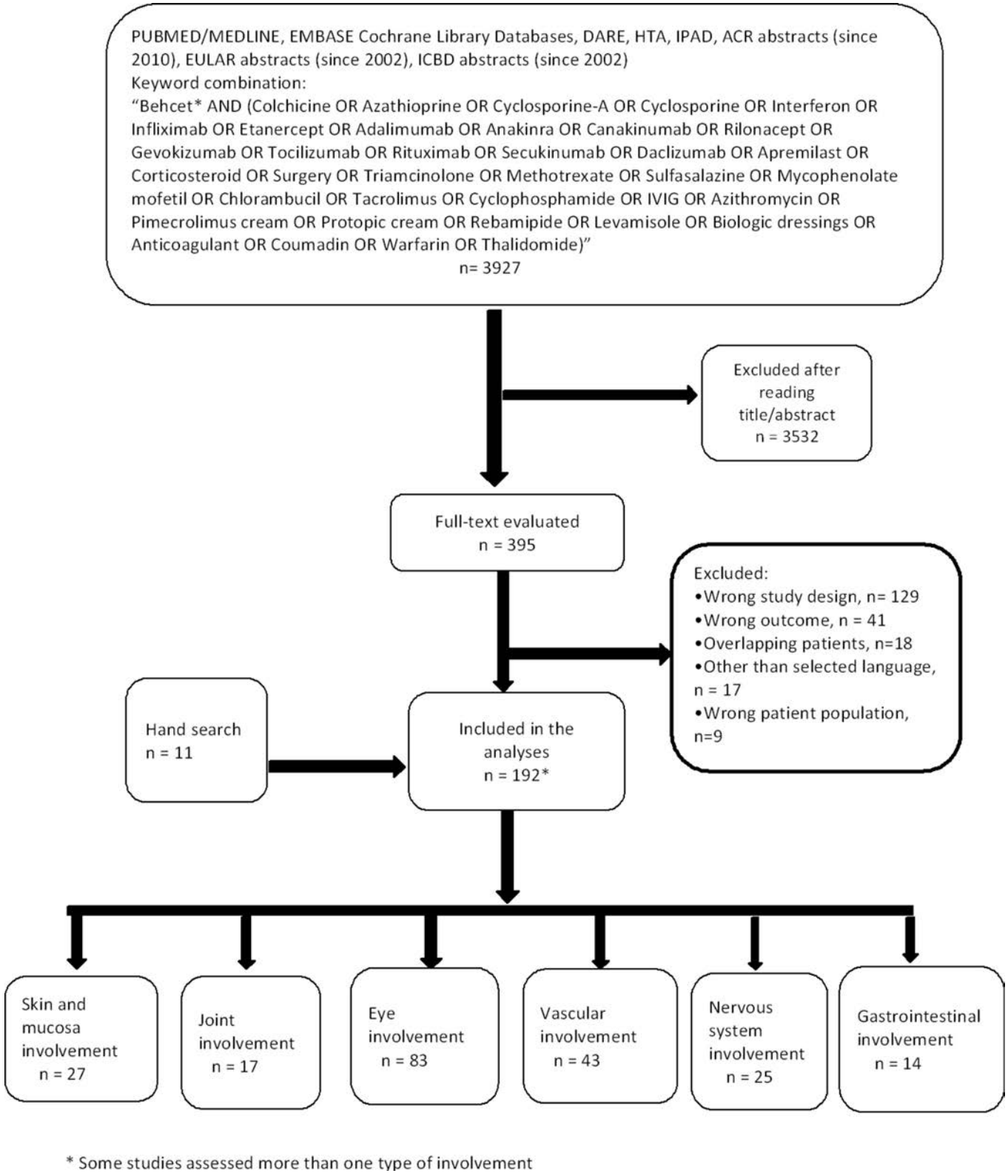

Figure 1 Flow chart of the study selection process. ACR, American College of Rheumatology; DARE, Database of Abstracts of Reviews of Effects; EULAR, European League Against Rheumatism; HTA, Health Technology Assessments; ICBD, The International Criteria for Behçet's Disease; IPAD, International Pharmaceutical Abstracts Database.

a dermatologist and vascular surgeon. (Level of evidence: IV; strength of recommendation: D)

Drugs such as azathioprine, thalidomide, interferon-alpha, tumour necrosis factor-alpha inhibitors or apremilast should be considered in selected cases. (Level of evidence: IB; strength of recommendation: A)

Several RCTs explored the efficacy of different immunomodulatory and immunosuppressive agents for mucocutaneous lesions. Colchicine was shown to be effective for genital ulcers and nodular lesions especially in women, but there was some controversy regarding its efficacy in oral ulcers.[6–8] The efficacy of colchicine and immunosuppressives for papulopustular or

Table 1 Updated European League Against Rheumatism recommendations for the management of Behçet's syndrome, with levels of evidence, grade of recommendations, voting rates and level of agreement

	Overarching principles and recommendations	Level of evidence*	Strength of recommendation †	Level of agreement
Overarching principles	► BS is a condition that typically runs a relapsing and remitting course and the goal of treatment is to promptly suppress inflammatory exacerbations and recurrences to prevent irreversible organ damage. ► A multidisciplinary approach is necessary for optimal care. ► Treatment should be individualised according to age, gender, type and severity of organ involvement and patient's preferences. ► Ocular, vascular, neurological and gastrointestinal involvement may be associated with a poor prognosis. ► Disease manifestations may ameliorate over time in many patients.	NA	NA	9.5±0.7
1. Mucocutaneous involvement	Topical measures such as steroids should be used for the treatment of oral and genital ulcers. Colchicine should be tried first for the prevention of recurrent mucocutaneous lesions especially when the dominant lesion is erythema nodosum or genital ulcer (IB). Papulopustular or acne-like lesions are treated with topical or systemic measures as used in acne vulgaris (IV).	IB/IV	A/D	9.4±0.8
	Leg ulcers in BS might be caused by venous stasis or obliterative vasculitis. Treatment should be planned with the help of a dermatologist and vascular surgeon.	IV	D	
	Drugs such as azathioprine, thalidomide, interferon-alpha, TNF-alpha inhibitors or apremilast should be considered in selected cases.	IB	A	
2. Eye involvement	Management of uveitis of BS requires close collaboration with ophthalmologists with the ultimate aim of inducing and maintaining remission. Any patient with BS and inflammatory eye disease affecting the posterior segment should be on a treatment regime such as azathioprine (IB), cyclosporine-A (IB), interferon-alpha (IIA) or monoclonal anti-TNF antibodies (IIA). Systemic glucocorticoids should be used only in combination with azathioprine or other systemic immunosuppressives (IIA).	IB/IIA	A/B	9.5±0.6
	Patients presenting with an initial or recurrent episode of acute sight-threatening uveitis should be treated with high-dose glucocorticoids, infliximab or interferon-alpha. Intravitreal glucocorticoid injection is an option in patients with unilateral exacerbation as an adjunct to systemic treatment.	IIA	B	9.4±0.7
3. Isolated anterior uveitis	Systemic immunosuppressives could be considered for those with poor prognostic factors such as young age, male sex and early disease onset.	IV	D	9.0±0.8
4.Acute deep vein thrombosis	For the management of acute deep vein thrombosis in BS, glucocorticoids and immunosuppressives such as azathioprine, cyclophosphamide or cyclosporine-A are recommended.	III	C	9.3±0.8
5. Refractory venous thrombosis	Monoclonal anti-TNF antibodies could be considered in refractory patients. Anticoagulants may be added, provided the risk of bleeding in general is low and coexistent pulmonary artery aneurysms are ruled out.	III	C	8.7±0.8
6. Arterial involvement	For the management of pulmonary artery aneurysms, high-dose glucocorticoids and cyclophosphamide are recommended. Monoclonal anti-TNF antibodies should be considered in refractory cases. For patients who have or who are at high risk of major bleeding, embolisation should be preferred to open surgery.	III	C	9.2±0.9
	For both aortic and peripheral artery aneurysms, medical treatment with cyclophosphamide and corticosteroids is necessary before intervention to repair. Surgery or stenting should not be delayed if the patient is symptomatic.	III	C	9.0±1.0
7. Gastrointestinal involvement	Gastrointestinal involvement of BS should be confirmed by endoscopy and/or imaging. NSAID ulcers, inflammatory bowel disease and infections such as tuberculosis should be ruled out.	III	C	9.2±0.9
8. Refractory/severe gastrointestinal involvement	Urgent surgical consultation is necessary in cases of perforation, major bleeding and obstruction. Glucocorticoids should be considered during acute exacerbations together with disease-modifying agents such as 5-ASA or azathioprine. For severe and/or refractory patients, monoclonal anti-TNF antibodies and/or thalidomide should be considered.	III	C	8.8±0.9
9. Nervous system involvement	Acute attacks of parenchymal involvement should be treated with high-dose glucocorticoids followed by slow tapering, together with immunosuppressives such as azathioprine. Cyclosporine should be avoided. Monoclonal anti-TNF antibodies should be considered in severe disease as first-line or in refractory patients.	III	C	9.1±1.2
	The first episode of cerebral venous thrombosis should be treated with high-dose glucocorticoids followed by tapering. Anticoagulants may be added for a short duration. Screening is needed for vascular disease at an extracranial site.	III	C	9.0±0.8

Continued

Table 1 Continued

	Overarching principles and recommendations	Level of evidence*	Strength of recommendation †	Level of agreement
10. Joint involvement	Colchicine should be the initial treatment in BS patients with acute arthritis. Acute monoarticular disease can be treated with intra-articular glucocorticoids. Azathioprine, interferon-alpha or TNF-alpha inhibitors should be considered in recurrent and chronic cases.	IB	A	9.0±1.0

*Level of evidence indicates evidence from: IA, meta-analysis of RCTs; IB, at least one RCT; IIA, at least one controlled study without randomisation; IIB, at least one type of quasi-experimental study; III, descriptive studies, such as comparative studies, correlation studies or case–control studies; IV, expert committee reports or opinions and/or clinical experience of respected authorities.
†Strength of recommendation is based on evidence: A, category I evidence; B, category II evidence or extrapolated recommendations from category I evidence; C, category III evidence or extrapolated recommendation from category I or II evidence; D, category IV evidence or extrapolated recommendation from category II or III evidence.
5-ASA, 5-aminosalicylic acid; BS, Behçet's syndrome; NA, not applicable; NSAID, non-steroidal anti-inflammatory drug; RCT, randomised controlled trial; TNF-alpha , tumour necrosis factor-alpha.

acne-like lesions seems to be limited. Mild forms of papulopustular or acne-like lesions are treated first by topical measures as used in acne vulgaris. However, chronic recurrent lesions or severe forms mimicking acne conglobata or acne cystica require systemic measures such as retinoids, sometimes together with surgical and physical therapy. Considering the safety and good tolerability of colchicine, the group agreed that it should be tried first in patients who have only mucocutaneous involvement. In patients who present with an acute exacerbation of mucocutaneous lesions, topical corticosteroids may help the rapid healing of these lesions. For patients whose lesions continue to recur despite colchicine, immunomodulatory or immunosuppressive drugs such as azathioprine, thalidomide, interferon-alpha, tumour necrosis factor alpha inhibitors (TNFis) or apremilast can be used.[9–13] The choice of immunomodulatory or immunosuppressive drug in such patients would depend on individual patient characteristics regarding safety, the cost and availability of these agents in each country, and patient preferences. Uncontrolled observational evidence suggests that lactobacilli lozenges may be a safe alternative.[14] Dapsone and azithromycin have also been tried with beneficial results.[15 16] Among the newer biological agents, interleukin (IL)-1 blockade with anakinra and canakinumab seems to provide a partial benefit in BS patients with mucocutaneous involvement, whereas IL-17 blockade with secukinumab was ineffective and IL-6 blockade with tocilizumab worsened mucocutaneous lesions.[17–22] A very recent manuscript published after the preparation of these recommendations suggests that ustekinumab may also be beneficial.[23] Management should be planned according to patients' preferences, depending on the burden of their mucocutaneous lesions weighed against the risk of adverse drug reactions with these agents.

The management of leg ulcers may be problematic since it is associated with venous stasis caused by deep vein thrombosis and/or obliterative vasculitis causing acute and chronic arterial ischaemia. Leg ulcers may occasionally be associated with pyoderma gangrenosum and require immunosuppressives. The systematic review showed no studies guiding the management of leg ulcers, thus this part of the recommendation was based solely on expert opinion. For each patient, treatment should be planned with a dermatologist and vascular surgeon experienced with such lesions as these may require the use of immunosuppressives, antibiotics if infection is present, debridement or occlusive measures such as the use of compression bandaging.

Recommendation 2: eye involvement

Management of uveitis of BS requires close collaboration with ophthalmologists with the ultimate aim of inducing and maintaining remission. Any patient with BS and inflammatory eye disease affecting the posterior segment should be on a treatment regime such as azathioprine (level of evidence: IB; strength of recommendation: A), cyclosporine-A (level of evidence: IB; strength of recommendation: A), interferon-alpha (level of evidence: IIA; strength of recommendation: B) or monoclonal anti-TNF antibodies (level of evidence: IIA; strength of recommendation: B). Systemic glucocorticoids should be used only in combination with azathioprine or other systemic immunosuppressives. (level of evidence: IIA; strength of recommendation: B)

Patients presenting with an initial or recurrent episode of acute sight-threatening uveitis should be treated with high-dose glucocorticoids, infliximab or interferon-alpha. Intravitreal glucocorticoid injection is an option in patients with unilateral exacerbation as an adjunct to systemic treatment. (Level of evidence: IIA; strength of recommendation: B)

Management of uveitis requires great caution with early recognition and evaluation of the severity of the involvement and frequent monitoring of drug response in order to prevent damage causing a permanent decrease in visual acuity and eventual blindness. Close collaboration with an expert ophthalmologist is essential.

Systemic, high-dose glucocorticoids are used for rapid suppression of inflammation during acute attacks. However, glucocorticoids should never be used alone in patients with posterior uveitis. Systemic immunosuppressives such as azathioprine, cyclosporine-A, interferon-alpha, infliximab or adalimumab should be used in such patients. RCTs have shown the efficacy of azathioprine and cyclosporine-A in preserving visual acuity and preventing relapses in patients with uveitis.[9 24–26] However, there are no RCTs to guide the management of patients who are refractory to these agents. Some experts have preferred interferon-alpha and others preferred monoclonal anti-TNF antibodies for such patients. A review of the literature for open-label, observational studies or retrospective case series with these agents hinted at certain differences such as a rapid response and improvement in visual acuity with infliximab, a sustained response with interferon-alpha as well as high remission rates with both of these agents.[27–63] The choice of treatment would depend on patient factors such as risk of infections including tuberculosis with monoclonal anti-TNF antibodies and tolerability of interferon-alpha, physician's experience with these agents and reimbursement policies of each country.

Among the monoclonal anti-TNF antibodies, although there is more accumulated experience with infliximab, adalimumab also seems to be an effective alternative.[63–67] Switching between these agents seems to be possible in patients with primary or secondary unresponsiveness or adverse events. A very recent manuscript published after the preparation of these recommendations

suggests that any drug-free, long-term remission after withdrawal of successful anti-TNF treatment combined with azathioprine given for 2 years is feasible in a good proportion of patients with sight-threatening ocular disease.[68] After the preparation of these recommendations, adalimumab has been approved for the treatment of non-infectious intermediate, posterior and panuveitis by the European Medicines Evaluation Agency and the US Food and Drug Administration based on two RCTs. However, results for patients with BS, which comprised a small portion of the study population in these trials, were not provided.[64 69]

Whether immunosuppressives such as azathioprine or cyclosporine-A should be used together with monoclonal anti-TNF antibodies was discussed. Although there are no controlled data, some experts felt that concomitant use of azathioprine and/or cyclosporine-A with monoclonal anti-TNF antibodies may improve the outcome. A retrospective case series of patients with BS who were prescribed monoclonal anti-TNF antibodies for different types of involvement suggested that concomitant use of these agents did not provide extra benefit.[63] Care should be taken since plasma concentrations of cyclosporine-A may be reduced by co-administration with azathioprine.[70]

Other biological agents such as IL-1 and IL-17 blockers have also been tried. The IL-1 blocker gevokizumab[71] and IL-17 blocker secukinumab[20] failed to meet their primary endpoints in RCTs.

Intravitreal glucocorticoid injections can be used in patients with an acute exacerbation in one eye.[72–76] However, this should be used only as an adjunct to systemic immunosuppressive therapy.

Vitrectomy should only be used in patients with complications such as vitreous condensation, coagulated vitreous haemorrhage, tractional retinal detachment, vitreoretinal or epiretinal membranes. There is no anti-inflammatory effect of this procedure in patients with uveitis.

Recommendation 3: isolated anterior uveitis

Systemic immunosuppressives could be considered for those with poor prognostic factors such as young age, male sex and early disease onset. (Level of evidence: IV; strength of recommendation: D)

Isolated anterior uveitis in patients with BS may be treated with topical agents. However, some patients may have hypopyon uveitis, which is a severe form of anterior uveitis, and some patients with isolated anterior uveitis develop posterior uveitis over time. Although it is not easy to predict which patients are at risk, it was shown that young men with an early age at disease onset have a higher risk of more severe disease. A systemic immunosuppressive such as azathioprine may be considered in such patients with the anticipation that it may have a protective effect. However, there are no data yet that show such an effect.

Recommendation 4: acute deep vein thrombosis

For the management of acute deep vein thrombosis in BS, glucocorticoids and immunosuppressives such as azathioprine, cyclophosphamide or cyclosporine-A are recommended. (Level of evidence: III; strength of recommendation: C)

In patients with BS, deep vein thrombosis is thought to result from inflammation of the vessel wall rather than hypercoagulability. Post-thrombotic syndrome is frequent especially with recurrent episodes of deep vein thrombosis and may result in leg ulcers that are very difficult to treat. One of the most controversial issues regarding the management of BS is whether deep vein thrombosis should be treated with immunosuppressives, anticoagulants or both.[77]

We performed a meta-analysis of the three retrospective studies that reported on the efficacy of immunosuppressives and/or anticoagulants for preventing recurrences of deep vein thrombosis in patients with BS.[78–80] A pooled estimate of the relapse risk of deep vein thrombosis in patients with BS treated with immunosuppressives and anticoagulants compared with those treated with only anticoagulants favoured the use of immunosuppressives with an relative risk (RR) of 0.17 (95% CI 0.08 to 0.35). On the other hand, treatment with anticoagulants and immunosuppressives compared with immunosuppressives alone did not provide a significant benefit in preventing relapses (RR 0.75, 95% CI 0.48 to 1.17).

There were no data to mandate the preference of one immunosuppressive agent over the others. Azathioprine, cyclophosphamide or cyclosporine-A are agents that can be preferred in such patients. Cyclophosphamide may be reserved for patients with extensive thrombosis of larger veins such as vena cava due to its potential adverse events.

Recommendation 5: refractory venous thrombosis

Monoclonal anti-TNF antibodies could be considered in refractory patients. Anticoagulants may be added, provided the risk of bleeding in general is low and coexistent pulmonary artery aneurysms are ruled out. (Level of evidence: III; strength of recommendation: C)

There were no data to guide the management of patients with refractory venous thrombosis. Monoclonal anti-TNF antibodies may be used since beneficial results have been obtained in BS patients with refractory arterial involvement. Interferon-alpha may be tried in selected cases.

Although our meta-analysis indicated that adding anticoagulants to immunosuppressives did not decrease the relapse risk, there is a retrospective study suggesting that not using anticoagulants may increase the risk of post-thrombotic syndrome (OR 3.8, 95% CI 1.04 to 14.1).[81] The task force felt that no recommendation against anticoagulant use can be made because of the lack of prospective controlled trial data demonstrating that anticoagulants do not decrease the relapse risk and the frequency of post-thrombotic syndrome in patients with BS.

However, great caution is required with respect to bleeding in anticoagulated patients with BS. This is especially important since arterial aneurysms are closely associated with deep vein thrombosis in BS. Patients need to be scrutinised for aneurysms when starting anticoagulants and physicians should be alert about the risk of developing aneurysms during the course of treatment since almost all BS patients with aneurysms have a history of deep vein thrombosis.[82]

Recommendation 6: arterial involvement

For the management of pulmonary artery aneurysms, high-dose glucocorticoids and cyclophosphamide are recommended. Monoclonal anti-TNF antibodies should be considered in refractory cases. For patients who have or who are at high risk of major bleeding, embolisation should be preferred to open surgery. (Level of evidence: III; strength of recommendation: C)

For both aortic and peripheral artery aneurysms, medical treatment with cyclophosphamide and corticosteroids is necessary before intervention to repair. Surgery or stenting should not be delayed if the patient is symptomatic. (Level of evidence: III; strength of recommendation: C)

The primary management of pulmonary artery aneurysms and thrombosis is with high-dose glucocorticoids and cyclophosphamide. Cyclophosphamide may be given as monthly intravenous pulses and glucocorticoids are usually given as three successive intravenous methylprednisolone pulses followed by oral prednisolone (or prednisone) at a dose of 1 mg/kg/day.[83 84] Observational, uncontrolled evidence showed that infliximab provided benefit in some of the refractory patients.[85] Mortality rate has been high in surgically treated patients and surgery should not be undertaken except for life-threatening situations.[84 86 87] Embolisation may be necessary in patients with a high risk of major bleeding.[83 87 88]

Peripheral artery aneurysms require emergency surgery or stenting unless they are small, asymptomatic and carry a low risk of rupture. Medical treatment with high-dose corticosteroids and cyclophosphamide may be sufficient for such small aneurysms. Observational studies show that medical treatment is necessary in addition to surgery or stenting in order to decrease the risk of postoperative complications and recurrences.[88–90] Medical treatment should ideally start before an aneurysm repair is attempted.

For both pulmonary and peripheral artery aneurysms, the choice of surgical intervention between graft insertion, ligation and bypass surgery can be made according to the size and location of the aneurysm and the surgeon's experience. Synthetic grafts should be preferred since venous grafts have a higher risk of thrombosis in patients with BS.

Recommendation 7: gastrointestinal involvement

Gastrointestinal involvement of BS should be confirmed by endoscopy and/or imaging. NSAID ulcers, inflammatory bowel disease and infections such as tuberculosis should be ruled out. (Level of evidence: III; strength of recommendation: C)

One of the most challenging issues regarding gastrointestinal involvement is to diagnose it correctly since abdominal pain, diarrhoea and intestinal ulcers may commonly be related to other reasons such as non-steroidal anti-inflammatory drug ulcers and gastrointestinal infections including tuberculosis, especially among patients receiving immunosuppressives.[91] Confirming the diagnosis is essential to prevent the unnecessary use of immunosuppressives that may be especially harmful if the patient has an infection.

Recommendation 8: refractory/severe gastrointestinal involvement

Urgent surgical consultation is necessary in cases of perforation, major bleeding and obstruction. Glucocorticoids should be considered during acute exacerbations, together with disease-modifying agents such as 5-ASA or azathioprine. For severe and/or refractory patients, monoclonal anti-TNF antibodies and/or thalidomide should be considered. (Level of evidence: III; strength of recommendation: C)

The evidence available for the management of gastrointestinal involvement relies on retrospective observational data since there are no controlled trials for this relatively uncommon type of involvement.[91] The choice of the initial treatment modality depends on the severity of gastrointestinal involvement. Glucocorticoids are thought to help the rapid healing of ulcers during acute exacerbations. There is some concern about the potential of high-dose glucocorticoids to facilitate perforation in patients who already carry a high risk of perforation; however, there are no data to show this. Milder gastrointestinal involvement may be treated with 5-aminosalicylate derivatives whereas more severe cases can be treated with azathioprine.[91–93] Retrospective data showed that infliximab, adalimumab and thalidomide may be beneficial in patients with severe involvement, refractory to azathioprine.[94–99] Infliximab and thalidomide may be used concomitantly in selected cases.

A cohort study of BS patients with gastrointestinal involvement showed that almost a third of these patients required emergency surgery due to perforation, major bleeding or obstruction.[91] Timely recognition of these complications is very important since they may be fatal if left untreated. Immunosuppressives seem to decrease the risk of postoperative recurrences and complications in such patients.

Recommendation 9: nervous system involvement

Acute attacks of parenchymal involvement should be treated with high-dose glucocorticoids followed by slow tapering, together with immunosuppressives such as azathioprine. Cyclosporine-A should be avoided. Monoclonal anti-TNF antibodies should be considered in severe disease as first line or in refractory patients. (Level of evidence: III; strength of recommendation: C)

The first episode of cerebral venous thrombosis should be treated with high-dose glucocorticoids followed by tapering. Anticoagulants may be added for a short duration. Screening is needed for vascular disease at an extracranial site. (Level of evidence: III; strength of recommendation: C)

The two types of central nervous system involvement, namely parenchymal involvement and cerebral venous thrombosis, rarely occur in the same patient. Cerebral venous thrombosis usually manifests as an extension of vascular involvement in BS. This obviates the need of screening for early and occult vascular lesions in patients diagnosed with cerebral venous thrombosis. There are differences in the management of these two types of nervous system involvement, and the recommendations for both are supported by only uncontrolled observational studies.

For the treatment of parenchymal involvement, high-dose glucocorticoids should be started together with an immunosuppressive such as azathioprine. A typical glucocorticoid regimen would be starting with daily pulses of intravenous methylprednisolone 1 g/day that may be continued for up to 7 days followed by oral prednisolone (or prednisone) at 1 mg/kg/day for 1 month and tapered by 5–10 mg every 10–15 days. Patients who have severe parenchymal involvement at onset, those who have persistent or relapsing disease despite corticosteroids and azathioprine and patients with chronic progressive nervous system involvement that is a more severe form of parenchymal involvement may benefit from monoclonal anti-TNF antibodies.[49 63 100–103] Limited observations with tocilizumab have also shown some benefit.[104]

The task force members agreed that an acute cerebral venous thrombosis episode should be treated with high-dose glucocorticoids to obtain a rapid remission. However, there are no data showing the benefit of adding immunosuppressives in the first episode of cerebral venous thrombosis and the group felt that this may not be necessary since relapses are not frequent in this type of involvement. Anticoagulants may be added for a short duration, especially in patients who have an additional prothrombotic condition.

A meta-analysis of observational studies with cyclosporine-A showed an increased risk of nervous system involvement in patients using this agent (RR 12.66, 95% CI 4.75 to 33.76).[105–108] Thus the task force recommended to avoid cyclosporine-A in BS patients with nervous system involvement, even if the nervous system involvement is no longer active.

Table 2 Research agenda

Eye involvement	Head-to-head trial comparing interferon-alpha to TNFis
	Controlled trials with IL-1 and IL-6 blockers
	Controlled trials assessing the comparative efficacy and safety of different TNFis
	Determining how long TNFis or interferon-alpha should be continued after remission is obtained
	Defining remission regarding a decision to switch to a maintenance therapy or considering treatment discontinuation for eye involvement
	Controlled trials determining whether glucocorticoids reduce the efficacy of interferon-alpha
Vascular involvement	Controlled trials to assess the efficacy and safety of anticoagulation for preventing relapses of venous thrombosis, post-thrombotic syndrome and recurrent arterial occlusive events
	Observational studies to identify individual differences (saccular/diffuse fusiform/large vs small) that guide the choice of surgical intervention
	Determining the optimal dose and duration of immunosuppressives after surgical intervention for peripheral artery aneurysms
	Determining the optimal treatment of postoperative recurrent anastomotic aneurysms (extra-anastomosis bypass vs local aneurysm repair)
	Determining the optimal management of intracardiac thrombosis
Nervous system involvement	Controlled studies for determining the optimal management of initial, refractory and recurrent parenchymal nervous system involvement and cerebral venous thrombosis
	Determining the role of MRI and other laboratory tests in making treatment decisions and follow-up of patients with nervous system involvement
Gastrointestinal system involvement	Controlled studies for determining the optimal management of initial, refractory and recurrent gastrointestinal system involvement
	Determining the role, optimal dose and duration of corticosteroids in acute relapses and whether they increase the risk of perforation
	Determining whether a control colonoscopy is needed in patients with clinical remission and the optimal timing for control colonoscopy
Overall	Controlled trials to assess the benefit of concomitant immunosuppressive use with TNFis
	Controlled trials assessing the efficacy of treatment modalities for patient important outcomes such as fatigue

IL, interleukin; TNFis, tumour necrosis factor alpha inhibitors.

Recommendation 10: joint involvement

Colchicine should be the initial treatment in BS patients with acute arthritis. Acute monoarticular disease can be treated with intra-articular glucocorticoids. Azathioprine, interferon-alpha or tumour necrosis factor alpha inhibitors should be considered in recurrent and chronic cases. (Level of evidence: IB; strength of recommendation: A)

Colchicine was shown to be beneficial for preventing arthritis episodes in RCTs.[6–8] Some members of the task force favoured the use of continuous low-dose corticosteroids in patients whose arthritis is not controlled with colchicine whereas others preferred azathioprine, interferon-alpha or TNFis.[9 29 34 41 63 96 109–111] Intra-articular glucocorticoids may be helpful during an acute monoarticular attack. However, this may not be necessary in many cases since the arthritis episodes are usually self-limiting and disappear in 2–3 weeks.

DISCUSSION

EULAR Recommendations for the management of BS were updated by notably revising the 2008 Recommendations and adding five overarching principles and one recommendation regarding the surgical management of arterial aneurysms. We also changed the title of the project to 'EULAR Recommendations for the Management of Behçet's Syndrome'. Some experts felt a designation of 'syndrome' was more accurate for Behçet's, actually a constellation of symptoms. The presence of geographic differences in disease expression, symptom clusters some of which are more frequent in familial cases and differences in drug response between different types of organ involvement especially with different cytokine inhibitors support this contention.[112] There was a discussion among the authors, and the disagreeing colleagues suggested that these considerations are also true for several complex and multifactorial diseases such as systemic lupus erythematosus, systemic sclerosis or antineutrophil cytoplasmic autoantibodies-associated vasculitis, none of which is called a 'syndrome'. A separate online vote was held among the authors. In total, 10/23 members preferred 'syndrome', 7/23 preferred 'disease' and 6/23 voted 'neutral'. It was also commented that this issue needed to be further discussed among a larger group of experts.

Recommendations are especially important for conditions that require the collaboration of different specialties for management. The current recommendations aim to standardise the care of patients with BS; however, there will inevitably be differences in management across countries depending on the geographic variation of the disease, differences in healthcare systems, cultural differences leading to differences in the expectations and preferences of patients and reimbursement policies. Some examples of such differences are related to the dose and duration of glucocorticoid use, more frequent use of biologics in some centres, preference of interferon-alpha instead of TNFis, anticoagulation in patients with deep vein thrombosis and the type of surgical intervention used for arterial involvement. One of the strengths of the EULAR Recommendations for the management of BS is that the task force comprised experts from several countries and from all disciplines involved in the care of patients with BS allowing the incorporation of many perspectives regarding different aspects of the disease. Another strength was the involvement of two patients with BS who were actively involved in all stages including the selection of research questions for the systematic review. The task force tried to cover management issues in different settings and different types of patients. We anticipate that these recommendations would also be useful in parts of the world where BS is less prevalent and physicians rarely facing patients with this condition or a specific type of involvement of the condition. We also aimed to guide the

physicians on the appropriate timing for referral to a specialist centre.

Despite the continuous accrual of research data for BS management, the main limitation of these recommendations is that they were still relying on mostly observational and uncontrolled evidence and expert opinion for the treatment of vascular, gastrointestinal and nervous system involvement; as a consequence, strong recommendations were derived at by broadening the suggested management options. There were RCTs with several agents for mucocutaneous, joint and eye involvement, but very few were head-to-head trials. In potentially controversial cases any specific therapeutic option was only suggested conditionally. Moreover, the heterogeneity in study design, outcome measures and patient selection made it difficult to compare the efficacy of different agents. There is also a lack of studies evaluating the efficacy of different treatment strategies for BS such as a 'step-up' versus a 'step-down' approach. Another limitation of these recommendations is that we did not include economic considerations which can show important differences across countries.

Finally, after completing the recommendations we listed the research questions that need to be answered in the future for improving the management of patients with BS and proposed a research agenda (table 2). In particular, further research is warranted for controversial issues such as the role of anticoagulation in patients with thrombosis and the comparative efficacy of interferon-alpha and TNFis in patients with eye involvement.

In conclusion, we revised the EULAR Recommendations for the management of BS and developed 5 overarching principles and 10 recommendations related to the different types of organ and system involvement of BS. Implementation of these recommendations into clinical practice will be an important endeavour. The dissemination of the recommendations could be facilitated by translation into different languages and presentations in national meetings of different specialties involved in the management of patients with BS.

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Treating juvenile idiopathic arthritis to target: recommendations of an international task force

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ABSTRACT

Recent therapeutic advances in juvenile idiopathic arthritis (JIA) have made remission an achievable goal for most patients. Reaching this target leads to improved outcomes. The objective was to develop recommendations for treating JIA to target. A Steering Committee formulated a set of recommendations based on evidence derived from a systematic literature review. These were subsequently discussed, amended and voted on by an international Task Force of 30 paediatric rheumatologists in a consensus-based, Delphi-like procedure. Although the literature review did not reveal trials that compared a treat-to-target approach with another or no strategy, it provided indirect evidence regarding an optimised approach to therapy that facilitated development of recommendations. The group agreed on six overarching principles and eight recommendations. The main treatment target, which should be based on a shared decision with parents/patients, was defined as remission, with the alternative target of low disease activity. The frequency and timeline of follow-up evaluations to ensure achievement and maintenance of the target depend on JIA category and level of disease activity. Additional recommendations emphasise the importance of ensuring adequate growth and development and avoiding long-term systemic glucocorticoid administration to maintain the target. All items were agreed on by more than 80% of the members of the Task Force. A research agenda was formulated. The Task Force developed recommendations for treating JIA to target, being aware that the evidence is not strong and needs to be expanded by future research. These recommendations can inform various stakeholders about strategies to reach optimal outcomes for JIA.

INTRODUCTION

In the past two decades, there have been major changes in the management of juvenile idiopathic arthritis (JIA), which include earlier introduction of methotrexate (MTX), the more widespread use of intra-articular glucocorticoids, and most importantly the availability of biological disease-modifying antirheumatic drugs (DMARDs).[1] These advances have made remission, or at least minimal levels of disease activity, an achievable goal for most, if not all, children with JIA. Complete disease quiescence is regarded as the ideal therapeutic objective because its attainment is associated with less long-term articular and extra-articular damage and physical disability.[2]

This therapeutic progress has been paralleled by the development and validation of standardised assessment tools for clinical trials and clinical practice, such as the JIA American College of Rheumatology Paediatric (ACR) response criteria,[3] the definitions of clinical inactive disease (CID)[4 5] and low (or minimal) disease activity (LDA),[6] and the Juvenile Arthritis Disease Activity Score (JADAS).[7 8] Cut-offs in the JADAS that correspond to the states of CID, LDA, and moderate and high disease activity have been established.[9–11] The definitions of CID and LDA in JIA are presented in table 1.

Studies in adults with rheumatoid arthritis (RA) have shown that patient outcomes are improved if low levels of disease activity are aimed for by frequent adjustments of therapy according to quantitative indices, regardless of the therapeutic agent chosen.[12–14] These observations have suggested that the strategy of tight control, aiming for remission, is more important than the individual medications used to treat RA.[15] In recent years, the paradigm of explicitly defining a treatment target and applying tight control and necessary therapeutic adjustments to reach the target has been incorporated into 'treat-to-target' recommendations for RA,[16 17] axial and peripheral spondyloarthritis, including psoriatic arthritis,[18 19] systemic lupus erythematosus[20] and gout.[21] This principle has been also endorsed by the European and North American recommendations for the management of RA.[22–25]

It is currently agreed that disease remission should be an over-riding goal in the management of JIA.[26–30] However, the concept of targeted therapy has not yet been routinely implemented in paediatric rheumatology clinical care. For this reason, a Task Force was convened to discuss this issue and to reach a consensus on a set of recommendations aimed at defining a treat-to-target strategy for JIA, based on a systematic literature review (SLR).

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Table 1 Instruments and criteria used for the definition of clinical inactive disease and low (minimal) disease activity in JIA

	Items included							
	PhGA	Pa/ChGA	AJC	ESR/CRP	Systemic features	Uveitis	Morning stiffness	Requirements for classification as CID or LDA
Criteria for CID								
Wallace's preliminary criteria[4]	X		X	X	X	X*		Normal ESR/CRP and all other items at 0 or not present
ACR preliminary criteria[5]	X		X	X	X	X†	X	Normal ESR/CRP, morning stiffness ≤15 min, and all other items at 0 or not present
JADAS criteria[9]	X	X	X	X				JADAS≤1
cJADAS criteria[11]	X	X	X					cJADAS≤1
Criteria for LDA								
Magni-Manzoni criteria—Oligo[6]	X		X					PGA≤2.5, AJC=0
Magni-Manzoni criteria—Poly[6]	X	X	X					PGA≤3.4, Pa/PtGA≤2.1, AJC≤1‡
JADAS criteria[9]	X	X	X	X				Oligoarticular course: JADAS≤2.0 Polyarticular course: JADAS≤3.8
cJADAS criteria[11]	X	X	X					Oligoarticular course: cJADAS≤1.5 Polyarticular course: cJADAS≤2.5

*Inactive uveitis was not defined.
†Inactive uveitis as defined by the Standardization of Uveitis Nomenclature Working Group.
‡In systemic arthritis, absence of systemic features is required.
ACR, American College of Rheumatology; AJC, active joint count; CID, clinical inactive disease; cJADAS, clinical JADAS; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; JADAS, Juvenile Arthritis Disease Activity Score; JIA, juvenile idiopathic arthritis; LDA, low disease activity; Oligo, persistent oligoarthritis; Pa/ChGA, parent's/child's global assessment of child's overall well-being; PhGA, physician's global assessment of overall disease activity; Poly, extended oligoarthritis, polyarthritis and systemic arthritis.

METHODS

At the beginning of this endeavour, a paediatric rheumatologist (AR) and a methodologist (JSS) invited paediatric rheumatologists from Europe and North America, selected on the basis of publication records, expertise in treating JIA and previous participation in similar activities, to form a Steering Committee (JS, AR, GH, DJL, RML, NMW). The Steering Committee met on 6 March 2017 in Vienna to discuss unmet needs in the treatment of JIA and the potential for using treatment targets in the management of JIA.

During the meeting, it was unanimously agreed that defining therapeutic targets and an appropriate strategic treatment approach would be valuable, but there was concern that evidence for its validity could be lacking. It was therefore decided that—in line with respective recommendations[31 32]—a comprehensive SLR was a mandatory initial step to serve as the basis for achieving consensus on the definition of treatment targets. After extensive discussion among the members of the Steering Committee, it was agreed that the SLR should primarily explore the current evidence regarding the following themes: (1) Is a treat-to-target strategy preferable to a non-steered management? (2) What is the best outcome to be used as target and with which instrument? (3) Which time should elapse before escalating treatment in patients with active disease? (4) What is the potential role of biomarkers and imaging methods in decision-making? (5) Does disease duration and JIA heterogeneity influence the strategy and choice of the target? (6) Does a longer time spent in CID lead to a better long-term outcome? (7) What is the impact of treat-to-target in terms of cost, safety and treatment burden? (8) What is the effect of treat-to-target on comorbidities, including uveitis, psoriasis, depression, infections and adverse events? (9) Does improved patient/parent understanding on the disease improve the outcome? (10) What is the impact of treat-to-target on functional status, health-related quality of life, burden of disease and effect on patient's family life? After the definition of a series of search questions aimed to address all these issues, the SLR was performed by author AC. The databases used for the SLR and the methodology employed to screen articles and extract data are summarised in online supplementary file S1. The SLR was not limited to randomised controlled trials, but also included observational studies.

The SLR results were presented to the Steering Committee at a subsequent meeting, held in Munich on 24 August 2017. The literature search revealed that no strategic trials that addressed a target-oriented, steered therapy in comparison with conventional management had been published in JIA. Indirect evidence on optimal therapeutic approaches was, however, available to inform the next stages of the process. On this basis, the Steering Committee formulated a provisional set of recommendations in line with the European League Against Rheumatism (EULAR) standardised operating procedures (SOP).[31]

The day after the Steering Committee meeting in Munich, the SLR and the proposed recommendations of the Steering Committee were presented to a Task Force of 23 additional paediatric rheumatologists practising in various areas of the world (Africa, Asia, Australia, Europe, Latin America and North America). These invitations were a consequence of the individuals' contributions to the field and deliberations among members of the Steering Committee. After presentation, two breakout groups were formed, each chaired by a member of the Steering Committee: one group addressed the proposed overarching principles and proposed recommendations 7 and 8; the other group addressed the proposed recommendations 1–6 (see below). Further discussions took place during these breakout sessions and the suggested wording reformulated as deemed appropriate, with majority votes where controversy emerged.

The results obtained by the breakout groups were reported to the whole Task Force, which then discussed the proposals, amended them and arrived at final wordings that were subjected to an open voting process through a show of hands. Items that achieved at least a 75% majority vote were accepted as final recommendations in the same way as they had been worded. Items that did not attain such majority approval straight away were rediscussed, reformulated and revoted, until a 67% of majority vote or, subsequently, a >50% majority vote was achieved.

In line with the SOP, no representative of the company that provided the unrestricted grant was present to avoid any potential influence on the discussion or development of recommendations. This position has been a general principle in all treat-to-target procedures.

After the Munich consensus conference, all participants were asked to adjudicate via email their level of agreement with each overarching principle and recommendation on a 0–10 scale (0=no agreement at all; 10=full agreement).

The evaluation of the level of evidence (LoE) and strength of recommendation (SoR) was based on the Oxford Evidence-Based Medicine categorisation.[33]

RESULTS

The evidence base

The SLR revealed that no randomised controlled trial had evaluated a targeted therapeutic approach in comparison with conventional therapy in JIA. There was, therefore, no direct evidence that a treat-to-target strategy was preferable to non-steered management. However, a randomised trial of early aggressive therapy in polyarticular JIA had employed therapeutic targets and predefined time requirements as endpoints to escalate therapy, although this applied only to the MTX monotherapy comparator arm, which was allowed to escalate to combination therapy with MTX, a tumour necrosis factor inhibitor and prednisolone, or to escape in case of disease flare.[34] The primary outcome was the JIA ACR 70 improvement at 4 months and the achievement of CID at 6 and 12 months. Another randomised study of aggressive drug therapy in very early polyarticular JIA had set the JIA ACR 75 as the minimum level of improvement, below which the dose of MTX had to be doubled and the route of administration switched from oral to parenteral.[35] Taken together, these trials provide examples that strategies aimed at intensifying therapy enable a sizeable proportion of patients to achieve CID. In systemic JIA, the rapid attainment of CID with early administration of interleukin-1 inhibitors[36 37] has led to postulate that early intensive therapy may take advantage of a window of opportunity, in which disease pathophysiology can be altered to avoid the occurrence of chronic arthritis.[38] Indirect support regarding the time that should elapse before escalating treatment in a patient with persistently active disease was provided by several randomised clinical trials, non-controlled therapeutic studies and therapeutic recommendations formulated by expert panels.[36 39–53] Nevertheless, given the lack of studies evaluating target-steered versus non-steered treatment, the LoE for the development of recommendations was anticipated to be low and mainly based on expert consensus.

The members of the Task Force also recognised that in contrast to adult RA, the heterogeneity of JIA (eg, polyarticular, oligoarticular or systemic) needed to be addressed and accounted for in the development of recommendations.

The consensus

The individual statements that received a positive vote by the majority of the Task Force members comprise six overarching principles and eight recommendations. These items are shown in table 2, together with the percentage of positive votes obtained at the consensus conference, LoE, SoR and level of agreement, and are discussed in detail below.

Overarching principles

A. The treatment targets and the therapeutic strategy should be based on shared decisions between the parents/patient and the paediatric rheumatology healthcare team.

   It was recognised that involvement of the parents and, where appropriate, of the child in therapeutic decision-making is important and may lead to better adherence to treatment and potentially improve the outcome. The Task Force felt that the parents/patient must be informed about and agree with the selected target, the therapeutic options to reach the target and the reason for choosing the target, also in the light of the risks related to both the treatment and the disease. Parents/patient should be encouraged to participate fully in this discussion. The principle specifies that patient care should be delivered by a paediatric rheumatology healthcare team, recognising that the management of patients with JIA should be ideally conducted by a group of professionals with specific paediatric expertise. It was, however, argued that the healthcare team could vary in different countries. In this respect, because not all children will have access to paediatric rheumatology care, it was acknowledged that the formulated recommendations and principles should be also widely adopted by the adult rheumatology community when caring for children with JIA. This item achieved 90% of participants' votes; a few participants would have preferred the wording 'type of therapy' instead of 'therapeutic strategy'.

B. JIA is a heterogeneous group of diseases that requires distinct treatment approaches.

   It is well established that JIA is not a single disease, but constitutes a heterogeneous group of disorders, all manifesting joint inflammation, but with different clinical phenotype, disease course and outcomes, as well as with distinct genetic background and pathophysiology. This variability implies that the therapeutic choices, optimal targets and treatment strategy may be different across disease categories. Differentiation of therapeutic approaches based on the disease phenotype is in keeping with the ACR recommendations for the treatment of JIA.[45 50] It was also emphasised that the management of children with JIA requires the involvement of a multidisciplinary team of specialists, which should include, beside paediatric rheumatologists, ophthalmologists, physiotherapists, occupational therapists, orthopaedic surgeons, dermatologists, gastroenterologists, social workers, psychologists and others. This item was unanimously endorsed.

C. The goals of treating patients with JIA are to control signs and symptoms; to prevent structural damage; to avoid comorbid conditions and drug toxicities; and to optimise function, growth and development, quality of life, and social participation.

   This principle was modified several times by changing the order of the individual therapeutic goals. It was decided unanimously to give priority to control of inflammatory signs and symptoms, followed by prevention of structural damage to joints. There was, however, an intense discussion regarding the importance of considering comorbidities, such as uveitis, psoriasis, osteoporosis, depression and infections, as well as medication-related toxicity, in making clinical decisions. It was widely agreed that caution should apply particularly to systemic glucocorticoids, whose side effects may have a devastating impact in the paediatric age group. Optimisation of linear growth and pubertal development was added to the therapeutic goals to highlight this unique paediatric issue and the specificity of the recommendations. For patients of adolescent age, the therapeutic strategy should be tailored in accordance with the broader process of transition from paediatric to adult rheumatology

Table 2 Recommendations to treat juvenile idiopathic arthritis (JIA) to target

	Percentage of positive votes at consensus conference	Level of evidence	Strength of recommendation	Mean±SD level of agreement
Overarching principles				
A. The treatment targets and the therapeutic strategy should be based on shared decisions between the parents/patient and the paediatric rheumatology healthcare team.	90			9.8±0.5
B. JIA is a heterogeneous group of diseases that requires distinct treatment approaches.	100			10
C. The goals of treating patients with JIA are to control signs and symptoms; to prevent structural damage; to avoid comorbid conditions and drug toxicities; and to optimise function, growth and development, quality of life, and social participation.	100			10
D. Abrogation of inflammation is essential to achieve these goals.	100			9.8±0.5
E. Long-term use of systemic glucocorticoids to maintain the target should be avoided.	100			9.8±0.5
F. Treatment to target by regularly assessing disease activity and adapting therapy accordingly is important to achieve these goals.	100			10
Recommendations				
1. The primary target for treatment of patients with JIA is clinical remission, which means the absence of signs and symptoms of inflammatory disease activity, including extra-articular manifestations.	85	2b	C	9.7±0.5
2. Minimal (or low) disease activity may be an alternative target, particularly in patients with long-standing disease.	97	2c	B	9.7±0.6
3. Setting the target, selecting the tools and the therapeutic decisions should be based on individual patients' characteristics and agreed on with the parents/patient.	100	5	D	9.7±0.6
4. Disease activity should be assessed and documented regularly using a validated composite instrument.	100	2c	C	9.8±0.5
5. The frequency of assessments depends on the category of JIA, level of disease activity and presence of extra-articular manifestations. This may require weekly assessments, such as in systemic JIA with active systemic manifestations; monthly to every 3 months evaluations for patients who have high/moderate disease activity; and less frequent assessments, in states of persistent clinical remission.	93	5	C	9.6±0.7
6. In all patients, at least a 50% improvement in disease activity should be reached within 3 months and the target within 6 months. In patients with systemic JIA with active systemic manifestations, resolution of fever should be attained within 1 week.	93	2b	B	9.2±0.9
7. Treatment should be adjusted until the target is achieved.	100	2b	C	9.7±1.0
8. Once the treatment target has been achieved, it should be sustained. Ongoing monitoring should occur to ensure maintenance of the target.	100	2b	C	9.9±0.3

care. During this process, there should be direct communication between paediatric and adolescent rheumatologist teams. Self-management support was widely recognised as a key aim of treatment. The term social participation encompasses participation in social life and school attendance, as well as participation in extracurricular activities. The final wording of this principle was voted for by 100% of the participants.

D. Abrogation of inflammation is essential to achieve these goals.

This principle underscores the key role of the inflammatory process underlying JIA in causing signs and symptoms of the disease and disease-related damage. A number of other terms were suggested instead of abrogation (including suppression, abolition, inhibition, resolution, remission, disappearance), but in the end the majority of participants felt that abrogation was the most appropriate. The final wording of this principle was voted by 100% of the participants.

E. Long-term use of systemic glucocorticoids to maintain the target should be avoided.

High-dose glucocorticoid therapy may be necessary to control the acute or life-threatening manifestations of systemic disease, and a short course of low-to-moderate-dose glucocorticoids is often prescribed in children with polyarthritis to achieve a rapid control of inflammatory symptoms while awaiting the full therapeutic effect of a synthetic or biological DMARD.[54] Long-term administration of glucocorticoids to maintain the target is inappropriate because it indicates that the selected DMARD therapy is not sufficient to control the disease. This principle was added to the list of those originally formulated by the Steering Committee during the consensus meeting to highlight the serious side effects related to the prolonged administration of glucocorticoids in children, and was endorsed unanimously.

F. Treatment to target by regular assessment of disease activity and adapting therapy accordingly is important to achieve these goals.

Although the SLR had produced only indirect evidence for the utility of the treat-to-target strategy in JIA, the participants unanimously agreed that regular measurement of disease activity and the adjustment of therapy with persistently active disease were an overarching principle. This principle was endorsed by 100% of the participants.

Recommendations

1. The primary target for treatment of patients with JIA is clinical remission, which means the absence of signs and symptoms of inflammatory disease activity, including extra-articular manifestations.

To date, no clinical trial has compared outcomes of JIA for progression of structural changes or improvement in quality of life when clinical remission (CR) rather than another state is targeted. However, there is indirect evidence to suggest that progression of damage is more effectively inhibited in states of CID/CR.[2] Considering that the recent therapeutic advances have made CR a realistic goal for potentially all patients with JIA, CR was set as the primary therapeutic target. The definition of CR was intended to be quite strict, that is, as complete absence of all signs and symptoms of inflammatory disease activity, in line with the Wallace criteria for CID in JIA[4 5] or the JADAS criteria.[9 11] The emphasis on the stringency of the criteria led to the elimination of the adjective 'significant' before inflammatory disease activity, as included in the preliminary version of this recommendation. Abrogation of inflammation should extend to extra-articular manifestations, such as fever and rash of systemic JIA, uveitis, enthesitis or psoriasis. Recognising that patients often require continued therapy to achieve and maintain a state of CR, ongoing treatment was considered acceptable. That achievement of the treatment target should not depend on the chronic use of glucocorticoids was not addressed here, since this important aspect has already been included as an overarching principle. The participants discussed in depth whether the adjective 'clinical' should be removed to leave only the term remission, owing to the potential role of biomarkers or imaging techniques in defining disease remission more reliably than clinical assessment. However, despite emerging evidence for biomarkers reflecting subclinical inflammation[55 56] and several studies that indicate that there may still be residual active synovitis by MRI or sonographic evaluation in patients in CR,[57–59] at present there is no established role for imaging in defining remission. Nevertheless, the definition of remission may have to be reconsidered based on emerging data in a future update of the recommendations. This statement was approved by 85% of the participants, with contrary votes being mostly explained by the disagreement about adding the adjective 'clinical' to remission.

2. Minimal (or low) disease activity may be an alternative target, particularly in patients with long-standing disease.

   Although the Task Force did not intend to replace the target of CR by that of LDA, it was recognised that stringent remission, as defined in point 1, may be difficult to achieve in some patients, especially those with long-standing disease. These patients are generally those with the most aggressive systemic or polyarticular forms who have experienced persistently active disease, received multiple drug therapies or accumulated substantial joint damage or comorbidities. It was agreed on by 97% of the participants that in such patients, LDA[6 9] is an alternative and valid target. LDA is differentiated from the state of CR by the existence of residual signs and symptoms. However, it is assumed that physical function and quality of life would not be substantially worse than in CR and that progression of structural damage, while possibly not halted, would be minimal.[19] Importantly, by stating that LDA is an alternative goal to remission, the Task Force implied that any other, higher state, even moderate disease activity, would not be acceptable and its presence should prompt therapeutic adaptation.

3. Setting the target, selecting the tools to define the target and the therapeutic decisions should be based on individual patients' characteristics and agreed on with the parents/patient.

   This recommendation emphasises the need to individualise the therapeutic target, the method used for its assessment and the therapeutic decisions based on patients' characteristics, which include the disease category (eg, oligoarthritis, polyarthritis or systemic arthritis), severity of arthritis, distribution of affected joints (eg, involvement of cervical spine or hip), and presence of extra-articular manifestations (eg, systemic features, uveitis, psoriasis, impending macrophage activation syndrome, MAS) or comorbidities (eg, osteoporosis, growth failure, infection). Therapeutic decision-making may be guided by the recent treatment recommendations for JIA issued by the ACR, which were tailored according to JIA phenotype, level of disease activity and the presence of features of poor prognosis.[45 50] The rationale for choosing a particular treatment target and the means to achieve it should be properly communicated to the parents and the patient, and agreed on with them, in combination with appropriate information on the disease and the benefits and risks of different therapies (see also overarching principle A). This communication should include the explanation of the characteristics of the tools used to define the target and the indication that parent/patient-reported outcomes are an essential component of patient assessment and therapeutic decisions. In this regard, it may be difficult for parents to understand the need for this approach in patients with early disease or relatively mild symptoms. To this end, educational programmes supporting this initiative and involvement of parent and patient organisations were unanimously endorsed.

4. Disease activity should be assessed and documented regularly using a validated composite instrument.

   There was full consensus that the use of composite measures of disease activity is the best way to estimate disease activity and response to therapy. Furthermore, it was agreed on that this assessment should be performed at each clinic visit. Two categories of composite measures are currently available to evaluate disease activity in JIA: those based on multiple criteria and the composite disease activity scores. The first group comprises the criteria for CID[4 5] and LDA[6 11]; the second includes the JADAS[7] and its reduced version that lacks the acute phase reactant, the clinical JADAS (cJADAS).[8] The definitions based on multiple criteria are suited to establish the presence of a disease state (ie, CR or LDA) at a particular visit, but cannot be used to quantify disease activity. Conversely, the JADAS and cJADAS are aimed to quantify the absolute level of disease activity by providing a number on a continuous scale. The JADAS and cJADAS cut-offs that correspond to CR and LDA in JIA were determined.[9 10 60] Recently, the cJADAS was found to be potentially suitable to guide a treat-to-target strategy in JIA.[61] During the consensus meeting, there was debate about the relative measurement properties and suitability for the treat-to-target strategy of the various tools. It was decided not to recommend the use of a specific instrument. Hence, to leave the choice open for the clinician, the neutral term 'composite instrument' was endorsed unanimously. These instruments are shown in table 1.

5. The frequency of assessments depends on the category of JIA, level of disease activity and presence of extra-articular manifestations. This may require weekly assessments, such as in systemic JIA with active systemic manifestations; monthly to every 3 months evaluations for patients who have high/

moderate disease activity; and less frequent assessments, in states of persistent clinical remission.

Owing to the clinical heterogeneity of JIA, the intervals between evaluations vary in relation to the disease phenotype, level of disease activity and presence of extra-articular manifestations. In the active stage of systemic arthritis, which is a highly inflammatory condition that is accompanied by high fever and may lead to potentially serious complications, such as pleuritis, pericarditis and MAS, there is a need for frequent assessment of the disease status (even weekly) to adjust treatment accordingly. Patients with non-systemic categories and high-to-moderate disease activity require less frequent evaluations, which may occur monthly in patients with severe polyarthritis or enthesitis-related arthritis with active sacroiliitis or every 3 months in patients with oligoarthritis. Patients in sustained remission should be assessed at certain intervals to ensure maintenance of the outcome and, in those who are still receiving therapy, to verify the lack of adverse events and avoid overtreatment. Most experts felt a 3-month interval to be unnecessary for this population of patients and the majority considered every 6 months sufficient. In the process of shared decision-making, patients and parents should be advised to return to the paediatric rheumatologist earlier than at the predetermined time point if they are concerned about a change in disease status. This recommendation was approved by 93% of the participants.

6. In all patients, at least a 50% improvement in disease activity should be reached within 3 months and the target within 6 months. In patients with systemic JIA with active systemic manifestations, resolution of fever should be attained within 1 week.

   The analysis of the recent JIA clinical trials identified in the SLR showed that the maximum clinical benefit, expressed in terms of percentage of improvement, was usually not achieved before 3 months of treatment. In these clinical trials, which were performed for regulatory approval, the primary outcome measure has been a JIA ACR 30 response. However, because paediatric rheumatology practitioners are no longer satisfied with merely reaching a 30% change, a minimum improvement of 50% should be achieved. Thus, if an individual patient does not attain a minimum decrease of 50% in signs and symptoms of disease within 3 months from starting therapy, treatment should be adjusted. There was wide agreement that the attainment of CID or LDA before 6 months may not be realistic in patients with the most severe forms of JIA. A recent trial of early aggressive therapy in polyarticular JIA set the assessment of the primary outcome of CID at 6 months.[34] There was extensive discussion about whether the time intervals for drug therapy adjustment should vary in relation to the disease category or disease duration (early vs established). It was finally agreed that the above intervals should remain the same for all disease phenotypes, with the sole exception of patients with systemic JIA and active systemic manifestations, in whom resolution of fever should be obtained within 1 week. This tighter time frame was justified by the risk of patients with systemic arthritis to develop potentially serious complications, such as pleuritis, pericarditis and MAS, and by the recent demonstrations of dramatic improvement of systemic features within 1 week in many patients treated with appropriate therapy.[36 47] Approval of this item was provided by 93% of the participants.

7. Treatment should be adjusted until the target is achieved.

   Indirect evidence suggests that early clinical response or the achievement of CID is associated with improved long-term outcome.[9 10 62] It is, thus, likely that pursuing the best possible target through treatment adjustment improves prognosis. Some participants argued that the word 'adjusted' sounds ambiguous and that 'modified' or 'escalated' could be more appropriate. It was, however, noted that the term adjustment covers both the modification and escalation of therapy. Several Task Force members emphasised the importance of non-pharmacological interventions, particularly physiotherapy and occupational therapy, optimisation of bone health, management of pain and psychological support. A concern was also raised that some targets may not be achievable for patients living in low-income countries, where costly biological DMARDs may not be available or affordable; however, others noted that the adaptation of treatment would have to be done with those options that are available. This recommendation was supported by 100% of the participants.

8. Once the treatment target has been achieved, it should be sustained; ongoing monitoring should occur to ensure maintenance of the target.

   Once the agreed therapeutic target has been achieved, it should be maintained continuously. Both evidence and rationale exist in chronic arthritis that sustained/persistent remission leads to an optimal quality of life, enhances physical function and stops progression of structural joint damage.[2 63–65] Conversely, an increase in disease activity during follow-up may lead to reduced quality of life and progression of the destructive process.[63–65] Maintenance of the treatment target does not necessarily imply maintenance of treatment. However, the decision regarding whether therapy should be stopped or gradually tapered should be based on available evidence. A number of studies on tapering of therapy, especially dose reduction, spacing of administration intervals and even withdrawal of drugs have been performed in children with JIA who had achieved a state of CR.[66] The relapse rate after termination of both MTX and biological DMARDs is substantial. Unfortunately, there is currently a lack of evidence-based data from clinical trials and clinical care and of guidelines to aid in the withdrawal of medications after disease remission in JIA. A recent survey among North American paediatric rheumatologists conducted by the Childhood Arthritis and Rheumatology Research Alliance has shown a large variability in the preferences of medication withdrawal for CID.[67] No reliable clinical, biomarker or imaging indicators are currently available to identify patients at higher risk of experiencing disease flare after treatment discontinuation. Adherence to therapy has to be carefully monitored because non-adherent patients may be exposed to a high risk of flares. Safety aspects and drug cost should also be taken into account in designing the strategies for treatment tapering or discontinuation. This item was unanimously approved.

Adjudication of the level of agreement after the consensus meeting

The level of agreement on overarching principles and recommendations adjudicated by the Task Force members after the Munich consensus meeting was very high, as all items achieved

an average score greater than 9 and only one item had an average score lower than 9.6 (table 2).

DISCUSSION

In recent years, several sets of treatment recommendations have been developed for JIA.[45 50 68–70] However, none of them has addressed specific treatment targets or described the strategy to reach the therapeutic goals. These objectives have been achieved in the present recommendations, which are primarily intended to provide expert guidance on general treatment approaches. A notable difference with the previous treatment recommendations is the absence of suggestions or advice regarding specific medications in any of the overarching principles or individual recommendations, apart from the avoidance of long-term glucocorticoid use. Consequently, these recommendations should be applicable and ideally adhered to in all regions and countries, irrespective of medication availability. Importantly, the recommendations are aimed at improving patient care in standard clinical practice and do not tackle the issue of registration trial design and conduct. However, the recommendations should be tested in respective strategic clinical trials.

The process was initiated by a Steering Committee, which followed the EULAR SOP for the development of recommendations.[31] It was accomplished after discussions among the members of the Steering Committee and a Task Force of 23 additional international paediatric rheumatologists. In light of the wide international representation within the Task Force, the very high level of agreement for all statements supports the conclusion that the result of the efforts gained broad international consent.

The recommendations are aimed at paediatric rheumatology practitioners and other health professionals involved in the care of patients with JIA; official bodies, such as health authorities or payers, who may wish to use this document as a reference for the assessment of success in treating patients with JIA; and regulatory agencies, owing to the increasing interest of pharmaceutical companies for strategic trials. Parents and patients are another important audience that should be informed on these statements and their potential role in preventing or minimising damage and disability. In this respect, we recognise that the lack of participation of parent or patient representatives is a limitation of the project. However, the dissemination of the recommendations to parent/patient organisations and the request of their feedback are planned in the near future. An update of these recommendations will likely be required once parts of the research agenda have been addressed. With the next iteration, parents/patients and healthcare partners will be included.

Ideally, treatment recommendations should be based on available evidence. As mentioned, strategic therapeutic trials, in which therapy was consistently adapted to reach a prespecified treatment target and compared with a non-steered approach, are currently not available in JIA. While the SLR has provided indirect evidence from clinical trials which targeted specific endpoints,[34 35] and thus supplied some information to the Task Force, the individual recommendations can only be regarded as consensus-based expert opinion and, therefore, call for further research in the field.

In spite of the lack of evidence, the Task Force felt that the definition of treatment targets and strategy for JIA was necessary and timely for three main reasons: (1) the remarkable therapeutic advances of the past two decades have greatly improved the probability of achieving excellent outcomes and have, thus, mandated the establishment of more stringent treatment targets; (2) JIA had not been previously addressed by 'treat-to-target' initiatives, such as those in RA and spondyloarthritis, for which treat-to-target recommendations were defined many years ago, have already been updated[16–19] and adopted in management recommendations[24 71]; and (3) the proposals originating from the consensus meeting and the formulation of a research agenda will likely foster and accelerate investigations towards providing the necessary evidence.[17 19]

The present recommendations were aimed at defining treatment targets that would lead to the optimal outcome for the individual patient, but do not account for potential financial constraints or access to particular therapies. The Task Force raised the concern that different accessibility to certain medications may lead to disparities in the proportion of patients who are able to attain the desired target across countries or regions. However, studies in adult patients with RA have shown that a good outcome can be obtained in a large proportion of patients with easily accessible and affordable therapies, provided that a strategic treatment approach is pursued.[12 72]

Looking at specific items, it is worth highlighting some important differences with the recommendations formulated for adult-onset diseases. First, the heterogeneity of JIA was accounted for by stating that therapeutic approaches, frequency of assessments and timeline for evaluation of improvement may be different across categories. It was, in particular, recognised that children with systemic arthritis and active systemic manifestations, particularly fever, require closer assessments and should have resolution of fever within 1 week. Another key point is that long-term administration of glucocorticoids to maintain the target is inappropriate, due to the devastating side effects related to prolonged administration of glucocorticoids in children. A further item specific to paediatric patients is the inclusion of the optimisation of linear growth and pubertal development in the therapeutic goals. Finally, the Task Force emphasised that care of adolescent patients be tailored in accordance with the broader process of transition from paediatric to adult rheumatology care. While this aspect was not specifically mentioned in the bullet point, it is mentioned in the accompanying text, which is part and parcel of the recommendations.

All Task Force members agreed unanimously that abrogation of inflammation (overarching principle D) is the most important goal in the treatment of JIA. Although there was also full agreement that this objective should be pursued by aiming at the state of disease remission, some experts were not in favour of adding the adjective 'clinical' to remission, in the light of the potential superiority over clinical assessment of more stringent targets, such as remission by biomarkers or ultrasonography. However, the majority considered that at this time remission should be defined on clinical grounds through the use of one of the existing criteria, as the evidence for other methods is scant.

Participants discussed that remission may not be achievable in all patients and, hence, formulated an alternative treatment target, especially for patients with long-standing disease, namely LDA (recommendation 2). Importantly, this conclusion implies that disease activity states other than CR or LDA should not be acceptable, unless justified for other reasons, such as comorbidity, parent/patient choice or treatment-related toxicity (recommendation 3).

There is ongoing discussion of the relative value of composite instruments for assessment of disease activity. There was concern that a recent study had shown that current criteria to capture CID and LDA do not always identify the same groups of patients.[73] A leading reason for the discordance was the inclusion of parent/patient global assessment in the JADAS[7 8] but not in the criteria

Box 1 Objectives to be included in the future research agenda

- Implementation of strategic trials aimed to show the superiority of a steered treatment approach based on treat-to-target over a non-steered approach.
- Acceptance and applicability of treat-to-target strategies in clinical practice.
- Acceptance and applicability of treat-to-target strategies in low-income countries.
- Evaluation of whether treat-to-target strategies should have different characteristics in adolescent patients.
- Impact of parent/patient evaluation, particularly in the presence of particular pain sensitivity, in the assessment of targets.
- Comparison of remission defined clinically versus remission based on imaging methods or biomarkers in relation to structural and functional outcomes.
- Analysis of the best modalities of tapering and/or withdrawing treatments in patients with juvenile idiopathic arthritis reaching inactive disease or complete remission.
- Revision of treat-to-target recommendations in relation to the revision of the classification criteria for juvenile idiopathic arthritis, currently in progress.

for CID in JIA.[4 5] However, it has been argued that integration of the parents' and children's perspective into clinical assessment may help with the physician's decisions and improve adherence to treatment.[74] It was finally decided not to recommend the use of a specific instrument, leaving the choice to the clinician.

This process highlighted the foremost importance of future research to underpin the next iteration of the recommendations. Some objectives that should be prioritised in the research agenda are listed in box 1.

In conclusion, the recommendations to treat JIA to target are presented. The Task Force is convinced that transferring them into clinical practice will significantly improve the outcomes in patients with JIA (LoE 5, SoR D).

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Common language description of the term rheumatic and musculoskeletal diseases (RMDs) for use in communication with the lay public, healthcare providers and other stakeholders endorsed by the European League Against Rheumatism (EULAR) and the American College of Rheumatology (ACR)

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ABSTRACT

A European League Against Rheumatism-American College of Rheumatology working group consisting of practising and academic rheumatologists, a rheumatology researcher and a patient representative created a succinct general statement describing rheumatic and musculoskeletal diseases (RMDs) in adults and children in language that can be used in conversations with the lay public, media, healthcare providers and other stakeholders. Based on the literature review, several elements were deemed important for inclusion in the description of RMDs. First, RMDs encompass many different diseases that can affect individuals at any age, including children. Second, there are various pathophysiological pathways underlying different RMDs. Third, the impact of RMDs on individuals and society should be emphasised. The working group agreed that the language should be comprehensible to the lay public. Thus, the following description of RMDs has been developed: 'Rheumatic and musculoskeletal diseases (RMDs) are a diverse group of diseases that commonly affect the joints, but can affect any organ of the body. There are more than 200 different RMDs, affecting both children and adults. They are usually caused by problems of the immune system, inflammation, infections or gradual deterioration of joints, muscles and bones. Many of these diseases are long term and worsen over time. They are typically painful and limit function. In severe cases, RMDs can result in significant disability, having a major impact on both quality of life and life expectancy.' This description can be used by rheumatology groups, researchers and those who work in advocacy and education related to RMDs.

The field of rheumatology encompasses a wide range of medical conditions that affect many organ systems. These conditions reflect diverse pathogenic mechanisms and result in functional limitations, diminished quality of life and increased patient mortality. In addition, although rheumatic conditions in total are among the most common of all medical problems, many of the individual diseases are uncommon or even rare. This situation results in an ever-present dilemma for the field. Most of the public and policymakers around the world do not know about many of the rheumatic and musculoskeletal diseases (RMDs) and even if they have heard of them, there is broad lack of awareness about the complexity and enormous importance of this area of medicine.

To further public awareness and support policies directed towards lessening the impact of these diseases on patients and society, a working group from the European League Against Rheumatism (EULAR) and the American College of Rheumatology (ACR), consisting of practising and academic rheumatologists, a patient representative and a rheumatology health professional, has developed a formal description of these conditions. The goal of this effort was to create a succinct general statement describing RMDs in adults and children in language that can be used in conversations with the general population with and without RMDs; media; healthcare providers; policymakers at local, national and international levels; health insurance companies; charities; employers and other stakeholders.

Several elements were deemed important for inclusion in the description of RMDs by the group. First, it should be emphasised that RMDs encompass many different diseases that can affect persons at any age, including children. Second, it should be clear that there are various pathophysiological causes of RMDs. Third, the impact of RMDs on individuals and society should be emphasised. Finally, the language should be easily understood by the lay public. Here we will discuss various aspects that provide relevant background information, which can be used during the discussion about the importance of RMDs with the relevant stakeholders.

METHODOLOGY

The participants of the working group were selected based on their position in the respective organisations. For EULAR this was the president, the chair of the EULAR standing committee of clinical affairs, the EULAR liaison to the ACR and a patient representative. For the ACR, the president and president-elect of the ACR, the president of the Association of Rheumatology Health Professionals and the president of the Rheumatology Research

Foundation were included. The group met once in person, had one teleconference TC) and all other exchanges were conducted by email. There was a scoping review of the literature with emphasis on grey literature such as reports from the European Union (EU).

RMDS ENCOMPASS MANY DIFFERENT DISEASES THAT CAN AFFECT PERSONS AT ANY AGE, INCLUDING CHILDREN

Data suggest that there are over 200 RMDs; some conditions are very common, while others are rare. Lists of RMDs have been compiled in a number of publications and survey results. For example, the ACR website features a detailed list as does the EULAR website.[1 2] The latter is considered the official list as applied by the European Union of Medical Specialists (EUMS) (see online supplementary appendix 1). In addition, the Arthritis Foundation, a patient organisation in the USA, and the ACR maintain patient-oriented lists of RMDs.[3 4]

Well-known and prevalent examples of RMDs are rheumatoid arthritis (RA), osteoarthritis (OA) and gout. According to a conservative estimate of the United Nations, symptomatic OA, or degenerative joint disease, affects 15% of people worldwide, and it is estimated that by 2050, over 130 million people will suffer from OA worldwide and 40 million will be severely disabled.[5]

RA is the most common autoimmune inflammatory form of arthritis and affects approximately 1 in 100 persons worldwide, with women affected twice as commonly as men.[6]

Gout is the most common cause of inflammatory arthritis in men and has a prevalence in the USA and Europe of about 4%.[7 8]

Many other RMDs are less common, but cause significant morbidity and mortality. For example, systemic lupus erythematosus, which affects women approximately nine times more frequently than men, is a systemic autoimmune disease that frequently causes arthritis and dysfunction of connective tissues among many other systemic manifestations.[9 10] The overall lifetime risk for developing an inflammatory RMD including RA, gout, lupus and others for an adult in the USA has been calculated as 1 in 12 for women and 1 in 20 for men.[11] Many RMDs are uncommon or rare, which contributes to the lack of familiarity and/or experience with many RMDs on the part of general practitioners. RMDs frequently affect joints resulting in arthritis, and also frequently involve other internal organs and the skin. Although arthritis is commonly considered as a disease of ageing, many RMDs—including many that cause disabling arthritis—occur in children. Lack of awareness of these conditions in both children and adults can lead to excessive and unnecessary damage and disability.[12–15]

THERE ARE VARIOUS PATHOPHYSIOLOGICAL PATHWAYS OF RMDS

Review of these many diseases and conditions indicates that they develop through a diverse range of pathogenic pathways, most of which are not completely understood. Many result from dysregulation and activation of immune mechanisms that lead to inflammation and tissue damage. Some of these are classified as autoimmune diseases. Other RMDs result from acute or chronic damage to musculoskeletal structures including bone, cartilage, muscle, tendon, ligament and blood vessels. Other primary metabolic, endocrine, neurologic and infectious diseases can lead to secondary dysfunction and damage of musculoskeletal tissue. For example, prolonged hyperglycaemia in diabetes can result in changes in the structure of tendons and other soft tissues resulting in impaired mobility and joint function. The metabolic changes of the iron-storage disease haemochromatosis can result in degenerative arthritis in the hands. In addition, an increasing number of genetic variations and mutations are associated with the development of RMDs.

RMDS RESULT IN A MAJOR BURDEN FOR BOTH THE INDIVIDUAL AND THE SOCIETY

Many of the diseases are chronic and as they can start as early as during childhood (eg, juvenile idiopathic arthritis) or young adulthood (eg, spondyloarthritis), patients suffer with their disease for decades. Moreover, most RMDs worsen over time with increasing impact on both the physical and psychological conditions of the patient. Some patients die prematurely as a result of the condition or comorbidities, although if appropriately treated, mortality is relatively low in most of these conditions.

Large population studies emphasise that RMDs are highly prevalent worldwide. The eumusc.net project is a collaboration of 22 organisations in 17 member states of the EU investigating musculoskeletal health in Europe.[16] Their report summarises the epidemiology of major RMDs, impact on the individual and society, and management and health services utilisation. They concluded that musculoskeletal problems are the most common cause of severe long-term pain and disability in the EU and lead to significant healthcare and social support costs. Moreover, RMDs are a major cause of loss of work productivity resulting in significant economic costs and may have serious impact on quality of life, affecting those with the conditions and their relatives.

Musculoskeletal pain is prevalent in the EU with just over one-fifth (22%) of the population reporting current or long-term muscle, bone and joint problems in a survey performed by the EU in 2007.[17] Exactly a quarter of all EU respondents say that at some point in their life they have experienced chronic (lasting for at least 3 months) restrictive pain affecting muscles, joints, neck or back which affected their ability to carry out activities of daily living. Such pain is reported more by women than by men (28% vs 22%). Musculoskeletal pain is the second most common complaint underlying long-term treatment contributing to major healthcare costs.[17] Twenty-four per cent of the respondents to the survey received long-term treatment for RMDs (second after hypertension with 36% of respondents). It is likely that the overall burden of arthritis is underestimated in virtually every population. A recent study from the USA using national data from a health interview survey and doctor-diagnosed arthritis and symptoms revealed that arthritis affected 91.2 million (36.8%) of the adult population, including about 29% of men and 55% of women between the ages of 18 and 65.[18]

RMDs were the most frequent reason among non-infectious diseases to consult the primary care physician in the UK in 2003, and this was increasing with age and higher in female patients in all age categories.[16] In Germany, 11.2% of the total cost of illness in 2008 was spent on musculoskeletal and connective tissue diseases.[16] These numbers appear to be rapidly increasing. For example, a recent report provides data on doctor-diagnosed OA in the USA between 2013 and 2015.[19] A total of 54.4 million Americans (22.7%) had doctor-diagnosed OA and this percentage was even higher among adults with heart disease (49.3%), diabetes (47.1%) and obesity (30.6%). In 2012, 54% of people in the USA over age 18 reported suffering a musculoskeletal problem and the prevalence approached 75% for those aged 65 and older (Burden of Musculoskeletal Diseases in the United States).[20] Several factors have an impact on the prevalence of RMDs, including sex, age, body mass index and physical

activity. As the population is ageing this has a major impact on the prevalence of RMDs. For example, the EU will have 58 million more people aged 65 and over in 2050 in comparison to 2004.[16] Similarly, obesity is increasing, which again will lead to a higher prevalence of RMDs.[16]

RMDs have led to significant reduction in function and quality of life as well as increased disability. Among those with OA, 43.5% of the adults experienced limitations in activity attributable to OA, and there was a significant increase of 20% in the proportion of adults reporting these limitations since 2002. The disability-adjusted life year (DALY) is a measure to compare impact of various diseases on disability and can be interpreted as the loss of 1 year of healthy life. The WHO listed OA as the eighth leading cause of impact measured by DALYs in their report on global burden of disease.[21] Another way of assessing the impact on disability is the years lived with disability (YLD). The WHO listed musculoskeletal diseases as the third cause for disability among non-communicable diseases assessed by YLDs. And among musculoskeletal diseases, OA was the most common disease followed by RA, 'other musculoskeletal diseases' and gout.[22] As common and impactful as musculoskeletal diseases are, such surveys do not always include the entire range of RMDs. This further underscores the high prevalence and cost of these conditions and emphasises the need for a unifying definition of RMD. An example of defining the global burden of 'other musculoskeletal disorders' was presented in a large study in 2010.[23 24]

The International Quality of Life Assessment project examined the effect of multiple chronic conditions on populations in Denmark, France, Germany, Italy, Japan, the Netherlands, Norway and the USA using the 36-item Short Form Health Survey (SF-36). This showed that arthritis, chronic lung disease and congestive heart failure were the conditions with the highest impact on SF-36 physical component summary score. RA had a significant negative effect on the SF-36 mental component summary score. Arthritis had the highest impact on health-related quality of life (HRQoL) in the general population.[25] A large survey study in the Netherlands which compared HRQoL (using SF-36 or SF-24) across a wide range of long-term conditions showed that people with musculoskeletal conditions (included are back impairments, RA, OA/other joint complaints) reported the lowest levels of physical functioning, role functioning and pain.[26]

A Spanish study showed that rheumatic diseases are among the diseases that produce the largest impairment in HRQoL and daily functioning.[27] When the definition of the burden of disease includes a measure of function and of HRQoL that is weighted by the prevalence of disease, RMDs, as a group, may be considered on a par with other major diseases such as neurologic, cardiac or pulmonary diseases.[16]

For many of the RMDs, it is important to recognise the disease early to have the best option to start treatment early and prevent or limit long-term consequences. To achieve this, EULAR has started the awareness campaign 'Don't delay, connect today'. The best example is RA. Early diagnosis, improved treatment options and applying treatment to target principles have improved the percentage of patients in (sustained) remission, and improved the quality of life and work productivity.[28 29] Even overall excess mortality in patients with RA in comparison to the general population, which was apparent in previous decades, is lower and even no longer present when RA is diagnosed and treated early and intensively.[30 31]

Cause-specific morbidities, such as cardiovascular disease, are greater in many of the RMDs, and may also be declining with improved disease management.[32]

WORKFORCE TAKING CARE OF PATIENTS WITH RMDS

A range of practitioners manage musculoskeletal problems. These include medical specialists, general practitioners, community pharmacists, physical therapists (physiotherapists, chiropractors), occupational therapists and behavioural therapists (counsellors, psychologists and social workers). Rheumatologists, including paediatric rheumatologists, are the specialists with the most broad and specific training for diagnosing and treating RMDs. Rheumatology specialty training standardised by EUMS across the EU and the Accreditation Council for Graduate Medical Education in the USA requires proficiency in general internal medicine followed by detailed training in the pathogenesis, diagnosis and management of the entire range of RMDs.[1 2]

The number of practising rheumatologists varies widely. The average number of rheumatologists in EU is 1.7 per 100.000 inhabitants, ranging from 0.5 in Ireland to 4.2 in France.[16] Similarly, in the USA, the number of rheumatologists ranges from greater than 2 per 100 000 in heavily populated regions to less than 1.5 per 1 000 000 in more rural regions.[30] However, due to a variety of factors affecting physician workforce, including the increasing prevalence of RMDs, these numbers are changing rapidly. For example, recent workforce projections in the USA estimate that by 2025 the average number of rheumatologists in the large majority of the country will be 0.5–1.0 per 100 000 inhabitants.[33] There is also a severe shortage of paediatric rheumatologists as substantiated by a survey in the USA.[34] Other specialists caring for patients with RMDs are orthopaedic surgeons, internists and rehabilitation specialists. There is also a wide variation in the workforce of allied health professionals. The number of physiotherapists varies enormously across EU countries from 34 per 100 000 inhabitants in Ireland to 234 per 100 000 in Finland.[16] Similarly, the variation in occupational therapists ranges from 2 in Italy to 100 per 100 000 inhabitants in Sweden and Denmark.[16] In contrast, in the USA, there were about 114 600 occupational therapy jobs listed in 2014 for a population of close to 318 500 000 (360 per 100.000).[35]

Based on the above data and considerations, the following common language description of RMDs was endorsed by both EULAR and ACR.

RMDs are a diverse group of diseases that commonly affect the joints, but can affect any organ of the body. There are more than 200 different RMDs, affecting both children and adults. They are usually caused by problems of the immune system, inflammation, infections or gradual deterioration of joints, muscles and bones. Many of these diseases are long term and worsen over time. They are typically painful and limit function. In severe cases, RMDs can result in significant disability, having a major impact on both quality of life and life expectancy.

SUMMARY

The description of RMDs is a succinct statement in common language detailing many of the important aspects of these conditions. Given the prevalence and impact of RMDs as well as the availability of effective management options, it is important to be able to communicate clearly what RMDs are with the public and stakeholders. It is especially imperative to communicate the impact and importance of RMDs to healthcare policymakers.

The many unanswered questions about the causes of RMDs, the importance of improved diagnosis for RMDs and clear need for effective and safe treatments that are unavailable for many

of these diseases emphasise the importance of increased research on RMDs. At the same time, the fact that many recent advances have been made in developing new therapies for RMDs so that many people are now treated very effectively—with prevention of disability and comorbidity—emphasises how critical it is that patients have ready access to diagnosis and care for these conditions. We hope that the description of RMDs provided in this report will enable improved communication about and advocacy for these conditions and the patients who suffer from them.

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Designing effective graphs to get your message across

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ABSTRACT

Research is of little use if its results are not effectively communicated. Data visualised in graphs (and tables) are key components in any scientific report, but their design leaves much to be desired. This viewpoint focuses on graph design, following two general principles: clear vision and clear understanding. Clear vision is achieved by maximising the signal to noise ratio. In a graph, the signal is the data in the form of symbols, lines or other graphic elements, and the noise is the support structure necessary to interpret the data. Clear understanding is achieved when the story in the data is told effectively, through organisation of the data and use of text. These principles are illustrated by original and improved graphs from recent publications, completed by tutorial material online (appendices, web pages and film clips). The popular matrix form (multiple graphs in one frame) is discussed as a special case. Differences between publication (including the proofing stage) and presentation are outlined. Suggestions are made for better peer review and processing of graphs in the publication stage.

Effective communication of scientific data is arguably one of the most important skills of a scientist. If the intended audience does not get the message in the data (and acts on it), the research effort is wasted. The audience must first be drawn in by a concise and attractive title, a carefully written abstract and key messages. People will stay interested if choices between body text, tables and graphs have been optimised for audience and setting. Data visualisation in tables and graphs can convey complex relationships in a way unmatched by simple text, but wrong choices can lead to misinterpretation and wrong decisions.[1]

Despite the above, communication has traditionally received short thrift in scientific education, and it is not really stimulated by journals, scientific societies and so on responsible for dissemination of research. PhD programmes offer writing tutorage mostly focused on body text; journal instructions rarely go beyond specifying the maximum number of tables and figures.[2] At conferences, guidance is mostly limited to the time allowed for oral presentations and the size of posters. On submission, resolution of figures is routinely downgraded in the package accessible to reviewers. It is therefore not surprising that the quality of data visualisation is at best mediocre. A review of articles submitted to BMJ concluded that less than half of the tables and figures met their data-presentation potential.[3] Also, external peer reviewers and editors rarely commented on tables or figures.

For *Annals of Rheumatic Diseases* (*ARD*) and other journals, I have been working over the years to improve quality through better guidance, including brief tutorial videos.[4 5] Recently, I published an article on effective table design in *Heart*, like *ARD* a journal in the 'BMJ family'[6]; this is a companion article that focuses on graphs. Both articles expand on the guidelines for authors.[4] Content is mostly based on sound design principles and tradition, informed by the science of human visual perception. There is little empirical evidence to support specific recommendations, so there is room for experimentation and innovation to see what works best. My own experience in data visualisation has been greatly inspired by three sources: Tufte, Cleveland and Few.[7–9]

METHODS

For this Viewpoint, I searched recent issues of *ARD* to find standard graph examples of at least adequate quality as published.[10–12] I reconstructed the datasets by copying each graph image to the program GraphClick for Mac (Arizona software, V.3.0.2) and extracting the data points by hand. I imported the resulting dataset into Excel for Mac 2016 (Microsoft, V.16.10) to achieve the proper ordering and coding, and subsequently into Prism for Mac (GraphPad software, V.7.0d) that helped me design all graphs, including the journal Impact Factor graphs designed specifically for this Viewpoint. For the distribution graphs, I used the dataset and an existing graph from a recent meta-analysis.[13] Available as online appendices are the annotated Prism files (online supplementary appendices 1 and 2) (can only be opened by the Prism program) and preference settings I use (online supplementary appendix 3), a spreadsheet file to help calculate the 'null zone' (online supplementary appendix 4, explained in the caption to figure 4), a checklist with common issues in table and graph design (online supplementary appendix 5), and additional examples (online supplementary appendices 6 and 7).

GENERAL DESIGN PRINCIPLES

At the start of the design process, key questions to ask are four 'W's: Who is the audience? What are my messages (to this audience)? Which messages need visualisation in a table or graph? What would be the most effective form for each? Graphs are best to convey large amounts of information and to allow pattern recognition, whereas tables are best to display a limited amount of information with precision and simple relationships between variables.

Two design principles can be distinguished: clear vision and clear understanding. Clear vision is about maximising the signal to noise ratio in the visualisation. The signal is the data 'ink', that is, all pixels in a graph that depict or represent data. The

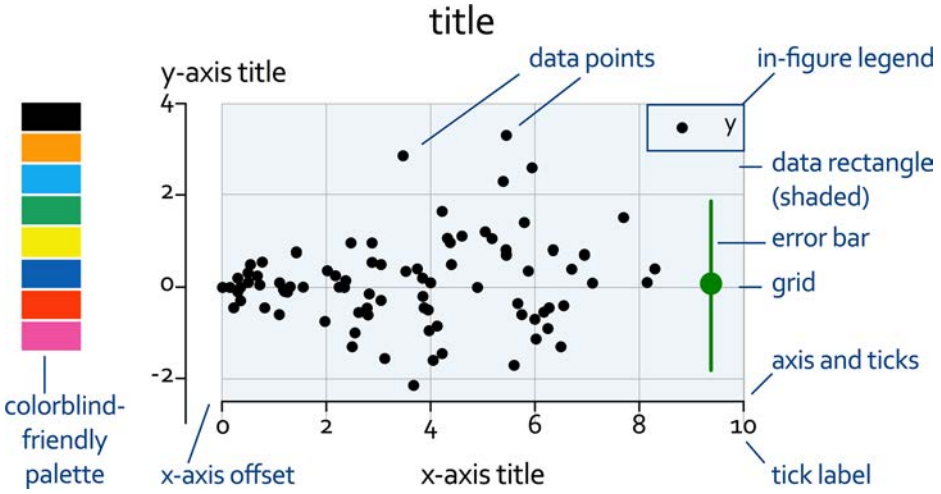

Figure 1 Graph nomenclature. This example shows most of the common elements that can be found on a graph. Apart from the data points and the error bar, all other elements are 'non-data ink' that may or may not be necessary. On the left, a colourblind-friendly colour palette is added. This current text is called the 'caption'.

noise ('non-data ink') is all of the supporting elements: axes, titles, labels, legends, and so on (figure 1).[14] Clear understanding is about telling the story in the data. This involves organising the data and optimising the use of text.

WHICH SOFTWARE?

A practical issue is the choice of software. Standard spreadsheet, presentation and statistical packages readily produce graphs of varying complexity, but optimisation for presentation or publication is a great challenge. Fortunately, there are now many dedicated graphing packages available that can fill the need. I have most experience with Prism and Deltagraph (Red Rock Software), but there are many more. For me, a key characteristic to choose between them is the availability to edit each separate graph component. The added learning curve is more than offset by the frustration avoided.

WHAT GRAPH?

Main purposes of scientific graphs are to describe: labelled measurements, time series, relations between two or more variables, distributions and statistical variation. Almost all of these can be captured by dot and bar plots (figure 2), step and line plots (figure 3 and figure 4), scattergrams (figure 5), histograms (not shown), box plots and combinations of these in matrix

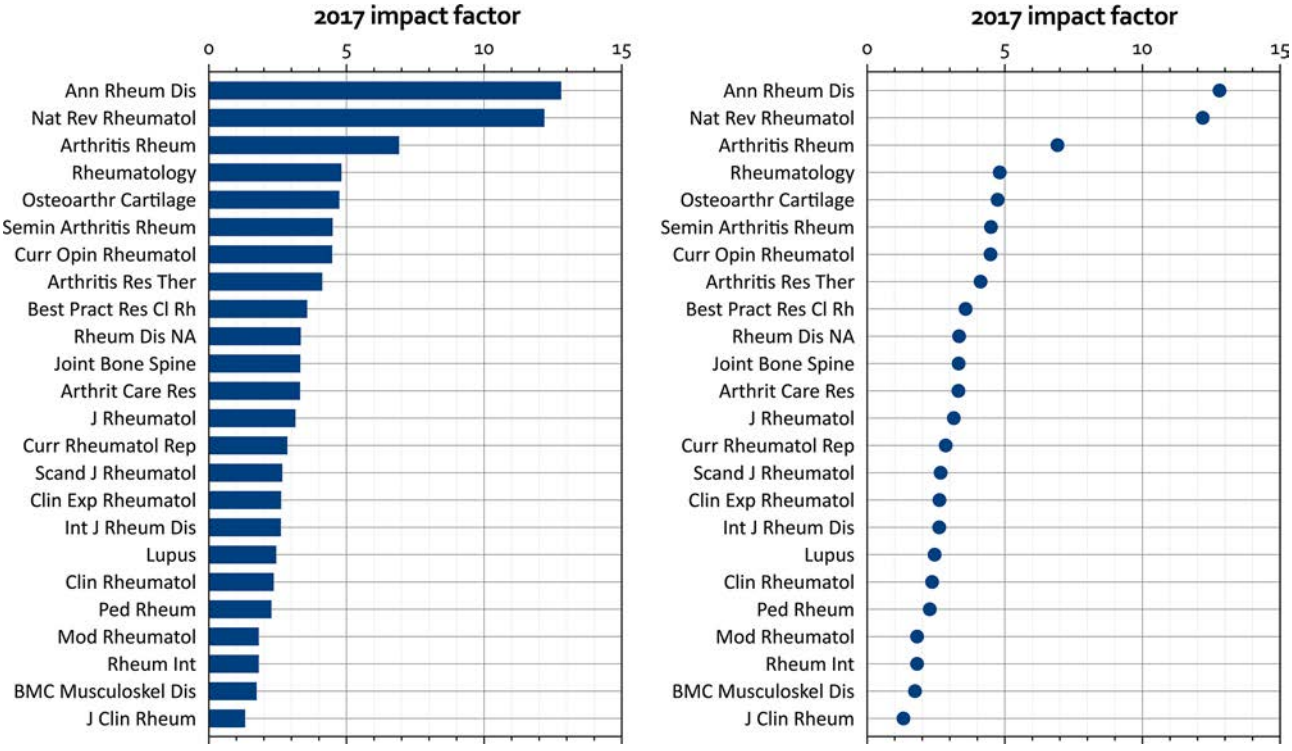

Figure 2 Labelled measurements. Impact factors of rheumatology journals in 2017, as bar (left) and dot plot (right), ranked by impact factor. A thin grid and repeated ticks allow exact reading. I prefer the dot plot because it communicates the same data with much less 'ink'. Bars are probably better for physical quantities, where the suggestion of real mass or volume must be conveyed.

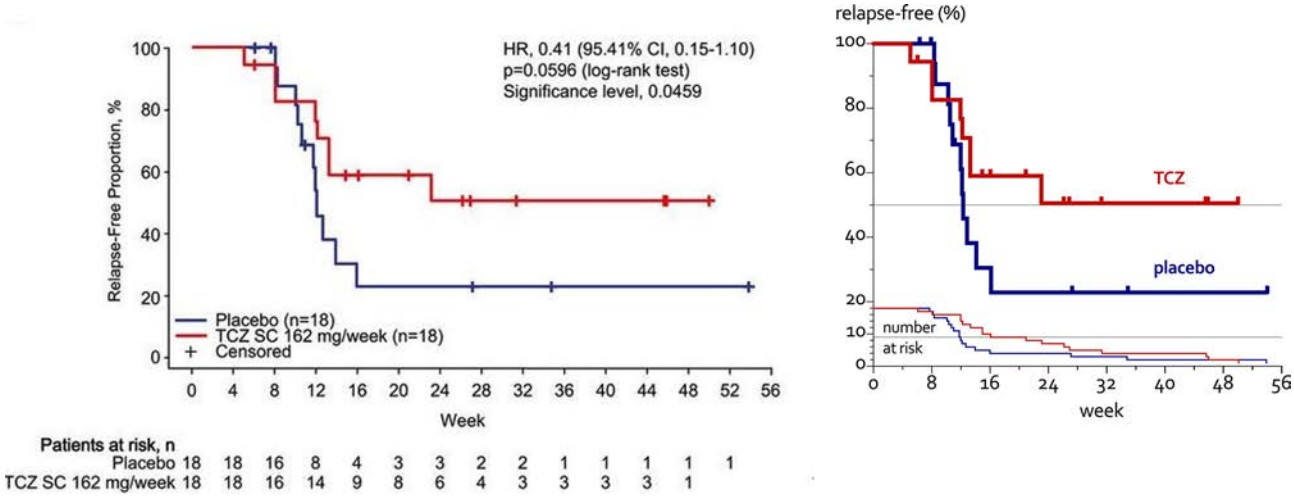

Figure 3 Survival curve (step plot). The original on the left[10] clearly depicts the data series. However, it has suboptimal resolution (grainy, fuzzy, artefacts on magnification) and a lot of text inside the data rectangle. In the suggested improvement on the right, the physical size is reduced: one column width and smaller vertically. Signal improvement: series lines made slightly more prominent. Noise reduction: axes and ticks thinner, y-axis title shortened and placed upright, number of x-axis tick labels reduced and number of patients at risk placed as a separate step plot under the main curve. 'Story' improvement: thin reference line placed at 50% in both curves; deleted text information (HR, p and also the in-figure legend) to be placed in the caption.

plots (figure 6). Each figure shows an example with suggestions for improvement discussed in more detail below. Bar plots are popular, but in most cases are best replaced by dot plots for labelled observations (figure 2) and scatter plots for distributions (figure 6). The following graph types are best avoided completely because they are less effective, prone to bias or both: pie, stacked bar, area, radar (spyder) and three dimensional.[9 15 16] Various new types of graphs have been developed to fit specific needs, but these fall outside of the scope of this Viewpoint.

GRAPH CONSTRUCTION

Graphing data should be an iterative, experimental process to optimise clear vision and clear understanding. Clear vision is achieved by highlighting the data. The first step is to minimise the 'non-data ink', the supporting elements surrounding the data. All unnecessary items must be avoided or removed, including garish colours and patterns ('chart junk'), and what is retained must be de-emphasised: axes and ticks must be thin, labels simple, and grids (if truly necessary) thin and light. Then the data ink must be maximised, the data must stand out through the use of visually prominent, non-overlapping symbols and a layout optimised for the final target—publication or presentation. Elements of this process are highlighted in figures 2–6.

Clear understanding means that your graph must tell a story. It goes beyond clear vision and implies choices for the right type of graph, selecting, organising, grouping and sequencing your data, sometimes plotting the same data repeatedly to bring out important relationships, and writing legends and captions that describe and explain clearly. Selecting your data also implies deleting non-essential information, as shown in figures 3 and 4, where summary results and excessive detail on the number of patients threaten to obscure the main message. Other elements of clear understanding are perhaps most clearly shown in the bottom panel of figure 4 where improved colour coding enhances the visibility of the two phases in the trial, so that the legend can be deleted. In figure 4 I have also added the 'null zone', an improvement on error bars (see next section): here, it shows no significant difference at any point in the trial.[17] Understanding also implies choices in scaling, determining the physical dimensions of the data rectangle, and for matrices, positioning and scaling of the multiple panels and accompanying labels (figure 6). Strategically placed labels and properly formulated captions enforce the message. The caption should explain the elements in the graph and complement the story.

SPECIFIC ISSUES

Scale of the axes should be chosen carefully and not be left to the program. I prefer the data to fill as much of the data rectangle as possible. A scale break is mandatory when the range does not include zero, and this works best with scatter plots. Alternatives include application of log scales, or plotting (part of) the data more than once, in separate panels with different scales. In that case, a cool trick is to draw a small reference bar next to each panel that is proportional to the scale used. When x-axis and y-axis have the same unit (eg, in receiver-operator characteristic curve plots), the plot should be square.

Symbols must be carefully chosen for readability, especially when there are many to plot. For difficult cases, characters can also be used as symbols: those best distinguished include 'S' and '<'. When there are many overlapping points or line segments, extra measures must be taken, such as introducing a small random component in the data, as shown in the unidimensional scatter plots (figure 6), or slightly offsetting overlapping series, as shown in the original of the line plot (figure 4, top panel). Axis offsets can help when data clusters around zero (figure 5). The order in which data series are presented is an important element of design.

Error bars are used to describe statistical variation. As shown in figure 6, these give much less information than unidimensional scatter or box plots. If retained, the error bars should preferably show the SD and perhaps the 95% CI of the mean rather than the SE; both can be shown in a 'two-tiered' error bar (figures 4 and 6). Error bars are also used to assess differences between means, but the amount of overlap is difficult to interpret. To solve this, I invented the 'null zone', which is the range where means fall if the difference between them is not significant (figure 4; online supplementary appendix 4 for a spreadsheet calculation tool).[17]

Colour used to be limited to presentations because of cost; now it is becoming mainstream for publications online, and many

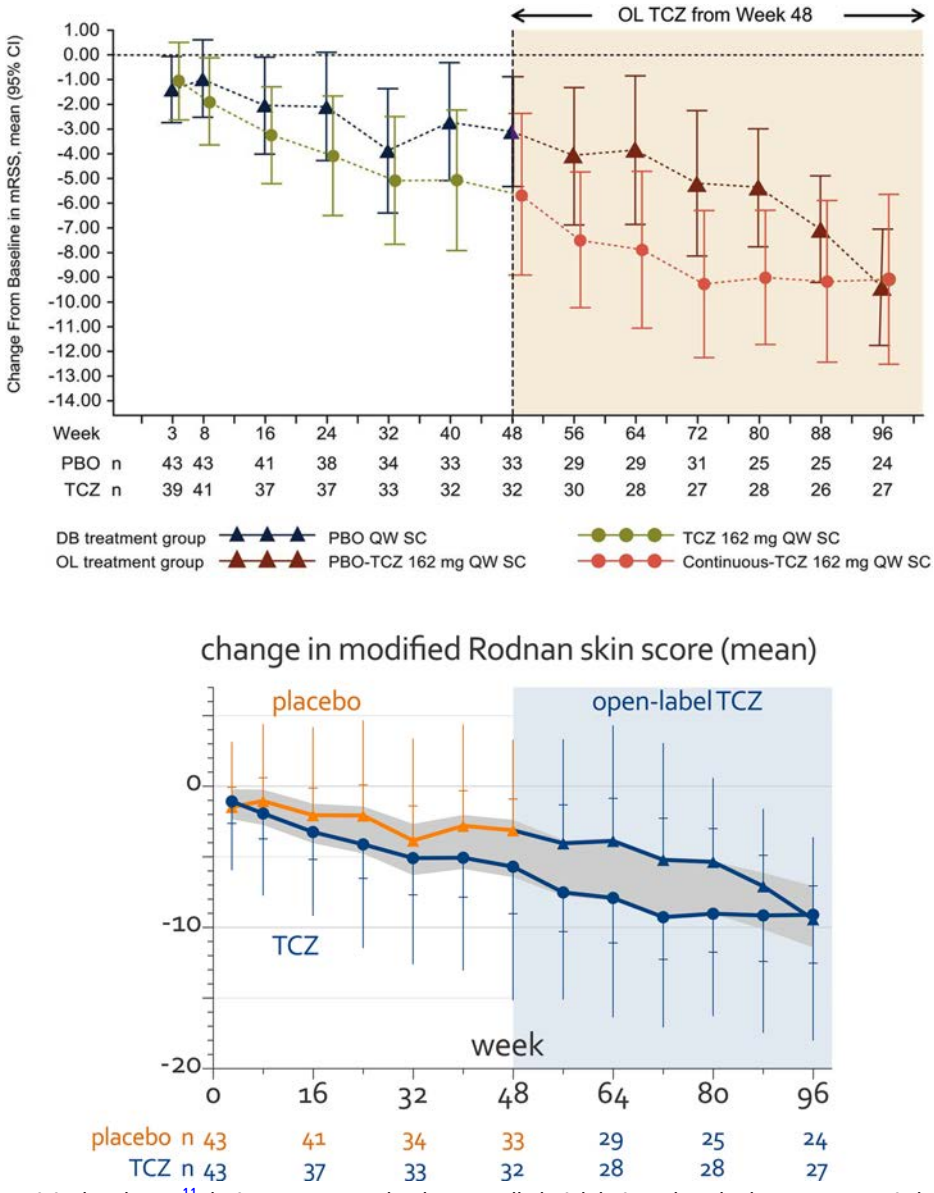

Figure 4 Line plot. The original at the top[11] depicts a common placebo-controlled trial design where both groups are switched to open-label active drug after a certain period. It clearly shows two data series, slightly staggered to prevent overlap, with change suggested at week 48 through a change of colour of the series, background shading, a label and arrows. The original figure also had a summary data table attached below (not shown here). The improved graph is shown with the same physical vertical size, but it could probably be shrunk to fit into one column. Signal improvement: as the treatment of the active group does not change, except for switch to open-label, this group is best depicted as one data series. To 'tell the story', I have used colour (blue for active and orange for placebo) for the series and carried this through to all the text labels and the shading. Also, the y-axis scale now better agrees with the uncertainty in the data. Further signal improvements: series lines are prominently thick and continuous; the thin error bars are double-tiered and show both the SD and the 95% CI of the mean (horizontal 'whiskers') on only one side of the mean, and I have added the 'null zone' that depicts the area in which the two means fall if the difference between them is not significant at the 5% level.[17] Noise reduction: a thin grid helps orient the eye (even thinner for the minor ticks), replacing the dashed horizontal line; the dashed vertical line and the arrows on top are also rendered superfluous. Supporting text is optimised: y-axis title placed upright as overall title, abbreviations are avoided as much as possible (except for 'TCZ', tocilizumab), and the amount of text (tick labels, patients in trial) reduced, but with increased font size. The legend below the figure is no longer necessary, but remaining details (such as the dose) can be included in the caption.

journals now offer colour printing at reduced cost or free (eg, *ARD!)*. When only the online version is in colour, all graphs should be checked (and if necessary redesigned) so that they will also work well in (greyscale) print. For such cases, I reiterate that patterns (eg, blocks, stripes, hashes, dashes) are relics of printing history: they create 'noise' and should be avoided at all cost!

Good colour design is not for the faint-hearted and takes commitment. Most software programs have standard colour palettes that are offensive to the eye, so one must look deeper or go online to find a palette that works well. As colour is readily detected by the visual system, colours can be muted ('unsaturated'), and the number of different colours should be limited to avoid chart junk. Likewise, 'graduated fills'—where a colour goes from dark to light in one direction, or one colour changes to another—are rarely a good idea. Finally, most palettes ignore colour blindness. For example, most

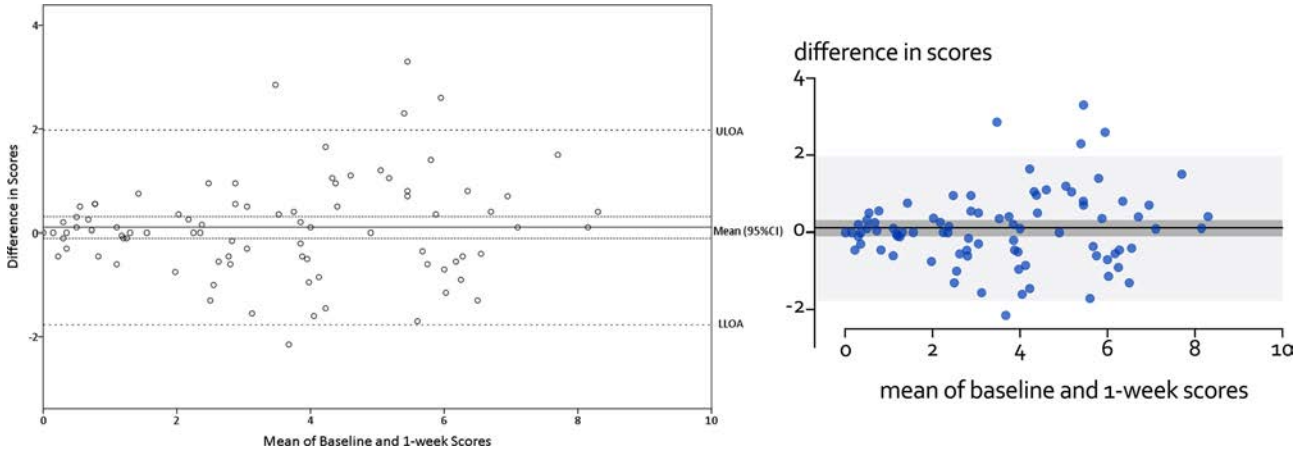

Figure 5 Scatter plot. The original on the left is a so-called 'Bland-Altman' plot where differences between two scores (y-axis) are plotted against the mean of the two scores (x-axis).[12] Horizontal lines depict the mean difference and its 95% CI, as well as the upper and lower limits of agreement. Although all elements are visible in the original, the resolution is suboptimal and the contrast is very low. The dimensions are suboptimal, falling between one and two columns width. In the improved figure on the right, signal is enhanced by enlarging and filling the data points (fill partially transparent to visualise overlap), offsetting the x-axis to improve visibility of data near zero and shading the areas of interest. Noise reduction: text labels are enlarged and placed upright; the dimensions are improved by shortening the x-axis and deleting the text on the right; this should be placed in the caption.

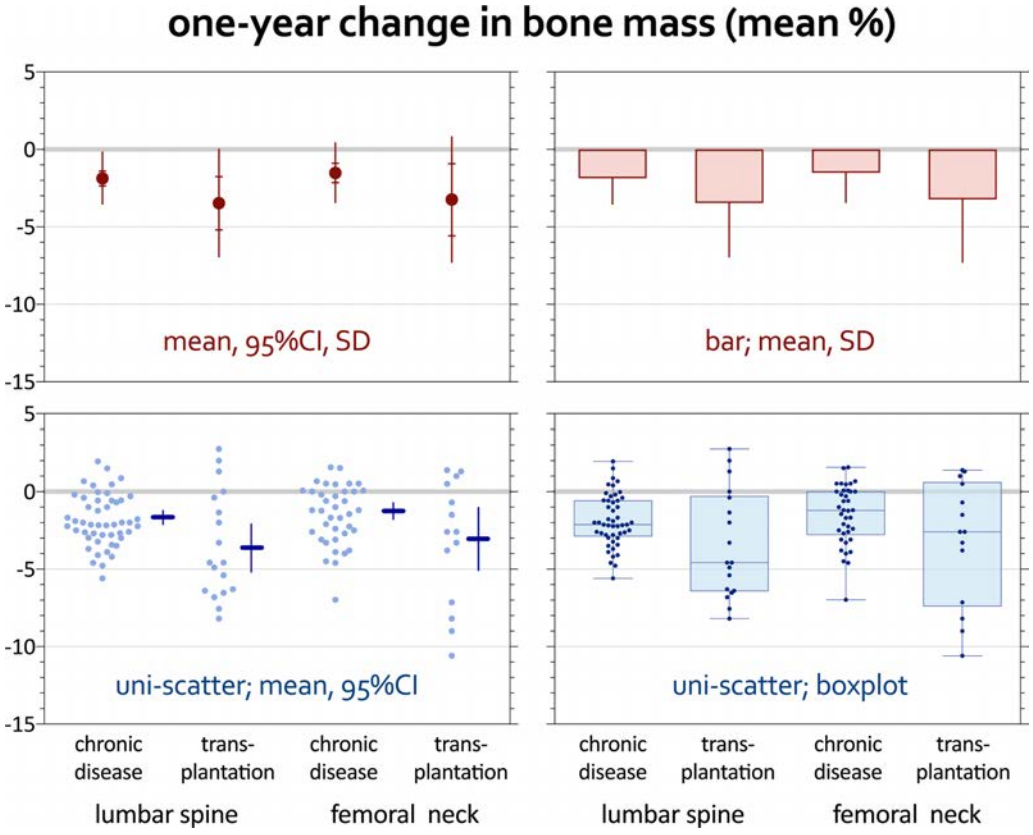

Figure 6 Matrix of distribution plots. The top two panels (in red) show a suboptimal double tier (left) and a poor bar graph representation (right) of a distribution. Double tiers are better than simple error bars, but still only show mean, 95% CI and SD, losing a lot of information. Bars should really be avoided: they only show mean and SD, and give a wrong visual impression of the form and location of the distribution. The improved bottom panels (in blue) show the distribution in a unidimensional scatter plot (individual observations spread out to prevent overlap) combined with a summary. In the left panel (figure as published[13]), the mean (horizontal line) and 95% CI (vertical line), drawn in by hand; in the right, a box plot designed to show the median, range and percentiles 25 and 75. The box plots are lightly shaded, and the data points are made smaller and darker to create a better balance. The story is enhanced by ordering the categories to bring out the comparison of health condition by anatomical location. For the matrix as a whole, noise is reduced by creating one title/y-axis label on top, printing the x-axis labels only below the bottom panels, unifying the y-axis scale and printing its labels only on the left panels. A thin y-axis main grid is added with a thicker line at zero. Panels are delimited by thin box lines, and tick marks are repeated on the y-axes on both sides to retain visual reference. In this way, the panels can be moved close together and redundant information is avoided as much as possible.

immunofluorescence micrographs (standard colouring scheme: red–green) are unreadable for colour-blind people. A palette exists that retains colour contrasts for everyone (figure 1). A special website explains the issues in great detail.[18]

Graphs should be truthful, and apart from the graph types noted above that are particularly prone to bias, improper scaling can often result in incorrect interpretation. For example, in a meta-analysis 'Forest plot' that depicts ratios (relative risks or odds ratios), a log scale is appropriate (not a linear scale). Correct labelling becomes even more important when difficult concepts and relationships are being graphed.

MATRIX GRAPHS

Matrix graphs are very popular in basic and translational science, where a lot of experiments need to be shown in a limited space or timeframe. These experiments often have an elegant stepwise approach with multiple negative and positive controls in a variety of settings. Unfortunately, the associated graph is often just a collection of suboptimal single graphs shrunk to miniature and placed on a matrix grid. A good matrix is a 'story of graphs' that requires meticulous design. An example of a published simple matrix graph and its optimisation is shown in one of the YouTube clips I made for *ARD*.[5] Clear vision is realised when the framework is predictably constant (through use of common graph types, scales, symbols, labels, etc) and repeating elements are minimised (figure 6). In basic science, generic labelling of series (eg, 'negative control', 'positive control' or logical abbreviations thereof) are much preferred above specific labels or abbreviations that make no sense outside the specific context. Clear understanding is realised when the ordering of the graphs follows the normal reading direction (left-to-right, top-to-bottom) and the steps described are in the optimum order. In addition, when the underlying process is complicated, graph titles and captions should be informative to help the story along. For example, instead of labelling the panels 'A', 'B' and so on, they could be labelled 'normal resting state', 'physiologic activation', 'activation after blocking pathway X' and so on. And the caption would not be: 'figure 1 Summary of activation experiments', but rather: 'figure 1 The ABC system in rest, after physiologic activation, and activation after blocking pathways X,Y,Z….'

PUBLISHING AND PRESENTING

For publishing, good quality starts with images uploaded in the correct format ('jpg', 'tiff' or 'gif', depending on the journal and your software capabilities) and in high resolution (minimum 300 dots per inch, but 600 or 1200 is better). Graphs should be designed with the typical journal page in mind: two columns on an A4 (or letter size) page. That means a figure will need to fit in one column, across two or fill the whole page. The author has a say in this! The journal production team must see to it that a full-page graph (or table) in landscape mode is rotated to be readable in the downloadable pdf. One can help the staff by placing remarks in the body text, for example, 'figure 1 about here, suggest to span two columns'. Non-standard sizes (often caused by labels or legends sticking outside the regular frame) will result either in unwanted size reduction or useless whitespace on one or both sides of the figure. One should check the image on screen and on print, also after setting the page size to 25%. This emulates what happens when the image is reduced in size to fit across one column. Many standard software programs have default settings that unacceptably downgrade resolution to limit file size; this also happens when one cuts and pastes images into a word processing document. Portable document format (pdf) is accepted by several journals, but quality on proof is not assured, and strange things can happen to the fonts; so image files are preferable, even though they are much larger. Proofreading is exceptionally important: not only to correct errors, but also to make sure the figures are reproduced as intended; multiple proofs may be necessary. In many cases the technical staff at the journal is less dedicated to the figures than the author. Common issues in the proof stage include resolution loss (even when the figures were submitted in the correct format; see online supplementary appendix 6),[17] suboptimal magnification and placement of figures in the text, font substitutions that reduce your symbols and labels to gibberish, partial reproduction (clipped graphs), suboptimal placement of captions and worse. Publishers should be held responsible for an optimal technical process. This includes the production of the figures for the journal web page which is currently completely out of the author's control.

For presenting, there are general guidelines that are outside the remit of this Viewpoint (eg, working with light background and dark letters for data projection, using sans serif fonts, using letter sizes that are large enough to read, etc). Importantly, tables and graphs (usually first designed for publication) should be optimised for presentation, given the much lower resolution and contrast of projection facilities. Graphs taken from publications should be redrawn (see online supplementary appendix 7).[19] They may need a redesign in view of the venue, size of the audience, projection facilities and host computer, especially when a (computer) platform switch is necessary. Poster presentation also raises specific issues not covered here.

PEER REVIEW

Peer reviewers should demand access to figures (and tables) at optimum resolution, and should study these just as critically as the text. The principles of design apply:

- Does this message require a graph (or does another message)?
- Is the message best conveyed with this graph?
- Is the graph optimal for clear vision, clear understanding?
- Is the graph truthful?

The review is more useful when suggestions for improvements are included.

CONCLUSION

Data visualisation through graphs (and tables) is essential in the scientific communication, but receives too little attention in the preparation (and production!) of scientific reports, publications and presentations. Most common flaws are easily avoided by staying away from suboptimal graph types and following design principles outlined in this article. Authors, editors and publishers should work together to improve data visualisation and stimulate innovation in design.

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OPEN ACCESS

EXTENDED REPORT

Open-label, multicentre, dose-escalating phase II clinical trial on the safety and efficacy of tadekinig alfa (IL-18BP) in adult-onset Still's disease

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ABSTRACT

Objectives Adult-onset Still's disease (AOSD) is a rare systemic autoinflammatory disease; its management is largely empirical. This is the first clinical study to determine if interleukin (IL)-18 inhibition, using the recombinant human IL-18 binding protein, tadekinig alfa, is a therapeutic option in AOSD.

Methods In this phase II, open-label study, patients were ≥18 years with active AOSD plus fever or C reactive protein (CRP) levels ≥10 mg/L despite treatment with prednisone and/or conventional synthetic disease-modifying antirheumatic drugs (DMARDs). Previous biological DMARD treatment was permitted. Patients received tadekinig alfa 80 mg or 160 mg subcutaneously three times per week for 12 weeks; those receiving 80 mg not achieving early predicted response criteria (reduction of ≥50% CRP values from baseline and fever resolution) were up-titrated to 160 mg for a further 12 weeks. The primary endpoint was the occurrence of adverse events (AEs) throughout the study.

Results Ten patients were assigned to receive 80 mg tadekinig alfa and 13 patients to the 160 mg dose. One hundred and fifty-five treatment-emerging AEs were recorded, and 47 were considered related to the study drug. Most AEs were mild and resolved after drug discontinuation. Three serious AEs occurred, one possibly related to treatment (toxic optic neuropathy). At week 3, 5 of 10 patients receiving 80 mg and 6 of 12 patients receiving 160 mg achieved the predefined response criteria.

Conclusions Our results indicate that tadekinig alfa appears to have a favourable safety profile and is associated with early signs of efficacy in patients with AOSD.

Trial registration number NCT02398435.

INTRODUCTION

Adult-onset Still's disease (AOSD) is a rare non-familial, non-monogenic systemic inflammatory disease, the aetiology and pathogenesis of which remain unknown.[1 2] AOSD belongs to the group of autoinflammatory disorders characterised by excessive innate immune responses. AOSD shares many similarities with systemic-onset juvenile idiopathic arthritis (SoJIA), but is approximately 10 times less frequent than its juvenile counterpart.[1 3] The course of AOSD is heterogeneous with patients experiencing a monocyclic phase with complete resolution, and others with persisting or recurrent bouts of arthritis and systemic inflammation.[4] The management of AOSD is largely empirical and includes the use of non-steroidal anti-inflammatory drugs (NSAIDs), systemic glucocorticoids and conventional synthetic (cs) disease-modifying anti-rheumatic drugs (DMARDs), such as methotrexate (MTX).[5] Randomised clinical trials in SoJIA have demonstrated the efficacy of anticytokine therapies, including interleukin (IL)-1 and IL-6 antagonists.[6 7] Similar strategies are used in AOSD, although the data are more scarce, including mainly retrospective studies,[8–11] and only one randomised open clinical trial.[12] The IL-1 antagonist, canakinumab, is indicated for the treatment of AOSD in patients who have responded inadequately to previous therapy with NSAIDs and systemic corticosteroids[13]; however, results from controlled clinical studies are not currently available.

IL-18 has been considered to play a major role among the inflammatory agents involved in AOSD pathogenesis.[14] IL-18 is a proinflammatory cytokine of the IL-1 family that is produced by various cell types, including monocytes/macrophages.[15] The biological activity of IL-18 is tightly controlled by IL-18 binding protein (IL-18BP), a naturally occurring inhibitor that binds IL-18 with high affinity.[16] In AOSD, circulating levels of IL-18 were associated with clinical status and laboratory markers of disease activity.[17 18] However, currently used immunoassays do not distinguish IL-18 complexed with IL-18BP (inactive) and unbound free IL-18 (active). Recently, by using a novel immunoassay that selectively measured biologically active IL-18, we showed that serum levels of free IL-18 were elevated in AOSD and correlated with clinical and biological markers of disease activity.[19] The aim of the current study was to determine the safety and efficacy of blocking IL-18 with the administration of recombinant human IL-18BP (tadekinig alfa) in the treatment of AOSD. This clinical trial was the first to determine if IL-18 inhibition is a therapeutic option in AOSD.

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METHODS

Study participants

The study (ClinicalTrials.gov, number NCT02398435) was conducted between March 2015 and July 2016. Eligible patients were age 18 or older at baseline with AOSD according to the Yamaguchi criteria.[20] Patients had active disease at baseline as defined by the presence of at least two Yamaguchi criteria at the screening visit plus either fever or elevated serum levels of C reactive protein (CRP ≥10 mg/L) despite being treated with prednisone at ≥5 mg daily for more than 1 month and/or csDMARDs (MTX at a dose of 10 mg per week for ≥3 months). Previous treatment with synthetic or biological DMARDs (bDMARDs) was allowed. All bDMARDs had to be discontinued before baseline, respecting specific washout periods (described in the online supplementary materials). Concomitant use of NSAIDs, prednisone and csDMARDs was allowed during the study. Specifically, the prednisone daily dosage could be maintained or tapered, but any increase was considered as treatment failure. Other inclusion and exclusion criteria are described in the online supplementary materials.

All patients provided written informed consent before study participation. The protocol, informed consent and any accompanying material were approved by the ethics committees or institutional review board at each centre before study initiation.

Study design

This international, multicentre, open-label, dose-escalating phase II study included patients from 20 centres in Switzerland, France and Germany. Patients were subdivided into two groups that were sequentially treated with subcutaneous injections of either 80 mg or 160 mg tadekinig alfa three times per week for 12 weeks. Tadekinig alfa was available at a concentration of 80 mg/mL. Two injections of 80 mg tadekinig alfa were given to patients receiving tadekinig alfa at a dose of 160 mg. All injections were administered by trained study nurses throughout the study period. After the first five patients were assigned to the 80 mg group, and after the independent Data Safety Monitoring Board (DSMB) approval, patients were randomly assigned 1:1 to receive either 80 mg or 160 mg tadekinig alfa. Allocation to receive 80 mg was terminated after the 10th patient was enrolled into this group. The decision to continue with the study drug and to up-titrate to a higher dose was at the discretion of the DSMB. Early predicted criteria of response at 3 weeks were normalisation of body temperature and decrease by 50% of the baseline CRP levels or normalisation of CRP values to <5 mg/L. Response to therapy at 12 weeks was predefined as an improvement of joint count (both Swollen Joint Count (SJC) and Tender Joint Count (TJC) according to a 44-joint assessment) by ≥20% from baseline values, and a 70% decrease of CRP levels compared with baseline values (or reduction to normal levels) or normalisation of ferritin.

After 3 weeks, patients receiving tadekinig alfa 80 mg who did not achieve early predicted criteria of response were up-titrated to the 160 mg dose for a further 12 weeks of treatment. Dose increases to 160 mg for the 80 mg group, or to 320 mg for the 160 mg group, were also allowed after 3 weeks. A third group, 320 mg, was planned to be included within the study; however, it was decided not to enrol patients into this group since early efficacy was achieved in the 80 mg and 160 mg groups. Any dose increases from 80 mg to 160 mg or 160 mg to 320 mg were made at the treating physician's discretion. Enrolled patients continued treatment through week 16, with a 4-week safety follow-up.

Assessments

Patients had regular scheduled visits at baseline (first tadekinig alfa administration) and weeks 1, 3, 6, 12 and 16.

The primary endpoint was the occurrence of adverse events (AEs) that were recorded throughout the study. The incidence, nature and severity of AEs, and abnormal laboratory tests were reported.

Secondary endpoints comprised clinical and biological signs of efficacy, including the evolution of body temperature, skin rash, number of SJC and TJC, patient global assessment, physician global assessment, pain assessment on a visual analogue scale (VAS), fatigue assessment on the VAS and dosage of prednisone with respect to baseline. Laboratory assessments included serum levels of CRP, serum amyloid A (SAA), ferritin, free IL-18, IL-6, tumour necrosis factor, IL-1 receptor antagonist (IL-1Ra), S100A8/9, S100A12 and blood leucocyte and granulocyte counts. All laboratory tests were performed in a centralised laboratory (MLM Medical Labs GmbH, Moenchengladbach, Germany; see online supplementary materials).

A small pharmacokinetic study was also performed (see online supplementary materials).

Statistical analysis

For AEs, the statistical analyses were descriptive and the reports included standard summary tables (including mean, SD, median, minimum, maximum or counts/percentages).

For secondary endpoints, to better assess efficacy, we used per-protocol analyses by imputing missing values only from week 6 to week 12 (last observation carried forward). If patients stopped earlier, they were excluded from the analyses. Continuous variables were compared over time using Wilcoxon signed-rank test and across dosages using Wilcoxon rank-sum test. Categorical variables were compared over time using exact McNemar's test and across dosages using Fisher's exact test. All analyses and tabulations were performed using R V.3.4.1.

RESULTS

Twenty-three of the 32 screened patients were included in the study. Ten and 13 patients were assigned to receive doses of either 80 mg (group 1) or 160 mg (group 2) tadekinig alfa three times per week, respectively (figure 1). The baseline characteristics of the patients included in the two groups are described in table 1. In the overall study population of 23 patients, arthritis and arthralgia (SJC and TJC) were present in 19 patients at baseline, neutrophilia in 14 patients, skin rash in 13 patients and fever in only 2 patients. Six patients had an early disease onset within 6 months prior to study inclusion, whereas all others had either chronic or polycyclic courses. Twenty-two patients had previously received glucocorticoids up to a median dose (IQR of 30 mg daily, 8.5–47.5). Thirteen patients had at least one course of treatment with a csDMARD and nine patients with at least one biological agent. One patient had received no prior glucocorticoids or csDMARDs. During the study, 21 patients received concomitant glucocorticoids at a median (IQR) baseline prednisone dosage of 15 mg daily (7.5–20), and 9 patients received a concomitant csDMARD, including 7 patients treated with MTX at a median (IQR) dosage of 17.5 mg weekly (15–22.5). By chance, patients recruited in group 2 were younger (35 vs 49.5 years) with shorter disease duration (11.6 vs 25.5 months) and lower prednisone daily dose (15 vs 35 mg) than in group 1.

One patient from group 2 discontinued after 1 week of therapy due to an injection site reaction (ISR) and was excluded from the efficacy analysis.

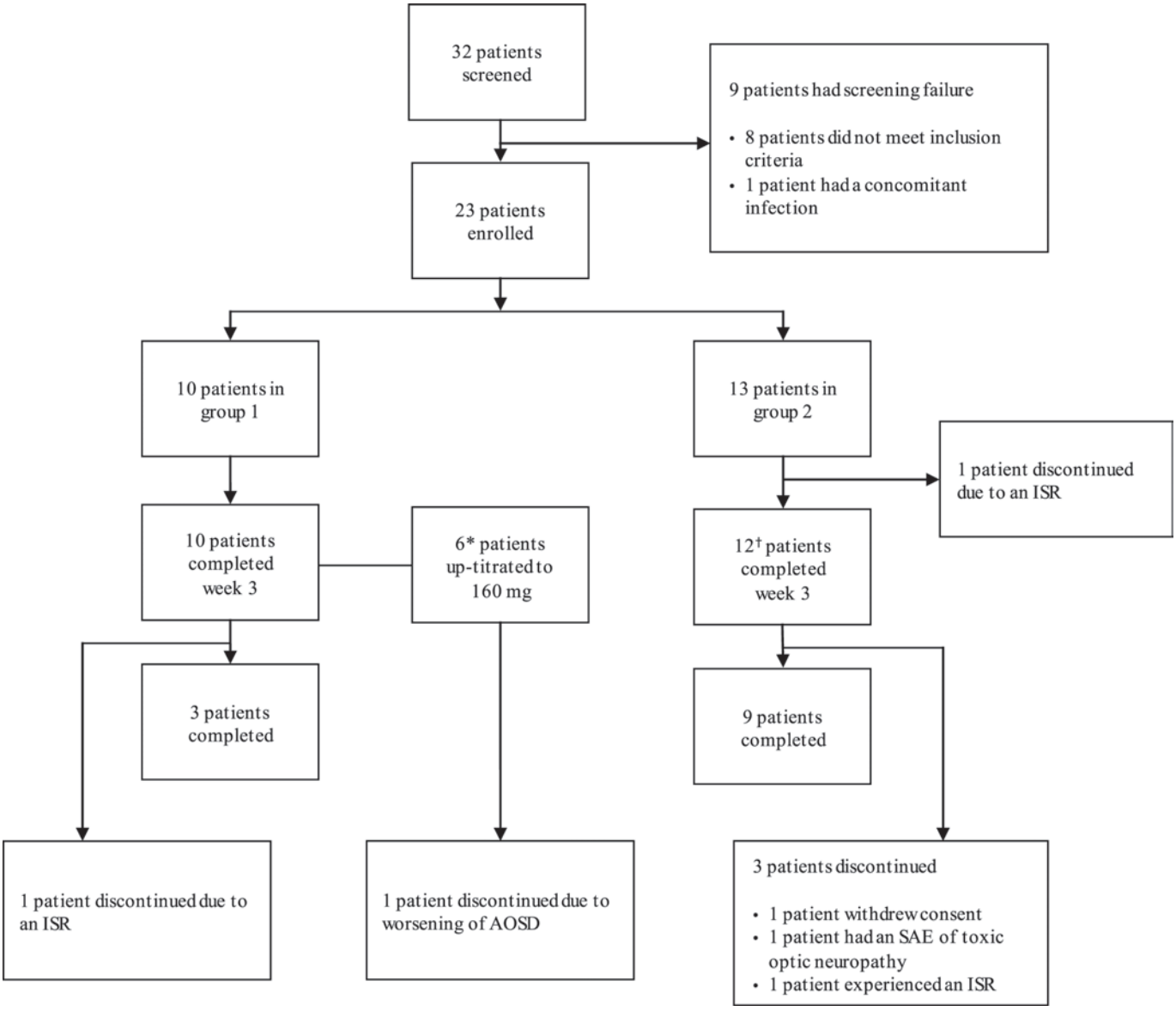

Figure 1 Patient disposition. *One responder and five non-responders. †One patient (responder) was up-titrated to 320 mg. AOSD, adult-onset Still's disease; ISR, injection site reaction; SAE, serious adverse event.

Safety analyses

The description of AEs by System Organ Class is shown in table 2. One hundred and fifty-five treatment-emergent AEs were recorded; 47 were considered related to the study drug by the treating physicians. ISRs, upper airway infections and arthralgia were the most common AEs. Three and 10 patients showed manifestations of local ISRs in groups 1 and 2, respectively. Some patients had more than one episode and most were considered mild. Two patients in group 2 had moderately severe ISR.

Three serious AEs (SAEs) occurred during the study; two were considered, by the treating physicians, as not related to tadekinig alfa (one episode of gastroenteritis and one severe back pain due to spondylolisthesis) and one as possibly related (toxic optic neuropathy). Case details of the toxic optic neuropathy SAE are provided within the online supplementary materials.

AEs led to the permanent discontinuation of the study in four patients, including three cases of ISR, one with 80 mg and two with 160 mg tadekinig alfa doses, respectively, and one case of SAE due to toxic optic neuropathy (details within the online supplementary materials). The patient who was up-titrated from 160 mg to 320 mg tadekinig alfa experienced no AE besides mild ISR.

Efficacy analyses

Among the patients available for clinical evaluation, five patients treated with the 80 mg dose (group 1), and six patients treated with the 160 mg dose (group 2) achieved the predefined response criteria, including a reduction of ≥50% CRP values from baseline and resolution of fever (table 3). As per the study protocol, five patients initially treated with 80 mg tadekinig alfa who did not achieve the response criteria at week 3 were up-titrated to receive 160 mg tadekinig alfa for an additional 12 weeks. Two responders, according to predefined study criteria at week 3, were also up-titrated: one patient from 80 mg to 160 mg and one patient from 160 mg to 320 mg tadekinig alfa, at the discretion of the treating physician. Since at least four patients in the 160 mg group achieved early predicted criteria of response, no patients were assigned to receive the higher dose based on non-response.

Table 1 Demographic and baseline characteristics of patients with AOSD treated with tadekinig alfa

	All (n=23)	80 mg (group 1) (n=10)	160 mg (group 2) (n=13)
Age, years	41 (30–58.5)	49.5 (34.2–58.7)	35 (30–58)
Gender (male/female)	7/16	4/6	3/10
BMI, kg/m^2	25.33 (23–27.5)	24.5 (23.1–34.5)	25.4 (22.4–26.4)
Disease duration, months	15 (6.5–42.2)	25.5 (8.7–44.2)	11.6 (2.1–37.6)
No of previous flares, n (%)			
0	7 (30.4)	2 (20)	5 (38.46)
1	2 (8.69)	1 (10)	1 (7.69)
2	5 (21.73)	2 (20)	3 (23.07)
≥3	3 (13.04)	3 (30)	0
Unknown	6 (26.08)	2 (20)	4 (30.76)
Baseline disease manifestations, n (%)			
Skin rash	13 (56.52)	7 (70)	6 (46.15)
Arthritis/arthralgia	19 (82.6)	8 (80)	11 (84.6)
Swollen joints	16 (69.5)	6 (60)	10 (76.92)
Tender joints	18 (78.26)	8 (80)	10 (76.92)
SJC*	4 (0–5)	2.5 (0–4.75)	4 (3–5)
TJC*	6 (1–11.5)	7 (4–12)	6 (1–8)
Fever	2 (8.70)	0	2 (15.38)
Neutrophilia (>8 g/L)	14 (60.86)	5 (50)	9 (69.23)
Comorbidities	21 (91.3)	10 (100)	11 (84.61)
Previous treatments, n (%)			
NSAIDs	14 (65.21)	7 (70)	7 (53.84)
Glucocorticoids	22 (96)	10 (100)	12 (92)
Prednisone dose (mg/day)	30 (7.9–50)	35 (30–50)	15 (7.5–32.5)
csDMARDs	13 (56.5)	6 (60)	7 (53.8)
1 csDMARD	9 (39.1)	5 (50)	4 (30.8)
≥2 csDMARDs	4 (17.4)	1 (10)	3 (23)
Previous csDMARD therapy, n (%)			
MTX	11 (47.82)	5 (50)	6 (46.15)
LFN	1 (4.3)	0	1 (7.69)
MMF	1 (4.3)	1 (10)	0
Antimalarials	3 (13)	1 (10)	2 (15.4)
Patients with bDMARDs, n (%)	9 (39.1)	4 (40)	5 (38.5)
1 bDMARD	4 (17.4)	1 (10)	3 (23)
≥2 bDMARDs	5 (21.7)	3 (30)	2 (15.4)
Previous bDMARD therapy, n (%)			
Anakinra	6 (26.08)	4 (40)	2 (15.38)
Canakinumab	3 (13.04)	2 (20)	1 (7.69)
Etanercept	2 (8.7)	1 (10)	1 (7.69)
Tocilizumab	3 (13.04)	0	3 (23.07)
Rituximab	1 (4.3)	0	1 (7.69)
Abatacept	1 (4.3)	0	1 (7.69)
Concomitant treatments, n (%)			
NSAIDs	12 (52.2)	8 (80)	7 (53.84)
Glucocorticoids	21 (91.30)	10 (100)	11 (84.61)
MTX	7 (26.08)	5 (50)	2 (15.38)
LFN	1 (4.3)	0	1 (7.69)
MMF	1 (4.3)	1 (10)	0

Data are n/N (%) or median (IQR), unless stated otherwise.
*According to a 44-joint assessment.
AOSD, adult-onset Still's disease; bDMARDs, biological disease-modifying antirheumatic drugs; BMI, body mass index; csDMARDs, conventional synthetic disease-modifying antirheumatic drugs; LFN, leflunomide; MMF, mycophenolate mofetil; MTX, methotrexate; NSAIDs, non-steroidal anti-inflammatory drugs; SJC, swollen joint count; TJC, tender joint count.

In patients without arthritis at baseline, clinical response was solely based on the results of laboratory tests. The 12-week efficacy analysis was first carried out including all patients with data at 12 weeks of tadekinig alfa treatment (table 3). Of the four patients treated with tadekinig alfa 80 mg throughout the study, two achieved the response criteria. Of 12 patients initially treated

Table 2 Most frequent AEs, >5% of the patients, by SOC and preferred term

	Overall*		80 mg*		160 mg*	
SOC	Patients	Events	Patients	Events	Patients	Events
ISRs	13	25	3	5	10	20
Infections/infestations	11	19	3	7	9	12
Musculoskeletal and CT disorders	9	39	3	4	8	35
Gastrointestinal disorders	8	13	1	1	8	12
Nervous system disorders†	4	8	1	1	4	7
Condition aggravated‡	3	5	0	0	3	5
Asthaenia and fatigue	5	5	2	2	3	3
Fever	1	5	0	0	1	5

*Events were recorded according to the dose that the patient was exposed to at the moment of the event.
†Comprising headache, dysaesthesia, sciatica and tension headache.
‡A worsening of disease components that were present at the onset of the study or are components of the primary disease (comprising global aggravated condition (n=1 patient), worsening of arthralgia or myalgia (n=1 patient), and an increase of global pain and skin rash (n=1 patient)).
AEs, adverse events; CT, connective tissue; ISRs, injection site reactions; SOC, System Organ Class.

with 160 mg tadekinig alfa, 7 patients achieved the response criteria. Eight of the 18 patients who received 160 mg tadekinig alfa, either from the study start or after 3 weeks of treatment with 80 mg tadekinig alfa, achieved the response criteria at week 12 (table 3). Both patients who up-titrated to 160 mg or 320 mg continued to be responders after 12 weeks. None of the non-responding patients from group 1 achieved the response criteria after 12 weeks of therapy with tadekinig alfa 160 mg. Of note, the response rate in patients with systemic manifestations but without synovitis (seven patients) did not differ from the whole group. The patient without prior glucocorticoid or DMARD treatment responded to tadekinig alfa (see online supplementary materials).

Additional secondary endpoints

Skin rash showed improvement over time versus baseline (figure 2), with 13/23 patients having skin rash at baseline, and only 6 patients at 12 weeks (P=0.02). Patient-reported and physician-reported outcomes also showed a general trend towards improvement (online supplementary table S1).

Overall, median prednisone dosage was decreased from 12.5 mg/day at baseline to 10 mg/day at 12 weeks (P=0.01). The decrease in prednisone was larger among the responders at 12 weeks with a tapering of 12.8 mg, compared with only 1 mg in the non-responders, although this difference was not statistically significant (P=0.23).

Biomarker levels decreased at week 12 compared with baseline levels (figure 3A–I; online supplementary table S2). Serum levels of free IL-18 were detected in seven patients at baseline. Among these patients, four exhibited a clinical response. Free IL-18 was undetectable in all these patients at the final blood assessment, whereas free IL-18 remained elevated in two of three patients who failed to respond to tadekinig alfa. In the overall population, as well as in patients from group 2 and in patients from group 1 up-titrated to the 160 mg dosage, the levels of ferritin, IL-6, neutrophils, S100A8/9 and S100A12 significantly decreased at week 12, as compared with baseline levels. Levels of CRP, SAA, ferritin, IL-6, S100A8/9 and S100A12 were significantly decreased in patients from group 2 (figure 3; online supplementary table S2). Elevated transaminase levels were present at baseline in two patients (>3-fold upper normal limits) and normalised with tadekinig alfa therapy in both patients.

Results of the pharmacokinetic study are reported in the online supplementary materials.

Table 3 Clinical and biological signs of response

	Week 3 (80 mg) (n=10)	Week 3 (160 mg) (n=12)	Week 12 (80 mg) (n=4)	Week 12 (160 mg) (n=12)	Week 12 (160 mg)* (n=18)
CRP reduction ≥50%	4 (40)	5 (41.7)	1 (25)	6 (50)	7 (38.9)
CRP reduction ≥70%	2 (20)	2 (16.7)	1 (25)	6 (50)	7 (36.8)
CRP normalisation (≤5 mg/L)	2 (20)	3 (25)	1 (25)	4 (33.3)	4 (22.2)
Ferritin normalisation (≤150 mg/L)	2 (20)	6 (50)	2 (50)	6 (50)	8 (38.9)
SJC44† reduction ≥20%‡	5 (83.3)	5 (55.6)	2 (100)	8 (88.9)	10 (76.9)
TJC44† reduction ≥20%§	7 (87.5)	4 (44.4)	2 (100)	5 (50)	8 (50)
Both joint counts reduction ≥20%	5 (83.3)	3 (37.5)	2 (100)	5 (62.5)	7 (58.3)
Responders at week 3 (1 or 3+no fever)	5 (50)	6 (50)	NA	NA	NA
Responders at week 12 (2 or 3 or 4 and 7)	N/A	N/A	2 (50)	7 (58.3)	8 (44.4)

Data are n (%).
*Includes six patients who were up-titrated to the 160 mg dose and 12 patients initially included in the 160 mg group. For patients who discontinued before week 12, the data correspond to the last observation.
†According to a 44-joint assessment.
‡Patients with SJC44 equal to 0 at baseline were excluded since their reduction could not be computed, leaving 6 patients in group 80 mg (week 3), 10 patients in group 160 mg (week 3), 2 patients in group 80 mg (week 12) and 14 patients in group 160 mg (week 12).
§Patients with TJC44 equal to 0 at baseline were excluded since their reduction could not be computed, leaving 8 patients in group 80 mg (week 3), 10 patients in group 160 mg (week 3), 2 patients in group 80 mg (week 12) and 17 patients in group 160 mg (week 12). At week 12, four patients continued their treatment at the dose of 80 mg.
CRP, C reactive protein; NA, not applicable; SJC, swollen joint count; TJC, tender joint count.

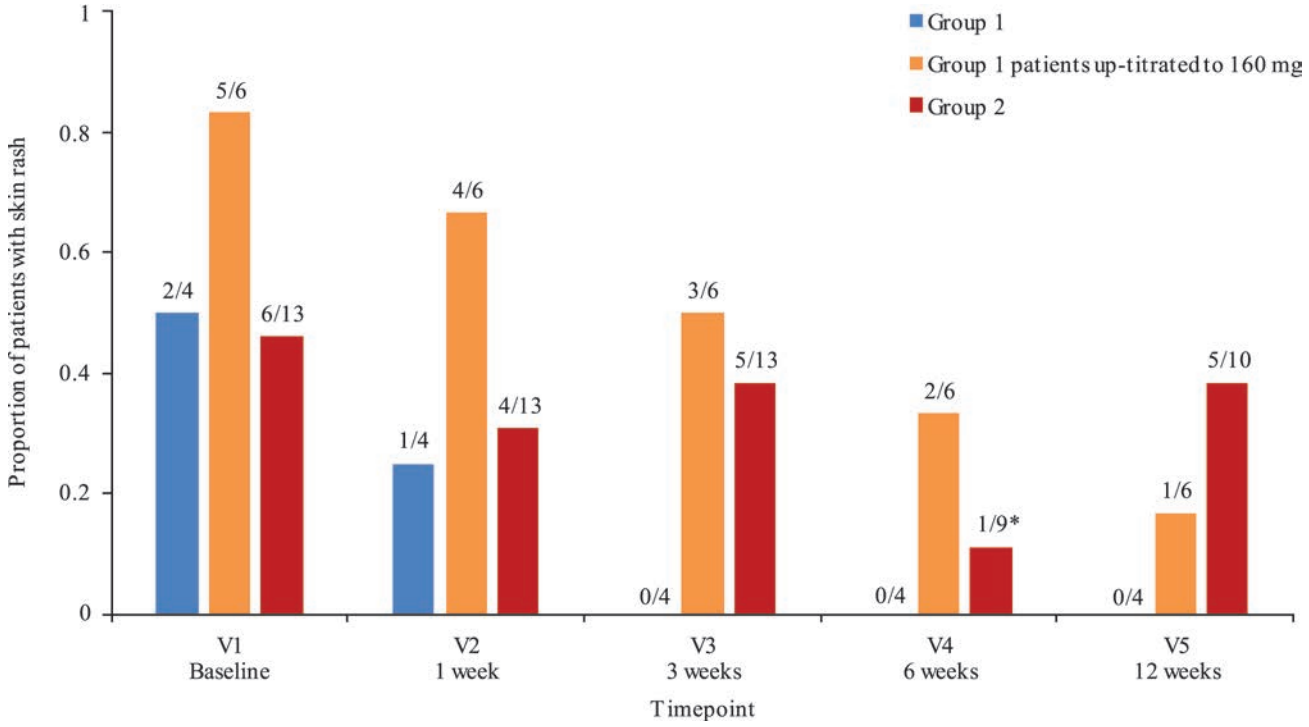

Figure 2 Evolution of skin rash over time. Presented fractions show number of patients with rash at each V/total number of patients. *Four patients did not have V4, but had V5 or V6 (early termination V). V, visit.

DISCUSSION

This phase II clinical trial examined for the first time the safety and efficacy of IL-18 blockade in patients with AOSD. Most patients had previously used glucocorticoids; approximately half had received csDMARDs and one-third bDMARDs. The results show that tadekinig alfa at doses of 80 mg and 160 mg three times per week appeared to have a favourable safety profile. In addition, we observed early signs of clinical and laboratory marker efficacy with response rates of 50%, irrespective of the tadekinig alfa dosage in this heterogeneous population of difficult-to-treat patients.

Most of the AEs were mild and resolved after drug discontinuation. The 60-year-old patient in which toxic optic neuropathy occurred suffered from hypertension, pulmonary emphysema and had serious thrombotic episodes prior to study participation. This AE was unexpected according to results of previous clinical studies. The DSMB reviewed the case and questioned the physician's conclusion that it was possibly drug related, suggesting that other, more likely, explanations had not been ruled out. The DSMB considered that there was insufficient information to draw any firm conclusion since there had been insufficient exploration to discard the diagnosis of ophthalmic vein thrombosis in this patient. ISRs were more frequently observed in patients receiving tadekinig alfa 160 mg.

The safety and efficacy of tadekinig alfa has been examined in two double-blinded, placebo-controlled, phase Ib clinical trials in 36 patients with rheumatoid arthritis and 35 patients with psoriasis (unpublished results). The safety profile showed that the most commonly reported AEs were ISRs that were mild in most cases. Local tolerability tended to worsen with increasing doses. Tadekinig alfa was administered subcutaneously at doses ranging from 80 mg to 350 mg three times per week based on the 30-hour half-life of tadekinig alfa for 6 weeks. These studies did not show any definite signs of efficacy.

In the current study, the results at week 3 did not show any difference in response between 80 mg and 160 mg tadekinig alfa doses. Furthermore, all non-responders who were up-titrated from 80 mg to 160 mg did not reach a subsequent clinical response. These results suggest that the tadekinig alfa 80 mg dose already has a meaningful clinical effect.

One patient was included despite not meeting the inclusion criteria of having been previously exposed to glucocorticoids, NSAIDS and/or csDMARDs since this case provides additional information on safety, our primary endpoint. This patient responded to tadekinig alfa without the addition of glucocorticoids or csDMARDs. Most importantly, following the discontinuation of tadekinig alfa at week 12, the patient experienced a disease flare.

Consistent with the positive effect of IL-1 or IL-6 targeting in SoJIA therapies, inhibition of these pathways has been studied in AOSD. Tocilizumab, a humanised monoclonal antibody against IL-6 receptor alpha, significantly reduced articular and systemic manifestations, acute-phase markers and prednisone dosage in patients with AOSD.[8] In 140 patients with AOSD treated with anakinra, a human recombinant IL-1Ra, systemic and articular manifestations improved in most patients, and a glucocorticoid-sparing effect and a significant reduction in the number of patients on csDMARDs were observed.[10] Our results show that IL-18 inhibition offers another possibility of therapy within the scope of anticytokine treatment for the management of AOSD.

The fact that only two patients had fever at baseline was unexpected. However, all patients had fever at some point during the disease course. It is plausible that the inclusion of some patients with long-standing disease may explain this observation. Some disease manifestations may also have been partly controlled by previous therapies. However, to avoid a carryover effect of former bDMARDs, a long washout period (ie, 6 months for canakinumab) was required prior to study inclusion.

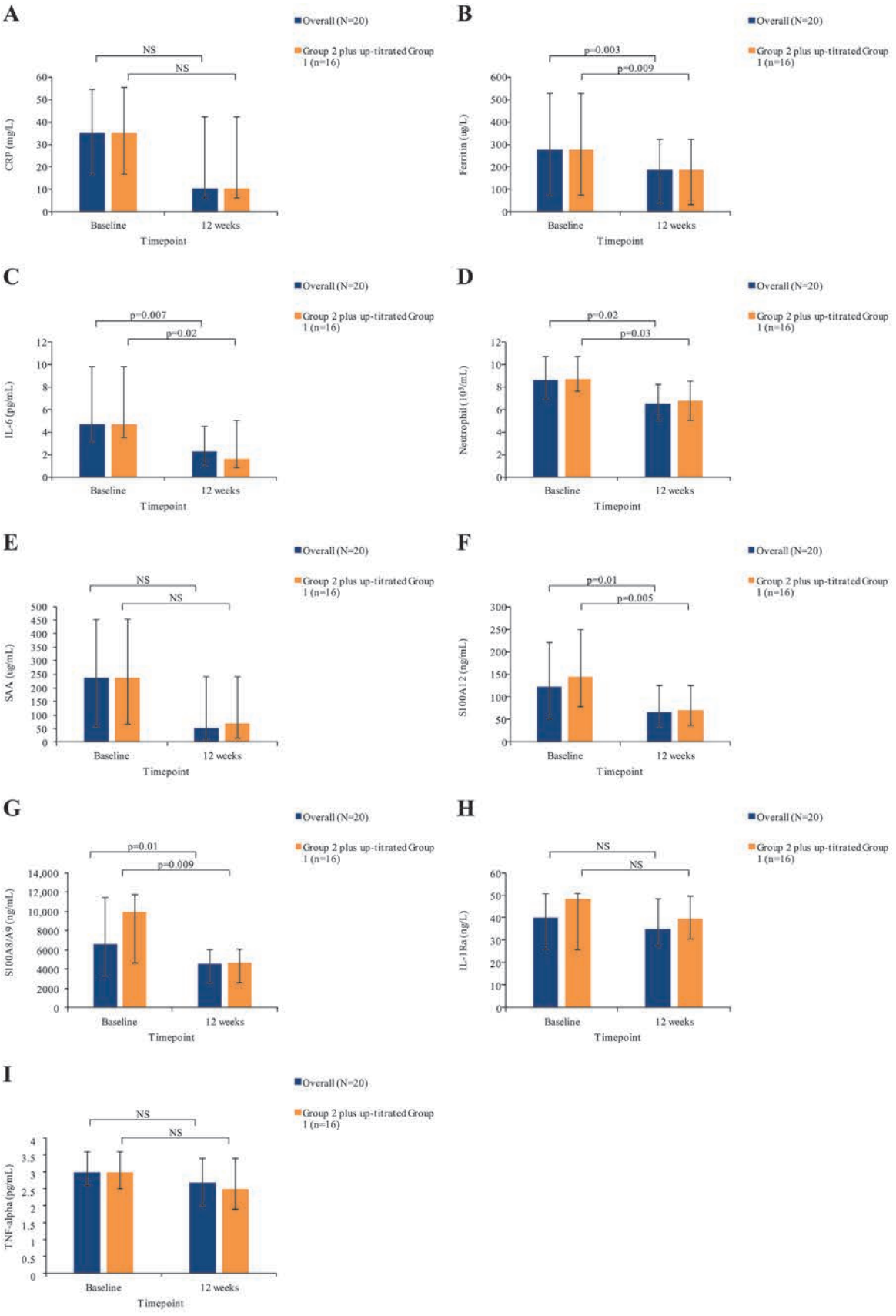

Figure 3 Evolution of serum biomarker levels by group and time point: (A) CRP; (B) ferritin; (C) IL-6; (D) neutrophil; (E) SAA; (F) S100A12; (G) S100A8/A9; (H) IL-1Ra; (I) TNF-alpha. Data are median (IQR). The 20 patients included in the overall group comprised 17 patients who had baseline values and at week 12, and 3 patients with values at week 6, but who discontinued thereafter. Three patients who discontinued at earlier time points were not included. Group 2 plus up-titrated group 1, n=16. CRP, C reactive protein; IL, interleukin; Ra, receptor antagonist; NS, not significant; SAA, serum amyloid A; TNF, tumour necrosis factor.

Our study is the first prospective study examining a drug with a completely new mode of action in AOSD. Another strength of our study is the inclusion of patients with 'difficult to treat' disease, including a large percentage of patients previously treated with bDMARDs. Furthermore, several patients had various comorbidities. Thus, our findings provide important results regarding the safety of IL-18 targeting with tadekinig alfa in AOSD. An open-label design, absence of a control group and heterogeneity within the study population are important limitations in the assessment of a treatment's efficacy. However, the clinical response in a group of patients with chronic disease despite the use of DMARDs and inclusion of objective measures, such as biomarkers of inflammatory responses, provide supportive data for treatment efficacy.

In conclusion, our results show that tadekinig alfa appears to have a favourable safety profile and is associated with early signs of efficacy in AOSD, thus warranting further clinical investigation.

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Contributors CG, BF, JR, JM, AS and EJS contributed to the conception and design of this study. CG, BF, JR, FS, EF, IK, EH, JM, TS, MAH, TM, BH, PL and HSK recruited patients into the study and participated in data collection. CG, FS, EH, DSC, AS and EJS contributed to the data analysis. All authors contributed to data interpretation, critically reviewed the article for important intellectual content and approved the final draft for submission.

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EXTENDED REPORT

Have the 10-year outcomes of patients with early inflammatory arthritis improved in the new millennium compared with the decade before? Results from the Norfolk Arthritis Register

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ABSTRACT

Objective To compare the 10-year outcome (disease activity, disability, mortality) of two cohorts of patients with inflammatory polyarthritis (IP) recruited 10 years apart.

Methods Patients with IP were recruited to the Norfolk Arthritis Register from 1990 to 1994 (cohort 1 (C1)) and from 2000 to 2004 (cohort 2 (C2)). Demographic and clinical data were collected at baseline and at years 1, 2, 3, 5, 7 and 10. Longitudinal disease activity (swollen/tender 51 joint counts (SJC51/TJC51)) and disability (Health Assessment Questionnaire (HAQ)) were compared between the cohorts using population-average negative binomial regression and generalised estimating equation analysis, respectively. Risk of 10-year mortality was compared between cohorts using Cox models. Risk of cardiovascular disease (CVD) mortality was compared between cohorts using competing risks analysis. Mortality rate ratios (MRR), adjusted for changes in mortality risk of the general population, were calculated using Poisson regression.

Results In total 1653 patients were recruited (C1=1022, C2=631). Patients in C2 had 17% lower SJC51 than C1 over 10 years (95% CI −23% to −10%), whereas TJC51 and HAQ were comparable. C2 patients had reduced risk of all-cause and CVD mortality compared with C1 (all-cause: HR 0.72, 95% CI 0.56 to 0.95; CVD: subhazard ratio 0.58, 95% CI 0.37 to 0.93). After accounting for changes in mortality risk in the general population, the difference in mortality was non-significant (all-cause: MRR 0.78, 95% CI 0.56 to 1.10; CVD: MRR 0.77, 95% CI 0.48 to 1.24).

Conclusion Disease activity significantly improved in the new millennium, whereas disability and mortality were unchanged.

Inflammatory polyarthritis (IP) and its subset rheumatoid arthritis (RA) are chronic conditions associated with synovial joint inflammation, progressive joint damage and premature mortality.[1 2] However outcomes can be improved by administration of appropriate therapy.[3 4] There have been significant advances in the available therapies to treat RA over the past two decades. Methotrexate was introduced as a treatment for RA in the 1990s and became the first-choice synthetic disease-modifying antirheumatic drug (sDMARD).[5 6] Since then biologic agents (biologic disease-modifying antirheumatic drugs (bDMARDs)) have been introduced and proven to be effective, but costly and are generally not used as first-line therapy.[7 8] Furthermore there has been a philosophical shift towards treating patients early after symptom onset, which is associated with improved physical function[9–11] and radiographic damage.[4 12 13]

A number of studies have compared patients during and post the treatment changes of the mid-1990s. An analysis of the Nijmegen early RA cohort compared the 5-year outcome of three subcohorts, based on the time period patients were recruited (subcohort 1=1985–1990, subcohort 2=1990–1995, subcohort 3=1995–2000). Patients in subcohort 3 had significantly lower mean Disease Activity Score (DAS28) at year 5 compared with the other two cohorts (mean DAS28: subcohort 1=3.7, subcohort 2=3.4, subcohort 3=3.2). However the Health Assessment Questionnaire (HAQ) scores were higher at year 5 in the most recent subcohort, although this was not statistically significant (mean HAQ: subcohort 1=0.49, subcohort 2=0.44, subcohort 3=0.83).[14] Humphreys *et al* compared mortality rates during the first 7 years of follow-up of three cohorts of patients with early RA recruited to the Norfolk Arthritis Register (NOAR) (cohort 1=1990–1994, cohort 2=1995–1999, cohort 3=2000–2004). That analysis reported no significant differences between the mortality rates in each of these cohorts over 7 years of follow-up (mortality rate ratio (MRR): cohort 1=ref, cohort 2=1.13 (95% CI 0.84 to 1.52), cohort 3=1.00 (95% CI 0.70 to 1.43)).[15]

The natural history of IP and RA is becoming less severe[16 17]; therefore, it is difficult to infer whether any improvements in long-term outcome (ie, 10 years) are associated with less severe disease or with the changes in treatment strategy. Thus the aim of this study was to compare the 10-year outcome of two cohorts of patients recruited 10 years apart, controlling for disease severity of the cohorts at baseline. Specifically, the objectives were to compare the baseline and 10-year characteristics of two cohorts of patients, one recruited from 1990 to 1994 and the other from 2000 to 2004, then to compare the disease activity, disability and mortality of the two cohorts over the course of 10 years.

PATIENTS AND METHODS

The NOAR began recruiting patients with IP registered with a primary care physician (general practitioner (GP)) in the former Norwich Health Authority region, Norfolk, UK in 1990. Incident cases of IP were recruited from GPs or rheumatologists. The inclusion criteria were ≥16 years old and ≥2 swollen joints lasting ≥4 weeks. In this study we included patients recruited from 1990 to 1994 (cohort 1) and those recruited from 2000 to 2004 (cohort 2). Patients were excluded from this analysis if their baseline assessment took place >2 years after symptom onset. Patients were also excluded if they were subsequently diagnosed with a condition other than RA, psoriatic arthritis, postviral arthritis or undifferentiated arthritis. Patients gave written informed consent. More details about NOAR can be found elsewhere.[18]

Assessments

Patients were assessed at baseline and at 1, 2, 3, 5, 7 and 10 years thereafter. Patients were only assessed beyond year 5 if they had documented swollen joints on two or more occasions or had received disease-modifying antirheumatic drugs (DMARDs) or oral corticosteroids by the fifth year assessment. Demographics were collected at baseline. A research nurse performed a 51 swollen and tender joint count (SJC51/TJC51), from which 28 joint counts were derived. Blood samples were taken at baseline, separated and frozen for future analysis. C reactive protein level (CRP; mg/L), rheumatoid factor positivity (RF; latex test, positive cut-off 40 units/mL) and anticyclic citrullinated peptide antibody positivity (anti-CCP2; tested using the Axis-Shield Diastat Anti-CCP Kit, Dundee, UK; cut-off 5 units/mL) were determined from these samples. The three-component DAS28-CRP was calculated.[19] The 2010 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) criteria for RA were applied retrospectively to the baseline characteristics of the patients.[20] Patients reported smoking status, and the start and stop dates for all sDMARDs, bDMARDs and oral steroids at each assessment.

Outcomes

Disease activity was assessed using SJC51 and TJC51 at each assessment, other than assessments 5 and 7 at which joint counts were not performed. Disability was self-reported at each assessment using the HAQ adapted for British use.[21] This is a validated self-report measure of functional disability that yields a score from 0 (no disability) to 3 (maximum disability). Patients were flagged with the Office for National Statistics (ONS), who provided copies of death certificates as patients died, including date and cause of death, coded using the International Classification of Diseases (ICD) V.9 and V.10. ICD9 codes were recoded to the corresponding ICD10 codes. Initially all-cause mortality was assessed, before assessing death from cancer (ICD10 codes C00–D48), cardiovascular disease (CVD; ICD10 codes I00–I99) or respiratory disease (ICD10 codes J00–J99) as the underlying cause of death. For mortality analysis patients were censored 10 years after symptom onset or on emigration from the country (n=7). Due to ONS flagging, mortality data were complete, regardless of whether patients stopped attending follow-up assessments. The ONS also provided age-specific and sex-specific all-cause and CVD specific mortality rates by calendar year for the Norfolk population (1990–2013) (online supplementary table 1).

Statistical analysis

The baseline and 10-year demographic, clinical and treatment characteristics of each cohort were summarised using descriptive statistics. Quantile, logistic or negative binomial regression was used to compare the baseline and 10-year scores between cohorts, depending on the type and distribution of the data. Age at symptom onset and gender were controlled for initially; then other baseline variables were included in the tenth year outcome models (age, gender, symptom duration at baseline, smoking status, SJC51, TJC51, RF, anti-CCP2, CRP, HAQ score and being on sDMARDs/steroids).

To analyse longitudinal disease activity and disability, the median SJC51, TJC51 and HAQ scores over 10 years are displayed using fractional polynomial smoothed plots. Population-average negative binomial regression was used to compare the SJC51 and TJC51 of the two cohorts over the repeated measures. HAQ scores were compared between cohorts over follow-up using generalised estimating equation analysis using the identity link function. All models were initially adjusted for baseline age and gender, before controlling for other baseline characteristics (listed above). Time-varying smoking status was then included in the model to control for differences in prevalence of smoking between the cohorts. Lastly, time-varying DMARD and steroid exposure were included to assess whether differences in medication usage between the two cohorts accounted for any difference in the outcomes. To analyse mortality, Kaplan-Meier survival curves were plotted. A Cox proportional hazards model was used to compare the risk of mortality between cohorts, with the same adjustments made as above. The proportional hazards assumption was met. An MRR was calculated using Poisson regression, which allows comparisons of mortality rates between cohorts, adjusted for the background age-specific and gender-specific mortality rates of the Norfolk population. This model was initially adjusted for baseline age and gender, before adjusting for further baseline characteristics (see above). For this analysis, patients were censored after 10 years of follow-up or at the end of 2013, whichever came first. To analyse risk of specific causes of death, competing risks regression analyses were applied, with each of the three causes of death (cancer, CVD, respiratory disease) as the outcome of interest in turn, with competing risks being other causes of death.[22] An MRR was also calculated for CVD mortality, using the same methods as above.

Multiple imputation using iterative chained equations was used to account for missing data at assessments which patients attended. Imputed variables were only used as covariates in regression analyses. In sensitivity analysis, we restricted the analyses to those patients fulfilling the 2010 criteria for RA at baseline (n=961). All analyses were performed using Stata V.13.1.

RESULTS

In total 1653 patients were included in this study: 1022 patients in cohort 1 and 631 in cohort 2 (table 1). Patients in cohort 2 were older at symptom onset and had longer symptom duration at baseline (median (IQR) age (years): cohort 1=54 (41–67), cohort 2=58 (47–70), median difference=4 (95% CI 2 to 6); median (IQR) symptom duration (months): cohort 1=5.1 (2.7–9.4), cohort 2=6.6 (3.9–11.3), median difference=1.5 (95% CI 0.9 to 2.2)). Cohort 2 had lower SJC51 and TJC51 at baseline (median (IQR) SJC51: cohort 1=6 (2–13), cohort 2=3 (1–8), relative difference=−40% (95% CI −46% to −33%); median (IQR) TJC51: cohort 1=7 (3–16), cohort 2=4 (1–12), relative difference −25% (95% CI −33% to −16%)). However, a greater proportion of patients in cohort 2 were on sDMARDs at the time of the baseline assessment (n (%) on sDMARDs at baseline: cohort 1=153 (15.0%), cohort 2=278 (44.1%), OR 4.47 (95% CI 3.54 to 5.65)). Nevertheless patients in cohort 2

Table 1 Baseline characteristics of patients with inflammatory polyarthritis included in the analysis, stratified by cohort

	Cohort 1 (1990–1994)		Cohort 2 (2000–2004)		Median difference/OR/relative difference (95% CI)*
	N	Median (IQR)	N	Median (IQR)	
Age at symptom onset (years)	1022	54 (41–67)	631	58 (47–70)	4.00 (2.00 to 6.00)
Gender (n (%) female)	662 (64.8)		408 (64.7)		OR 0.99 (0.81 to 1.22)
Smoking status	1021		569		
Never, n (%)	323 (31.6)		181 (31.8)		
Ex-smoker, n (%)	424 (41.5)		245 (43.1)		RRR 1.03 (0.81 to 1.31)
Current smoker, n (%)	274 (26.8)		143 (25.1)		RRR 0.93 (0.71 to 1.22)†
Symptom duration (months)	1022	5.1 (2.7–9.4)	631	6.6 (3.9–11.3)	1.54 (0.89 to 2.18)
Swollen joint counts					
28	1022	5 (1–11)	631	2 (0–6)	– 39% (–45% to –31%)
51	1022	6 (2–13)	631	3 (1–8)	– 40% (–46% to –33%)
Tender joints counts					
28	1022	5 (2–12)	631	2 (0–8)	–29% (–37% to –20%)
51	1022	7 (3–16)	631	4 (1–12)	– 25% (–33% to –16%)
CRP (mg/L)	817	5 (0–16)	521	9.5 (3–22)	4.50 (2.87 to 6.13)
DAS28-CRP	817	3.95 (2.88–5.02)	521	3.60 (2.65–4.53)	–0.35 (–0.56 to –0.15)
HAQ	1010	0.75 (0.25–1.38)	616	0.88 (0.38–1.63)	0.13 (0.01 to 0.24)
RF status	891		553		
Positive, n (%)	252 (28.3)		201 (36.4)		OR 1.45 (1.15 to 1.82)
Anti-CCP2 status	759		511		
Positive, n (%)	178 (23.5)		161 (31.5)		OR 1.50 (1.17 to 1.93)
Met 2010 RA criteria, n (%)	614 (60.1)		347 (55.0)		OR 0.81 (0.66 to 0.99)
Current sDMARDs use, n (%)	153 (15.0)		278 (44.1)		OR 4.47 (3.54 to 5.65)
Treatment delay, months	565	9 (4–22)	471	6 (3–12)	–3.2 (–4.6 to –1.9)

*Quantile/logistic/negative binomial regression was used to compare the two cohorts on each variable depending on the type of variable. Cohort 1 is the reference category.
†Multinomial logistic regression used to compare smoking status between cohorts. Never smoking is the base outcome and cohort 1 is the reference category.
anti-CCP2, anticyclic citrullinated protein antibodies; CRP, C reactive protein; DAS28, Disease Activity Score (28); HAQ, Health Assessment Questionnaire; n, number of patients with available data; RA, rheumatoid arthritis; RF, rheumatoid factor; RRR, relative risk ratio; sDMARD, synthetic disease-modifying antirheumatic drugs.

had higher functional disability at baseline (median (IQR) HAQ score: cohort 1=0.75 (0.25–1.38), cohort 2=0.88 (0.38–1.63), median difference=0.13 (95% CI 0.01 to 0.24)) (see online supplementary table 2 for the baseline characteristics of patients with RA).

Cross-sectional analysis at 10 years

In total 947 (57.3%) patients attended the tenth year assessment (cohort 1=607 (59.4%), cohort 2=340 (53.8%)) (table 2) (see online supplementary table 3 for summary statistics regarding the reasons why patients left the cohort). Patients in cohort 2 had lower SJC51 (median (IQR) SJC51: cohort 1=1 (0–4), cohort 2=0.5 (0–2.5)) compared with cohort 1. After controlling for baseline characteristics, patients in cohort 2 had 33% lower SJC51 compared with cohort 1 at 10 years (95% CI −47% to −15%). The median TJC51 at 10 years were comparable between cohorts, while cohort 2 had a slightly higher median HAQ score; after adjusting for baseline characteristics,

Table 2 Characteristics at 10 years and median change from baseline, stratified by cohort

	Cohort 1 (1990–1994)		Cohort 2 (2000–2004)		Median difference/ OR/relative difference (95% CI)*	Median difference/ OR/ relative difference (95% CI)†
	N	Median (IQR)	N	Median (IQR)		
Age at 10-year assessment (years)	607	62 (51–72)	340	66 (56–75)	4.00 (1.55 to 6.45)	–
Gender (n (%) female)	422 (69.5)		238 (70.0)		OR 1.02 (0.77 to 1.37)	–
Swollen joint counts (51)	601	1 (0–4)	340	0.5 (0.0–2.5)	–36% (–49% to –20%)	–33% (–47% to –15%)
Median change from baseline	601	–4 (–10 –−1)	340	–2 (–6– 0)		
Tender joints counts (51)	601	2 (0–11)	340	2 (0–8)	–5% (–24% to 19%)	2% (–20% to 30%)
Median change from baseline	601	–3 (–11–1)	340	–1 (–6–2)		
HAQ	597	0.88 (0.25–1.63)	336	1.00 (0.25–1.88)	0.01 (–0.18 to 0.20)	–0.01 (–0.16 to 0.14)
Median change from baseline	588	0.13 (–0.25–0.75)	330	0.00 (–0.38–0.63)		
Current sDMARDs use, n (%)	195 (32.1)		209 (61.5)		OR 3.35 (2.53 to 4.43)	OR 2.71 (1.91 to 3.86)

*Quantile/logistic/negative binomial regression was used to compare the two cohorts on each variable depending on the type of variable. Regressions comparing SJC, TJC, HAQ and current DMARD use between cohorts controlled for age and gender. Cohort 1 is the reference category.
†Quantile/logistic/negative binomial regression used to compare SJC, TJC, HAQ and number on sDMARDs between cohorts at 10 years. These models controlled for baseline: age, gender, symptom duration before baseline, smoking status, SJC (51), TJC (51), RF, anti-CCP, CRP, HAQ score and being on sDMARDs/steroids. Cohort 1 is the reference category.
anti-CCP2, anticyclic citrullinated protein antibodies; CRP, C reactive protein; DMARD, disease-modifying antirheumatic drug; HAQ, Health Assessment Questionnaire; n, number of patients with available data; sDMARD, synthetic disease-modifying antirheumatic drug; SJC, swollen joint count; TJC, tender joint count.

Table 3 Number and percentage of patients at each follow-up on different treatments and smoking status, stratified by cohort

	Follow-up assessment						
	0	1	2	3	5	7	10
Patients at assessment							
Cohort 1, n	1022	948	875	832	780	626	607
Cohort 2, n	631	588	530	533	498	407	340
Smoking status							
Cohort 1							
Never, n (%)	323 (31.6)	302 (31.9)	281 (32.1)	265 (31.9)	252 (32.3)	204 (32.6)	193 (31.8)
Ex-smoker, n (%)	424 (41.5)	416 (43.9)	392 (44.8)	385 (46.4)	361 (46.3)	288 (46.0)	287 (47.3)
Current, n (%)	274 (26.8)	229 (24.2)	202 (23.1)	180 (21.7)	167 (21.4)	134 (21.4)	127 (20.9)
Cohort 2							
Never, n (%)	233 (37.1)	218 (37.2)	201 (38.1)	203 (38.2)	195 (39.2)	157 (38.7)	134 (39.5)
Ex-smoker, n (%)	252 (40.1)	244 (41.6)	225 (42.6)	231 (43.5)	211 (42.5)	186 (45.8)	161 (47.5)
Current, n (%)	143 (22.7)	124 (21.2)	102 (19.3)	97 (18.3)	91 (18.3)	63 (15.5)	44 (12.9)
sDMARD*							
Cohort 1, n (%)	153 (15.0)	276 (29.1)	274 (31.3)	269 (32.3)	239 (30.6)	207 (33.1)	195 (32.1)
Cohort 2, n (%)	278 (44.1)	356 (60.5)	327 (61.7)	321 (60.2)	288 (57.8)	256 (62.9)	209 (61.5)
Methotrexate							
Cohort 1, n (%)	12 (1.2)	56 (5.9)	73 (8.3)	93 (11.2)	116 (14.9)	120 (19.8)	129 (21.3)
Cohort 2, n (%)	186 (29.5)	251 (42.7)	231 (43.6)	231 (43.3)	209 (42.0)	196 (48.2)	163 (47.9)
bDMARD*							
Cohort 1, n (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.2)	11 (1.8)
Cohort 2, n (%)	2 (0.3)	5 (0.9)	17 (3.2)	19 (3.6)	27 (5.4)	29 (7.1)	40 (11.8)
Oral steroids							
Cohort 1, n (%)	78 (7.6)	113 (11.9)	109 (12.5)	111 (13.3)	93 (11.9)	70 (11.2)	66 (10.9)
Cohort 2, n (%)	132 (20.9)	124 (21.1)	96 (18.1)	91 (17.1)	83 (16.7)	63 (15.5)	39 (11.5)

Percentages are given as a percentage of the number of patients in the corresponding cohort at the corresponding follow-up.
*sDMARDs included intramuscular gold salts, penicillamine, sulfasalazine, (hydroxy)chloroquine, methotrexate, azathioprine, cyclophosphamide and leflunomide. bDMARDs included etanercept, infliximab, adalimumab and rituximab.
bDMARD, biologic disease-modifying antirheumatic drug; sDMARD, synthetic disease-modifying antirheumatic drug.

the differences were not statistically significant (median (IQR) TJC51: cohort 1=2 (0–11), cohort 2=2 (0–8), relative difference 2% (95% CI −20% to 30%); median (IQR) HAQ: cohort 1=0.88 (0.25–1.63), cohort 2=1.00 (0.25–1.88), median difference −0.01 (−0.16 to 0.14)) (table 2). Similar results were seen when restricting the results to patients with RA who met the 2010 ACR/EULAR criteria (online supplementary table 4).

Longitudinal analysis over 10 years

Consistently more patients in cohort 2 were taking sDMARDs at each follow-up than cohort 1 (table 3). Oral steroid use was also consistently higher in cohort 2 compared with cohort 1. Very few patients in cohort 1 took bDMARDs at any time point as many would have reached their tenth anniversary assessment before bDMARDs were available, whereas 11.8% of patients in cohort 2 were taking bDMARDs at the tenth year follow-up. Data restricted to patients with RA only are shown in online supplementary table 5.

Figure 1A,B shows the unadjusted median SJC51 and TJC51 over the course of 10 years, stratified by cohort. Patients in cohort 2 had consistently lower median SJC51 over the course of 10 years and had lower median TJC51 at all follow-ups other than at follow-up 10, at which median TJC51 were comparable. Patients in cohort 2 had, on average, 17% lower SJC51 over 10 years than patients in cohort 1, after adjusting for baseline characteristics (relative difference −17%, 95% CI −23% to −10%), whereas TJC51 were comparable between cohorts over follow-up (relative difference 1%, 95% CI −7% to 10%). At baseline there were 274 current smokers in cohort 1 (26.8%) and 143 in cohort 2 (22.7%). Over the course of follow-up, 78 of these patients quit in cohort 1 (28.5%), whereas 62 quit in cohort 2 (43.4%). However, the inclusion of time-varying smoking status did not substantially alter the results; the inclusion of time-varying treatment also did not alter the results (table 4).

Figure 1C displays the median HAQ score over the course of 10 years, stratified by cohort. Patients in cohort 2 had on average 0.09 higher HAQ score over follow-up compared with cohort 1 (95% CI 0.03 to 0.16), after controlling for age and gender. However after controlling for baseline variables, the HAQ scores between the cohorts over 10 years were comparable (table 4). The same was true after including time-varying smoking and treatment into the model.

During 15 185 person-years of follow-up, 291 patients died (cohort 1=179 (17.5%), cohort 2=112 (17.8%)). Figure 1D shows the Kaplan-Meier curves for the two cohorts. Patients in cohort 2 had reduced risk of mortality compared with cohort 1 after adjusting for age and gender (HR 0.76, 95% CI 0.60 to 0.96) and after adjusting for baseline variables (HR 0.72, 95% CI 0.56 to 0.95). However, there was no significant difference in risk of mortality between the cohorts when restricting the analysis to patients who met the 2010 ACR/EULAR RA criteria at baseline (table 4). Including time-varying smoking and treatment into the model did not substantially alter the results (HR 0.77, 95% CI 0.58 to 1.02). The MRR for cohort 2 compared with cohort 1 was 0.96 (95% CI 0.75 to 1.22) after adjusting for age and gender. After further adjustment, the MRR was 0.78 (95% CI 0.56 to 1.10), indicating no significant difference in mortality

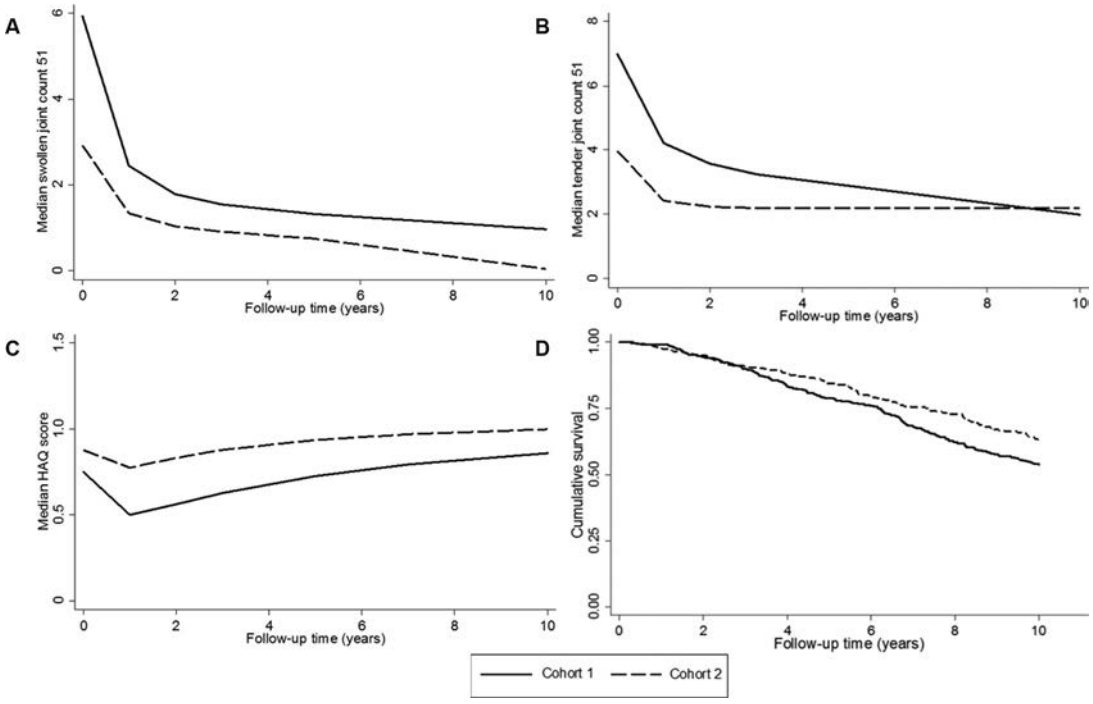

Figure 1 Outcome over 10 years stratified by cohort: (A) median swollen joint count 51, (B) median tender joint count 51, (C) median Health Assessment Questionnaire (HAQ) score and (D) Kaplan-Meier survival curve (adjusted for age and gender (age centred at 70 years)).

in cohort 2 after accounting for differences in the background risk of death between the cohorts.

The proportion of patients dying from CVD was lower in cohort 2 than in cohort 1 (CVD n (% total died): cohort 1=72 (40.2%), cohort 2=34 (30.4%)), and this was statistically significant, after adjusting for age and gender (subhazard ratio (SHR) 0.61, 95% CI 0.40 to 0.93) and after adjusting for baseline variables (SHR 0.58, 95% CI 0.37 to 0.93). Adjustment for time-varying smoking and time-varying treatment did not substantially alter the estimate (SHR 0.61 (95% CI 0.37 to 0.99)). The MRR for CVD mortality was 0.90 (95% CI 0.59, 1.37) when adjusting for age and gender. After further adjustment, the MRR was 0.77 (95% CI 0.48 to 1.24), indicating no significant difference in CVD mortality in cohort 2 compared with cohort 1, over the secular change of background risk of CVD mortality in the general population. The proportions of patients dying from cancer and respiratory disease were slightly higher in cohort 2 (cancer, n (% total died): cohort 1=53 (29.6%), cohort 2=38 (33.9%); respiratory disease, n (% total died): cohort 1=22 (12.3%), cohort 2=21 (18.7%)), and the risk of death from these causes was not significantly different between the two cohorts (table 4).

DISCUSSION

This analysis shows that patients with early IP with symptom onset in the new millennium had lower 10-year mortality risk than patients with symptom onset 10 years earlier. However, after controlling for the background mortality risk in the general population, the difference in mortality risk between the cohorts was non-significant. In addition, SJCs were significantly lower in patients with IP in cohort 2 compared with cohort 1. However, TJC and disability over 10 years were not improved for patients in cohort 2 compared with cohort 1.

A meta-analysis of 11 longitudinal studies of patients with RA reported decreasing mortality rate from 1955 to 1995.[23] Furthermore, a recent analysis of data from 31 countries assessing mortality with RA as the underlying cause reported a reduction in mortality rates over the period between 1987 and 2011 (mean pooled age-standardised rate: 1987–1989=7.1/million person-years, 2009–2011=3.7/million person-years).[24] However, our analysis did not demonstrate a significant reduction in 10-year mortality in patients with early IP, after accounting for secular changes in the background mortality risk of the general population. Our results are in line with a study of patients with RA from Ontario, Canada, which reported no significant change in MRR over the period 1996–2009.[25]

A recent analysis from a cohort of patients recruited in Olmsted County, Minnesota, reported a reduction in CVD mortality over a 10-year period for patients recruited from 2000 to 2007 compared with patients recruited from 1990 to 1999.[26] While the effect estimate from our analysis illustrated a reduction in CVD mortality, the CI overlapped 1, meaning our analysis cannot confirm the conclusions of the Olmsted County study.

The results of the disease activity and disability analyses extend previous literature looking at outcome of patients over 5 years.[14] However, our analysis is the first study to directly compare the longitudinal clinical outcome over 10 years between two cohorts of patients recruited 10 years apart. Including treatment variables as covariates tempered the association between cohort inclusion and long-term disability and SJC51, but disability remained comparable between cohorts and SJC51 remained significantly lower for cohort 2 compared with cohort 1. While this suggests that there may be factors other than treatment influencing the association between cohort and outcome, it is possible that there is residual confounding by treatment given and treatment response as we were only able to adjust for whether or not the patient was on a DMARD at each assessment.

Different patterns of device usage between the cohorts could be influencing the HAQ scores. However, recalculating the HAQ to remove the device adjustment did not alter the results (data not shown). An explanation for why there were no improvements

Table 4 Comparison of mortality risk, swollen joints, tender joints and functional disability between cohort 1 and cohort 2 over time

	N (number of events)	Age-adjusted and gender-adjusted, HR/SHR (95% CI)	Adjusted for baseline variables*, HR/SHR (95% CI)	Adjusted for baseline variables* and time-varying smoking, HR/SHR (95% CI)	Adjusted for baseline variables* and time-varying treatment/smoking, HR/SHR (95% CI)
Mortality risk†					
Patients with IP	1653 (291)	0.76 (0.60 to 0.96)	0.72 (0.56 to 0.95)	0.73 (0.56 to 0.95)	0.77 (0.58 to 1.02)
Patients with RA	961 (178)	0.87 (0.64 to 1.19)	0.83 (0.59 to 1.18)	0.85 (0.60 to 1.20)	0.95 (0.65 to 1.38)
Cancer mortality risk†					
Patients with IP	1653 (91)	0.97 (0.64 to 1.49)	1.06 (0.66 to 1.73)	1.06 (0.65 to 1.72)	1.04 (0.60 to 1.77)
Patients with RA	961 (55)	1.03 (0.60 to 1.79)	1.25 (0.67 to 2.32)	1.26 (0.67 to 2.35)	1.26 (0.60 to 2.62)
CVD mortality risk†					
Patients with IP	1653 (106)	0.61 (0.40 to 0.93)	0.58 (0.37 to 0.93)	0.53 (0.33 to 0.84)	0.61 (0.37 to 0.99)
Patients with RA	961 (68)	0.69 (0.41 to 1.18)	0.61 (0.34 to 1.12)	0.61 (0.34 to 1.12)	0.79 (0.41 to 1.52)
Respiratory disease mortality risk†					
Patients with IP	1653 (43)	1.26 (0.67 to 2.35)	1.33 (0.67 to 2.66)	1.11 (0.54 to 2.28)	1.01 (0.45 to 2.25)
Patients with RA	961 (25)	1.63 (0.75 to 3.53)	1.50 (0.61 to 3.69)	1.54 (0.64 to 3.72)	1.57 (0.62 to 4.00)

	N	Age-adjusted and gender-adjusted, relative difference (95% CI)	Adjusted for baseline variables*, relative difference (95% CI)	Adjusted for baseline variables* and time-varying smoking, relative difference (95% CI)	Adjusted for baseline variables* and time varing treatment/smoking, relative difference (95% CI)
Swollen joint counts (51)†					
Patients with IP	1653	−34% (−39% to −29%)	−17% (−23% to −10%)	−16% (−23% to −10%)	−15% (−21% to −8%)
Patients with RA	961	−30% (−36% to −23%)	−12% (−20% to −3%)	−11% (−20% to −2%)	−5% (−14% to 6%)
Tender joint counts (51)†					
Patients with IP	1653	−3% (−11% to 5%)	1% (−7% to 10%)	1% (−7% to 10%)	−2% (−10% to 7%)
Patients with RA	961	0% (−10% to 12%)	−4% (−14% to 7%)	−4% (−14% to 8%)	−5% (−15% to 6%)

	N	Age-adjusted and gender-adjusted, mean difference (95% CI)	Adjusted for baseline variables*, mean difference (95% CI)	Adjusted for baseline variables* and time-varing smoking, mean difference (95% CI)	Adjusted for baseline variables* and time-varying treatment/smoking, mean difference (95% CI)
HAQ scores†					
Patients with IP	1651	0.09 (0.03 to 0.16)	0.05 (−0.00 to 0.10)	0.05 (−0.00 to 0.10)	0.01 (−0.05 to 0.06)
Patients with RA	960	0.08 (−0.01 to 0.17)	0.01 (−0.06 to 0.08)	0.01 (−0.06 to 0.08)	−0.05 (−0.12 to 0.02)

Cohort 1 is the reference category for all models.

*Baseline variables controlled for age, gender, time from onset to baseline, rheumatoid factor, anticyclic citrullinated protein antibodies, smoking status, HAQ, swollen/tender joint counts (51), C reactive protein, taking sDMARDs and Disease Activity Score 28.

†Mortality risk modelled using Cox proportional hazards model, cancer/CVD/respiratory disease modelled using competing risks regression, swollen and tender joint counts modelled using population-average negative binomial regression, and HAQ score modelled using generalised estimating equations analysis.

CVD, cardiovascular disease; HAQ, Health Assessment Questionnaire; IP, inflammatory polyarthritis; RA, rheumatoid arthritis; sDMARD, synthetic disease-modifying antirheumatic drug; SHR, subhazard ratio.

in 'subjective' outcomes (TJC, HAQ) could be that the expectation of treatment efficacy was higher in patients in cohort 2 compared with cohort 1. Thus a similar level of disability may be rated as higher in cohort 2 compared with cohort 1.[27] A qualitative study published in 2004 reported that some patients had very high expectations of the efficacy of treatment,[28] and expectations of patients have been demonstrated to be associated with differences in pain following joint replacement surgery[29 30] and adherence to medication.[31]

This study has a number of strengths. The continuous recruitment and standardised assessment of patients in NOAR over 20 years meant that we were able to compare the long-term outcomes of patients treated in different treatment eras. Adjusting for the baseline characteristics of the patients controlled for the secular change towards reduced severity of IP and RA at presentation.[16 17] As with any study following patients over an extended period, there was attrition in both cohorts. However the characteristics of the patients leaving the study did not differ between cohorts, and therefore attrition is not biasing the analysis. Furthermore, as sDMARD dose was not routinely collected for these cohorts, we were unable to analyse changes in dose over follow-up.

In conclusion we have shown a 17% reduction in SJCs in patients with more recent symptom onset. However the 10-year mortality, TJCs and functional disability of patients did not significantly differ between the cohorts.

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EXTENDED REPORT

Effect of pregnancy on disease flares in patients with systemic lupus erythematosus

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ABSTRACT

Objective Prior studies found conflicting results about whether lupus is likely to flare during or after pregnancy. Using a large cohort of pregnant and non-pregnant women with lupus, we estimated the effect of pregnancy on disease flares in systemic lupus erythematosus.

Methods Data were collected in the Hopkins Lupus Cohort 1987–2015. Women aged 14–45 years with >1 measurement of disease activity were included. The time-varying exposures were classified as pregnancy, postpartum or non-pregnant/non-postpartum periods. Flares were defined as: (1) change in Physician Global Assessment (PGA)≥1 from previous visit and (2) change in Safety of Estrogens in Lupus National Assessment-Systemic Lupus Erythematosus Disease Activity Index (SELENA-SLEDAI)≥4 from previous visit. A stratified Cox model estimated HRs with bootstrap 95% CIs.

Results There were 1349 patients, including 398 pregnancies in 304 patients. There was an increased rate of flare defined by PGA during pregnancy (HR: 1.59; 95% CI 1.27 to 1.96); however, this effect was modified by hydroxychloroquine (HCQ) use, with the HR of flares in pregnancy compared with non-pregnant/non-postpartum periods estimated to be 1.83 (95% CI 1.34 to 2.45) for patients with no HCQ use and 1.26 (95% CI 0.88 to 1.69) for patients with HCQ use. The risk of flare was similarly elevated among non-HCQ users in the 3 months postpartum, but not for women taking HCQ after delivery.

Conclusions Our study supports and extends previous findings that the incidence of flare is increased during pregnancy and within the 3 months postpartum. Continuing HCQ, however, appeared to mitigate the risk of flare during and after pregnancy.

INTRODUCTION

Systemic lupus erythematosus (SLE) is characterised by fluctuations of disease activity, with periods of high disease activity (ie, flares) followed by periods of low activity. The effect of pregnancy on disease activity in SLE has long been debated. Previous research has found that 19%–68% of women with SLE experience a flare during pregnancy.[1–11] Risk factors for flares during pregnancy include active disease at conception, prednisone use, kidney disease and previous flares.[2 5 7]

There are conflicting results about the effect pregnancy has on the health of SLE women. Some studies report an increased rate of flares during pregnancy, while others report no difference in disease activity.[8 9 12 13] A study by Lockshin *et al*[14]analysed flare characteristics of pregnant and non-pregnant patients with SLE and did not find a difference between women who were and were not pregnant. In contrast, Petri *et al*[8] found the rate of flare was greater during pregnancy than in non-pregnant controls, and a subsequent analysis by Ruiz-Irastorza *et al*[9] found the flare rates during pregnancy and 6 weeks postpartum were increased compared with non-pregnant, age-matched controls. However, as these studies of flares during pregnancy were published over 20 years ago, an updated analysis is warranted.

A limitation of the current literature is the inconsistency in which flares were defined, making it difficult to make comparisons across studies. Many previous studies were also limited by small sample size, which reduced power to determine differences in the rate of flares between pregnant and non-pregnant patients. Understanding the effect pregnancy has on disease activity is clinically significant for the patient, as high disease activity during pregnancy is associated with preterm births and pregnancy loss.[4 15 16] Additionally, examining the rate of flares during the postpartum period is important in determining if patients need to be more closely monitored in the months following pregnancy. The objective of the current analysis was to estimate the effect of pregnancy on disease flares in SLE.

METHODS

Study population

The Hopkins Lupus Pregnancy Cohort is a subset of the Hopkins Lupus Cohort, which has prospectively followed patients with SLE since 1987, with data available through February 2015. Patients meeting the American College of Rheumatology (ACR) or Systemic Lupus International Collaborating Clinics (SLICC) criteria for SLE[17–19] were eligible for enrolment in the cohort following informed consent. Patients enrolled in the Hopkins Lupus Cohort and patients with SLE seen in the Hopkins Obstetrics Clinics were referred to the Hopkins Lupus Pregnancy Cohort. Other patients were referred by their rheumatologists, the Maryland Lupus Foundation and self-referral.[8] Pregnant women were seen every 4–6 weeks during pregnancy by a single rheumatologist. During each visit, lupus disease activity (Physician Global Assessment of disease activity (PGA)[20] and Safety of Estrogens in Lupus National Assessment-Systemic Lupus Erythematosus Disease Activity Index (SELENA-SLEDAI)[21–23] was measured, medications were updated, and laboratory tests were conducted. Pregnancy outcome data were collected from patients at the first postpartum

visit or by telephone or email, if a woman did not continue care at the Lupus Center.

Exposures

Exposure was classified as pregnancy (yes/no), postpartum period (yes/no) or non-pregnant/non-postpartum period (unexposed). The exposure variables were included as time-varying covariates, so as to include all observations for an individual (including prepregnancy observations on women who became pregnant). The postpartum period was analysed separately as lasting 3 months and 12 months.

Outcomes

Disease flares during follow-up were classified by PGA and SELENA-SLEDAI:

1. Change in PGA≥1 from the previous visit.
2. Change in SELENA-SLEDAI≥4 from the previous visit.

Subject selection

During the study period, there were 2417 patients with SLE observed in the Hopkins Lupus Cohort, of which 2229 were female. Fifteen patients were removed due to lack of complete information on pregnancies, and an additional 350 patients were removed because SLE diagnosis occurred after age 45. In order to calculate flares, at least two disease activity measurements were required. Of the remaining patients, 1426 had more than one study visit; however, 77 of patients were observed only during pregnancy and were removed from the study population due to likely being systematically different from patients routinely followed in the cohort. The final analytic cohort consisted of 1349 women, including 304 women who had 398 pregnancies. There were 381 observed postpartum periods, with at least one visit during the postpartum period.

Analysis

All women in the cohort between the ages of 14 and 45 were included in the analysis, regardless of pregnancy status. The time of entry into the cohort was considered the initial measurement for all women. Patients were right censored and removed from the risk set at age 45, menopause, loss to follow-up, death or the end of follow-up. If patients had a gap of more than 1 year in study visits, patients were considered lost to follow-up but were allowed to re-enter the cohort when study visits resumed.

If a woman had more than one pregnancy, all pregnancies and postpartum periods were included. Incidence rate ratios and 95% CI were calculated separately for pregnancy and postpartum periods compared with non-pregnant/non-postpartum periods. The HRs of flares were estimated using a stratified Cox model based on the Prentice, Williams and Peterson total time approach using the PHREG procedure.[24 25] A stratified Cox model is a conditional model that does not assume independence of multiple events of flare.[26] Instead, the model took into account that a patient was not at risk for a second flare without having experienced a first flare. Using the same model, relative hazard rates of flare were calculated between (1) pregnant and non-pregnant/postpartum periods and (2) postpartum and non-pregnant/postpartum periods. Due to repeated events of flares being counted in the same patient and patients being allowed to exit and re-enter the analytic cohort, 95% CI were estimated with 1000 bootstrap replications sampled with replacement.[27] Potential covariates of interest included patient race, age at diagnosis, age at baseline and duration of disease at baseline. Confounders were defined by a 10% change in beta estimates when included in the model. None of these covariates were found to be confounders and were not included in any models.

Prednisone and hydroxychloroquine (HCQ) were explored as time-varying covariates. To test whether effects were similar for HCQ users and non-users, as well as prednisone users and non-users, interaction terms between each medication with the exposure were included in the model. Models exploring effect modification by HCQ, for example, would (1) estimate the HR of flare for pregnant women taking HCQ compared with non-pregnant/non-postpartum women taking HCQ, (2) estimate the HR of flare for pregnant women *not* taking HCQ compared with non-pregnant/non-postpartum women *not* taking HCQ and (3) compare these two HR to see if the HR for women taking HCQ differed from the HR for women *not* taking HCQ. Effect measure modification was determined by likelihood ratio test (α=0.20).

To account for the time-varying exposures (pregnancy, postpartum period and medication use) and the possibility for each individual to experience multiple events, a new patient ID was created when the exposure changed for each patient to account for the censoring that occurred in the model with a change in exposure and clustering within an individual as well as within a certain exposure group.[28] Both the original ID, to account for correlation across all observations for an individual, and new ID, to account for correlation within each exposure period for an individual, were included in the models. In order to determine if all women in the cohort were an appropriate comparator group for women who became pregnant, we performed a sensitivity analysis that included only women with an observed pregnancy in the cohort (n=304). To determine if changes in clinical practice affected the results, models were stratified by time period: before and after 2000. All analyses were conducted in SAS V.9.3 (Cary, North Carolina, USA).

RESULTS

The median age at cohort entry was 30.6 years and 29.4 years at the first pregnancy (table 1). The median duration of SLE at cohort entry was 2.0 years, and the median follow-up was 3.9 years. Of the 398 pregnancies, 85% were live births, of which 29% were preterm. HCQ was taken during 58% of pregnancies, and 80% of patients took HCQ at some point during follow-up. Forty-five per cent of pregnancies occurred between 1987 and 2000. The median number of visits was 5 during pregnancy, 1 postpartum and 11 in non-pregnant/non-postpartum periods.

PGA flares were more common during pregnancy compared with outside of pregnancy (table 2; HR: 1.59; 95% CI 1.27 to

Table 1 Demographics for patients with SLE at baseline and pregnant women at time of first pregnancy in cohort in the Hopkins Lupus Cohort, 1987–2015

	Total cohort at baseline (n=1349)	Pregnant women at first pregnancy in cohort (n=304)
Race, n (%)		
White	656 (48.6)	173 (56.9)
Black	546 (40.5)	102 (33.6)
Other	147 (10.9)	29 (9.5)
	Median (IQR)	**Median (IQR)**
Age, years	30.6 (25.5–36.8)	29.4 (26.1–33.2)
Duration of SLE, years	2.0 (0.3–6.7)	4.8 (1.7–9.6)

SLE, systemic lupus erythematosus.

Table 2 Association of pregnancy and lupus flares defined by PGA*: the Hopkins Lupus Cohort, 1987–2015 (n=1349)

	Flares	PY	Crude incidence per 100 PY	Crude IRR (95% CI)	Stratified Cox† HR (95% CI)
12-month postpartum period					
All patients (n=1349)					
Not pregnant/postpartum	2246	5583.2	40.2	1.0 (ref)	1.0 (ref)
Pregnancy	134	220.8	60.7	1.51 (1.27 to 1.80)	1.59 (1.27 to 1.96)
12 months postpartum	148	370.9	39.9	0.99 (0.84 to 1.17)	1.02 (0.83 to 1.25)
Patients with ≥1 observed pregnancy in the cohort (n=304)					
Not pregnant/postpartum	642	1790.4	35.9	1.0 (ref)	1.0 (ref)
Pregnancy	134	220.8	60.7	1.69 (1.40 to 2.04)	1.88 (1.48 to 2.49)
12 months postpartum	148	370.9	39.9	1.11 (0.93 to 1.33)	1.24 (0.96 to 1.66)
3-month postpartum period					
All patients (n=1349)					
Not pregnant/postpartum	2336	5786.3	40.4	1.0 (ref)	1.0 (ref)
Pregnancy	134	220.8	60.7	1.50 (1.26 to 1.79)	1.57 (1.26 to 1.92)
3 months postpartum	58	95.6	60.7	1.50 (1.16 to 1.95)	1.48 (1.07 to 1.95)
Patients with ≥1 observed pregnancy in the cohort (n=304)					
Not pregnant/postpartum	732	1993.5	36.7	1.0 (ref)	1.0 (ref)
Pregnancy	134	220.8	60.7	1.65 (1.38 to 1.99)	1.79 (1.40 to 2.42)
3 months postpartum	58	95.6	60.7	1.65 (1.26 to 2.16)	1.71 (1.11 to 2.52)

*Flare defined as change in ≥1 from PGA score at previous visit.
†Stratified Cox model is a conditional model that does not assume independence of multiple events of flares and allows different baseline hazards based on the number of previous flares a patient experienced.
IRR, incidence rate ratio; PGA, Physician Global Assessment of disease activity; PY, person-years.

1.96). There was no evidence of an increased rate of flare during the 12-month postpartum period, but there was an increase in flare in the initial 3 months postpartum (HR: 1.48; 95% CI 1.07 to 1.95). In the sensitivity analysis of only patients with an observed pregnancy, the incidence of flares during non-pregnant/non-postpartum periods decreased, suggesting women who became pregnant while in the cohort had, on average, fewer flares than women without a pregnancy. During pregnancy, almost half of flares occurred during the 3rd trimester, while 24% occurred during the 1st trimester. One-third of flares during pregnancy were scored PGA 2 or higher, compared with 40% of flares during non-pregnant, non-postpartum times.

When flares were defined by SELENA-SLEDAI, results were comparable to PGA (table 3), with a higher rate of flare during pregnancy (HR: 1.57; 95% CI 1.25 to 1.92). There was no evidence of an increased rate 12 months postpartum,

Table 3 Association of pregnancy and lupus flares defined by SELENA-SLEDAI*: the Hopkins Lupus Cohort, 1987–2015 (n=1349)

	Flares	PY	Crude incidence per 100 PY	Crude IRR (95% CI)	Stratified Cox† HR (95% CI)
12-month postpartum period					
All patients (n=1349)					
Not pregnant/postpartum	2641	5583.0	47.3	1.0 (ref)	1.0 (ref)
Pregnancy	140	220.8	63.4	1.34 (1.13 to 1.59)	1.57 (1.25 to 1.92)
12 months postpartum	170	370.9	45.8	0.97 (0.83 to 1.13)	1.09 (0.89 to 1.32)
Patients with ≥1 observed pregnancy in the cohort (n=304)					
Not pregnant/postpartum	708	1790.4	39.5	1.0 (ref)	1.0 (ref)
Pregnancy	140	220.8	63.4	1.60 (1.34 to 1.92)	1.82 (1.34 to 2.38)
12 months postpartum	170	370.9	45.8	1.16 (0.98 to 1.37)	1.32 (1.03 to 1.69)
3-month postpartum period					
All patients (n=1349)					
Not pregnant/postpartum	2748	5786.3	47.5	1.0 (ref)	1.0 (ref)
Pregnancy	140	220.8	63.4	1.34 (1.13 to 1.58)	1.36 (1.06 to 1.69)
3 months postpartum	63	95.6	65.9	1.39 (1.08 to 1.78)	1.37 (0.94 to 1.82)
Patients with ≥1 observed pregnancy in the cohort (n=304)					
Not pregnant/postpartum	815	1993.5	40.9	1.0 (ref)	1.0 (ref)
Pregnancy	140	220.8	63.4	1.55 (1.30 to 1.86)	1.61 (1.16 to 2.16)
3 months postpartum	63	95.6	65.9	1.61 (1.25 to 2.08)	1.61 (1.02 to 2.40)

*Flare defined as change in ≥4 from SELENA-SLEDAI score at previous visit.
†Stratified Cox model is a conditional model that does not assume independence of multiple events of flares and allows different baseline hazards based on the number of previous flares a patient experienced.
IRR, incidence rate ratio; PY, person-years; SLE, systemic lupus erythematosus; SLEDAI, SLE Disease Activity Index.

Table 4 Modification* by HCQ of HRs for the association of pregnancy and lupus flares: the Hopkins Lupus Cohort, 1987–2015 (n=1349)

	Patients exposed to HCQ				Patients unexposed to HCQ			
	Flares	PY	Crude incidence per 100 PY	HR† (95% CI)	Flares	PY	Crude incidence per 100 PY	HR† (95% CI)
PGA‡								
12-month postpartum period								
Not pregnant/postpartum	1458	3843.0	38.0	1.0 (ref)	788	1740.3	45.3	1.0 (ref)
Pregnancy	52	123.4	42.1	1.26 (0.88 to 1.69)	82	97.3	84.3	1.83 (1.34 to 2.45)
12 months postpartum	75	212.2	35.3	1.02 (0.72 to 1.32)	73	158.7	46.0	0.98 (0.67 to 1.31)
3-month postpartum period								
Not pregnant/postpartum	1510	3967.3	38.1	1.0 (ref)	826	1819.0	45.4	1.0 (ref)
Pregnancy	52	123.4	42.1	1.24 (0.86 to 1.73)	82	97.3	84.3	1.84 (1.37 to 2.44)
3 months postpartum	23	51.6	44.6	1.25 (0.71 to 1.87)	35	44.0	79.5	1.63 (1.04 to 2.39)
SELENA-SLEDAI§								
12-month postpartum period								
Not pregnant/postpartum	1765	3843.0	45.9	1.0 (ref)	876	1740.3	50.3	1.0 (ref)
Pregnancy	64	123.4	51.9	1.35 (0.92 to 1.81)	76	97.3	78.1	1.59 (1.17 to 2.09)
12 months postpartum	100	212.2	47.1	1.13 (0.88 to 1.44)	70	158.7	44.1	0.91 (0.64 to 1.20)
3-month postpartum period								
Not pregnant/postpartum	1834	3967.3	46.2	1.0 (ref)	914	1819.0	50.2	1.0 (ref)
Pregnancy	64	123.4	51.9	1.32 (0.91 to 1.79)	76	97.3	78.1	1.61 (1.20 to 2.10)
3 months postpartum	31	51.6	60.1	1.53 (0.96 to 2.25)	32	44.0	72.7	1.45 (0.87 to 2.11)

*To test whether effects were similar for HCQ users and non-users, an interaction term between each medication with the exposure was included in the model. Effect measure modification was determined by likelihood ratio test (α=0.20).
†Estimated by stratified Cox model, a conditional model that does not assume independence of multiple events of flares and allows different baseline hazards based on the number of previous flares a patient experienced.
‡Flare defined as change in ≥1 from PGA score at previous visit.
§Flare defined as change in ≥4 from SELENA-SLEDAI score at previous visit.
HCQ, hydroxychloroquine; PGA, Physician Global Assessment of disease activity; PY, person-years; SLE, systemic lupus erythematosus; SLEDAI, SLE Disease Activity Index.

but there was a non-statistically significant increase in flares in the initial 3-month postpartum (HR: 1.37; 95% CI 0.94 to 1.82). Similar to models of PGA flares, the incidence of SELENA-SLEDAI flare decreased during non-pregnant/non-postpartum periods when only women with an observed pregnancy were included. SELENA-SLEDAI flares most commonly occurred during the 3rd trimester (54% of flares). Half of flares during pregnancy and 45% of flares during non-pregnant, non-postpartum time were mild, with a score of 4–8. Only 15% of flares during pregnancy and 20% of flares during non-pregnant, non-postpartum times were scored ≥12.

HCQ use was found to be an effect modifier in the association of pregnancy and flares. The increase in flares during pregnancy appeared to only be present in women not taking HCQ. When flares were measured by PGA, the HR of flares in pregnancy compared with non-pregnant/non-postpartum periods was 1.83 (95% CI 1.34 to 2.45) for patients with no HCQ use and 1.26 (95% CI 0.88 to 1.69) for patients with HCQ use (likelihood ratio P value: 0.04; table 4). While HCQ appeared to have a similar effect in the 3-month postpartum period, with the HR of flares 1.63 (95% CI 1.04 to 2.39) without HCQ and 1.25 (95% CI 0.71 to 1.87) with HCQ, the difference did not meet our statistical definition for modification. When flares were measured by SELENA-SLEDAI, there was a modest decrease in the association between pregnancy and flares for women taking HCQ, but not to the extent that HCQ would be considered an effect modifier. However, when limited to only patients with an observed pregnancy in the cohort, the HR of SELENA-SLEDAI flares in pregnancy compared with non-pregnant/non-postpartum periods was 2.09 (95% CI 1.39 to 2.97) for patients with no HCQ use and 1.49 (95% CI 0.92 to 2.08) for patients with HCQ use (likelihood ratio P value: 0.07). No differences in race, age at diagnosis, age at baseline and duration of disease were found between HCQ users and non-users.

When the cohort was limited to visits after the year 2000, results were similar for flares defined by PGA and SELENA-SLEDAI (online supplementary table 1). Prior to 2000, more person-time during pregnancy was unexposed to HCQ (63.2 person-years (PY) compared with 20.9 exposed to HCQ). After 2000, the majority of patients during pregnancy, as well as most patients during non-pregnant periods, were treated with HCQ (102.6 PY compared with 34.1 PY unexposed to HCQ during pregnancy). When flares were measured by PGA, the HR of flares during pregnancy remained higher in patients not taking HCQ in both time periods (online supplementary table 2). When flares were defined by SELENA-SLEDAI (online supplementary table 3), however, the HR of flares during pregnancy was increased for patients unexposed to HCQ in the time period prior to 2000, but an opposite effect was observed in the time period after 2000.

Prednisone use was only found to be an effect modifier in the association of pregnancy and flares in the sensitivity cohort of patients with an observed pregnancy when flares were defined by SELENA-SLEDAI. The HR of flares in pregnancy compared with non-pregnant/non-postpartum periods was 1.91 (95% CI 1.23 to 2.81) in patients with no prednisone use and 1.44 (95% CI 0.98 to 2.01) in patients with prednisone use (likelihood ratio P value: 0.16). There was no evidence for modification by prednisone use in PGA models or other SELENA-SLEDAI models.

DISCUSSION

Previous studies found conflicting results about whether lupus was more or less likely to flare in pregnancy.[8–10 13 14 29] The present analysis is the largest cohort study to date and includes data collected over almost 30 years. When compared with non-pregnant women with SLE, pregnant women and recently pregnant women did appear to flare more frequently. However, women taking HCQ did not appear to have an increased risk of lupus flare in pregnancy or the postpartum period. Prednisone may also play a role in decreasing disease activity during pregnancy, as it was found to be an effect modifier for SELENA-SLEDAI flares in the sensitivity cohort of patients with an observed pregnancy.

The results support what has previously been reported in the literature, both within the Hopkins Lupus Cohort and in other pregnancy cohorts.[4 8 9 13] The initial effort to determine the impact of pregnancy on lupus activity in this cohort was completed by Petri and colleagues in 1991[8] and found that among the first 40 pregnant patients in this cohort, the rate of flare was greater during pregnancy (1.6 flares per PY) compared with non-pregnant controls (0.7 flares per PY). A previous analysis in this cohort by Clowse *et al*[4] reported that among patients seen at least 6 months prior to pregnancy, 12.5% had high disease activity (PGA≥2) compared with 21.3% of patients during pregnancy. Additionally, lupus activity was greater among patients who discontinued HCQ during pregnancy compared with patients who continued.[3] The current study extended previous work in the Hopkins Pregnancy Cohort by analysing a longer follow-up period and including comparisons to postpartum periods. Interestingly, the rate of flares per PY has dramatically decreased from the initial study, with the crude flare incidence in the entire cohort averaged around 0.6 flares per PY, ranging from 0.4 with HCQ to 0.8 without HCQ, highlighting the improvements in management of lupus during pregnancy over the past 25 years. We found that the protective effect of HCQ remained for flares measured by PGA when results were stratified prior to and after 2000. Of interest, we did not observe a similar pattern for flares measured by SELENA-SLEDAI, with a protective effect of HCQ observed in the time prior to 2000 but not after 2000.

An increased rate of flare during pregnancy has been observed in other SLE cohorts. Ruiz-Irastorza *et al*[9] found that the rates of flare during pregnancy and 6 weeks postpartum were increased compared with non-pregnant, age-matched controls (table 5). A study of 29 pregnancies in Hong Kong estimated a higher rate of flares during pregnancy compared with non-pregnant patients.[13] However, in contrast to our results, other studies have found no evidence of an increased rate of flare during pregnancy,[10 14 29] potentially due to differences in patient ethnicity, study design, sample size or definition of flare.

Fewer studies have examined postpartum flares. In this same cohort, Petri *et al*[8] reported a lower mean rate of flare after delivery than during pregnancy among 42 patients, with the rate of flare decreasing from 1.6 flares per PY during pregnancy to 0.7 per PY in the year after delivery. A study in Argentina observed 19% of patients flared during pregnancy, compared with 4% in the puerperium.[6] Ruiz-Irastorza *et al*[9] estimated a rate of flare during pregnancy of 0.08 per person-month, compared with 0.15 per person-month 8 weeks after pregnancy outcome, which decreased to 0.05 1-year postpartum. In our analysis, we defined the postpartum period according to two definitions and observed an increase rate of flare during a 3-month postpartum period, yet no increased rate during a 12 month postpartum period, suggesting the increased risk of flare experienced during pregnancy remains for several months postpartum.

We estimated HRs using stratified Cox models, which take into consideration the order in which flares occurred and allowed different baseline hazards based on the number of previous flares a patient had in the cohort.[26] Given that a patient with no history of flares likely has a different baseline hazard of flare than a patient who has had multiple previous flares, a model that takes this into account seems more appropriate. A limitation of our study design was patients were censored in the model when the exposure changed. We accounted for this by creating a new ID variable when a patient's exposure changed and included the original and new IDs in the model. However, this caused a patient's stratum for previous flares to be limited to the current exposure period, which may result in residual confounding. Even so, we view this residual confounding to be preferable to the potentially biased estimates of a crude model or an unadjusted counting process Cox model that would not account for any previous flares.

The present analysis benefited from including two disease activity indices in the same analytic cohort, which allowed us to compare how results might differ depending on the flare index used. We found that, although more flares were observed by SELENA-SLEDAI, the HRs for both indices were similar. Additionally, using data from all women enrolled in the cohort allowed us to analyse more of the disease history of patients. Because all patients may not be the most appropriate comparator

Table 5 Incidence of flares in SLE pregnancy

Reference	Country	Pregnancies	Flare definition	Incidence of flare per person-month		
				Non-pregnant patients	Pregnancy	Postpartum
Mintz *et al* (1986)[29]	Mexico	102	Onset of new signs of active disease in a previously inactive organ system, measured by clinical and laboratory variables	0.04	0.06	–
Petri *et al* (1991)[8]	USA	40	Change of ≥1.0 in PGA score since the preceding visit or during the last 93 days	0.05	0.14	0.05
Wong (1991)[13]	China	29	Evidence of acute synovitis; pleuritis or pericarditis with radiographic or echocardiographic changes; new neurologic or psychiatric symptoms; thrombocytopenia (<100×10^9/L); leucopenia (<4×10^9/L); haemolytic anaemia (positive antiglobulin (Coombs') test); new cutaneous lesions or active kidney disease (with abnormal urinalysis results, increasing proteinuria and/or low C3 and C4 levels)	0.04	0.08	–
Ruiz-Irastorza *et al* (1996)[9]	UK	78	Increase of ≥0.26 or more from the minimum Lupus Activity (LAI) score during follow-up	0.04	0.08	0.15
Present analysis	USA	398	Change of ≥1.0 in PGA score since the preceding visit	0.03	0.05	0.03–0.05
			Change of ≥4 in SELENA-SLEDAI score since the preceding visit	0.04	0.05	0.04–0.06

PGA, Physician Global Assessment of disease activity; SLE, systemic lupus erythematosus; SLEDAI, SLE Disease Activity Index.

group for women who became pregnant, we conducted a sensitivity analysis restricted to women with an observed pregnancy. We found that non-pregnant/non-postpartum flare rates do change depending on the group of women analysed, with women who had a pregnancy having a lower incidence of flare during non-pregnant/non-postpartum periods. We also considered patients who had more than a 1 year gap between study visits to be considered lost to follow-up, although patients were allowed to re-enter the analytic cohort. This was done to include patients who were under routine care and to allow for an appropriate comparator score for the calculation of disease flare. The disease activity of these patients during unobserved periods is unknown, and if a gap in visits was due to remission of the disease, it is possible we underestimated the person-time for low disease activity periods. Although we did not find any differences between HCQ users and non-users, there remains a possibility that non-users were patients with an allergy to HCQ, intolerant to HCQ or refused to take the medication. Additionally, flares captured in the analysis were based on flares observed at the Lupus Center; therefore, we were unable to include flares that occurred during hospitalisations.

Our study supports prior data suggesting HCQ may prevent lupus flares during pregnancy and now suggests that it also may prevent postpartum flares. While in prior decades many women with lupus were expected to flare during or after pregnancy, more recent data suggest that a large proportion of women have minimal disease activity throughout the period. We hypothesise that routine continuation of HCQ in modern lupus pregnancies may be the driving force for the diminution in lupus activity during and following pregnancy. The results suggest we can be more optimistic with many women with lupus: they do not need to expect a lupus flare during or after pregnancy, particularly if they continue HCQ.

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Patient consent Detail has been removed from this case description/these case descriptions to ensure anonymity. The editors and reviewers have seen the detailed information available and are satisfied that the information backs up the case the authors are making.

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EXTENDED REPORT

Are MRI-detected erosions specific for RA? A large explorative cross-sectional study

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ABSTRACT

Objectives MRI is recommended in the diagnostic process of rheumatoid arthritis (RA) to detect joint damage early. MRI-detected erosions are also present in symptom-free controls, especially at older age. It is unclear if RA-specific MRI-detected erosions can be distinguished from 'physiological' erosions in symptom-free individuals. This study compared MRI-detected erosions of patients with RA with healthy controls and with other arthritides.

Methods 589 newly presenting patients with early arthritis (238 RA, 351 other arthritides) and 193 symptom-free controls underwent contrast-enhanced 1.5T MRI of unilateral metacarpophalangeal and metatarsophalangeal (MTP) joints. Total erosion score (according to the Rheumatoid Arthritis MRI Scoring System), number, severity, location of erosions and simultaneous presence of MRI-detected inflammation (synovitis and/or bone marrow oedema) were compared; participants were categorised in three age groups (<40, 40–59, ≥60).

Results Patients with RA had statistically significant higher total erosion scores than controls but scores of individual persons largely overlapped. Grade ≥2 erosions and MTP5 erosions were specific for RA (specificity 98%–100% and 90%–98% for different age groups). MTP1 erosions were only specific if aged <40 (specificity 98%) and erosions with inflammation if aged <60 (specificity 91%–100%). ≥1 of the mentioned erosion characteristics were present in 29% of patients with RA. Comparing patients with RA with other arthritides revealed that grade ≥2 erosions and MTP5 erosions remained specific for RA (specificity ≥89%) as well as MTP1 erosions if aged <40 (specificity 93%), in contrast to erosions combined with inflammation (specificity 49%–85%).

Conclusions Total erosion scores of individual persons were largely overlapping. Erosion characteristics specific for RA were identified, but were infrequently present. Caution is needed not to overestimate the value of MRI erosions in the diagnostic process.

INTRODUCTION

Rheumatoid arthritis (RA) is characterised by joint inflammation that may lead to bone erosions. Traditionally, erosions are evaluated using conventional radiographs. Recently it has been recommended by the European League Against Rheumatism (EULAR) imaging task force that MRI is valuable to detect erosions early.[1] Indeed, MRI has shown to be more sensitive for structural damage in early RA than conventional radiographs.[2–9]

Radiographic erosions specific for RA are defined in the 2010 American College of Rheumatology/EULAR criteria[10] as erosions seen in at least three separate joints at the proximal interphalangeal (PIP), the metacarpophalangeal (MCP), the wrist and metatarsophalangeal (MTP) joints (specificity >80%, sensitivity 15%–29%).[11] However, for MRI-detected erosions a definition specific for RA has not yet been derived. Because MRI is more sensitive in detecting erosions than radiographic imaging, RA-specific MRI-detected erosions need to be characterised. Previously, it was shown that MRI-detected erosions are also observed in other rheumatic diseases and in healthy controls, especially at older age.[12–17] Thus, in order to prevent false-positive MRI results, it is important to distinguish RA-specific erosions from other erosions.

This cross-sectional study compared erosions in MCP and MTP joints as detected on MRI (evaluated using the Rheumatoid Arthritis MRI Scoring System (RAMRIS)) between patients with early RA at the time of diagnosis and symptom-free controls for different characteristics: besides the total erosion score, the number, severity and location of erosions and the simultaneous presence of MRI-detected inflammation (synovitis and/or bone marrow oedema (BME)) were compared. Second, patients with RA were also cross-sectionally compared with patients with early arthritis that presented with other diagnoses. Within patients who presented with undifferentiated arthritis (UA), erosions were compared between patients who did and did not progress to RA during the first year. All analyses were done with the ultimate aim to identify features of MRI-detected erosions that are specific for RA.

METHODS

Patients

Up to 598 patients who presented with early arthritis and were included in the Leiden Early Arthritis Clinic (EAC) between 2010 and 2014 were studied. The EAC is an inception cohort including patients with clinically confirmed arthritis and symptom duration <2 years. At baseline, questionnaires were administered, joint counts and blood samples were collected and MRI was performed.[18] Nine patients were excluded because no contrast agent was administered. Two weeks after inclusion, when results of regular investigations were known (this did not include information on MRI results), the initial diagnosis of the patients was documented by the rheumatologists. The clinical diagnosis of RA was verified by fulfilling the 1987 or 2010 criteria

at baseline.[10 19] Of the 589 patients, 238 had RA. The diagnoses of the remaining group with other arthritides (n=351) were UA (n=192), reactive arthritis (n=22), (pseudo)gout (n=15), psoriatic arthritis (n=34), inflammatory osteoarthritis (n=35), Lyme arthritis (n=3), paramalignant arthritis (n=1), systemic lupus erythematosus (n=4), other systemic disorder (n=7), mixed connective tissue disease (MCTD), vasculitis (n=2), sarcoidosis (n=3), spondyloarthropathy with peripheral arthritis (n=5), RS3PE (n=10) and other diagnosis (n=18).

In addition, 193 symptom-free controls were recruited by advertisements in local newspapers and websites as previously reported.[13] They had no history of RA or other inflammatory rheumatic diseases, no joint symptoms during the last month, no recent trauma (<1 year prior to MRI) and no arthritis at physical examination.

MRI and scoring

At baseline, MRI of the 2nd–5th MCP and 1st–5th MTP joints on the most painful side or in case of symmetric symptoms and in healthy controls on the dominant side was performed. MRI was performed on an MSK Extreme 1.5T extremity MRI system (General Electric, Wisconsin, USA). The MRIs of all subjects were made on the same scanner. Coronal T1-weighted fast spin echo (FSE) and contrast-enhanced coronal and axial T1-weighted FSE with frequency-selective fat suppression were obtained. Further details on the scan protocol are provided in the online supplementary methods. Erosions, BME and synovitis were scored according to the RAMRIS method, with the exception that BME was assessed on a contrast-enhanced T1-weighted fat-suppressed sequence.[20] According to the RAMRIS method, erosions were defined as sharply marginated bone lesions, with correct juxta-articular localisation and typical signal characteristics, which are visible in two planes with a cortical break seen in at least one plane. All bones were scored separately for erosions on a scale of 0–10, based on the proportion of eroded bone (0: no erosion, 1: 1%–10% of bone eroded, 2: 11%–20%, and so on). The total erosion score was calculated by summing the erosion score in the MCP and MTP joints (range 0–180). Each MRI was scored by two readers, blinded to any clinical data. Intrareader intraclass correlation coefficients (ICC) and inter-reader ICCs were ≥0.86 (see online supplementary methods).

Erosion characteristics

The total erosion score (hence a combination of number of erosions and severity), number, severity and location of erosions were studied on the person level. The presence of concomitant inflammation was studied. This comprised the presence of BME in the same bone or the presence of synovitis around the same bone as where the erosion was located. These analyses were done on person and on bone level. For the total erosion score the mean of two readers was used. When assessing number, severity, location and the combination of erosions with inflammation, MRI erosions were considered present when the mean of both readers was ≥1 at a specific bone. Grade ≥2 erosions indicate that >10% of the bone is eroded.

Statistical analyses

First, total erosion scores of patients with RA were compared with scores of controls. A linear regression analysis adjusted for age and gender was used with the total erosion score as outcome and group (RA/healthy control) as independent variable. Erosion scores were log transformed (log10(score+1)) to approximate a normal distribution. The reported effect sizes were back-transformed to the normal score and indicated how many times the erosion scores of patients with RA were higher than that of controls. Thereafter, patients were stratified in three age groups (<40, 40–59, ≥60 years) and frequencies of erosion characteristics were compared between groups. Test characteristics were determined. Similar analyses were performed comparing patients with RA with other arthritides. Finally, the diagnostic value of MRI-detected erosions in patients with UA was assessed. SPSS V.23.0 (IBM) was used. P values <0.05 were considered significant.

Table 1 Baseline characteristics of patients with RA, symptom-free controls and patients with other arthritides

	Patients with RA (n=238)	Symptom-free controls (n=193)	Patients with other arthritides (n=351)
Age in years, mean (SD)			
Female, n (%)	159 (67)	136 (70)	204 (58)
Symptom duration in weeks, median (IQR)	15 (8–29)	NA	9 (4–26)
66-SJC, median (IQR)	6 (2–11)	NA	2 (1–4)
68-TJC, median (IQR)	9 (5–15)	NA	3 (2–8)
CRP (mg/mL), median (IQR)	9 (3–21)	NA	4 (3–13)
RF positivity, n (%)	147 (64)	NA	39 (12)
ACPA positivity, n (%)	123 (52)	NA	14 (4)

Some serology data were missing as follows: in patients with RA: RF n=10, ACPA n=1; in patients with other arthritides: RF n=15, ACPA n=12.
ACPA, anti-citrullinated protein antibodies; CRP, C-reactive protein; NA, not applicable; RA, rheumatoid arthritis; RF, rheumatoid factor; SJC, swollen joint count; TJC, tender joint count.

RESULTS

Patient characteristics

Baseline characteristics of patients and symptom-free controls are presented in table 1.

At group level, patients with RA have slightly higher MRI-detected erosion scores than symptom-free controls, but on the individual level there is a large overlap

First, the total erosion scores were evaluated. In both the group of patients with RA and that of symptom-free controls the MRI erosion score was associated with age (figure 1A, online supplementary table 1). When comparing the erosion scores of patients with RA and controls, patients with RA had 1.20 (95% CI 1.08 to 1.33, P<0.001) times higher erosion scores than controls, independent of age and gender. This effect size indicates that patients with RA had in general a 20% higher total erosion score than controls. Despite the significant difference there was a large overlap of MRI erosion scores between patients with RA and controls, as visually no separate clustering of groups was observed (figure 1A). Thus, total erosion scores could not differentiate patients with RA from controls on the individual level.

Grade ≥2 MRI erosions are more prevalent in patients with RA than in symptom-free controls

Then other erosion characteristics were studied to search for RA-specific characteristics. Because of the association with age, all analyses were stratified for age group (<40, 40–59 and ≥60 years). Since the total erosion score is a combination of the number of erosions and severity, both characteristics were evaluated separately. The median total number of erosions

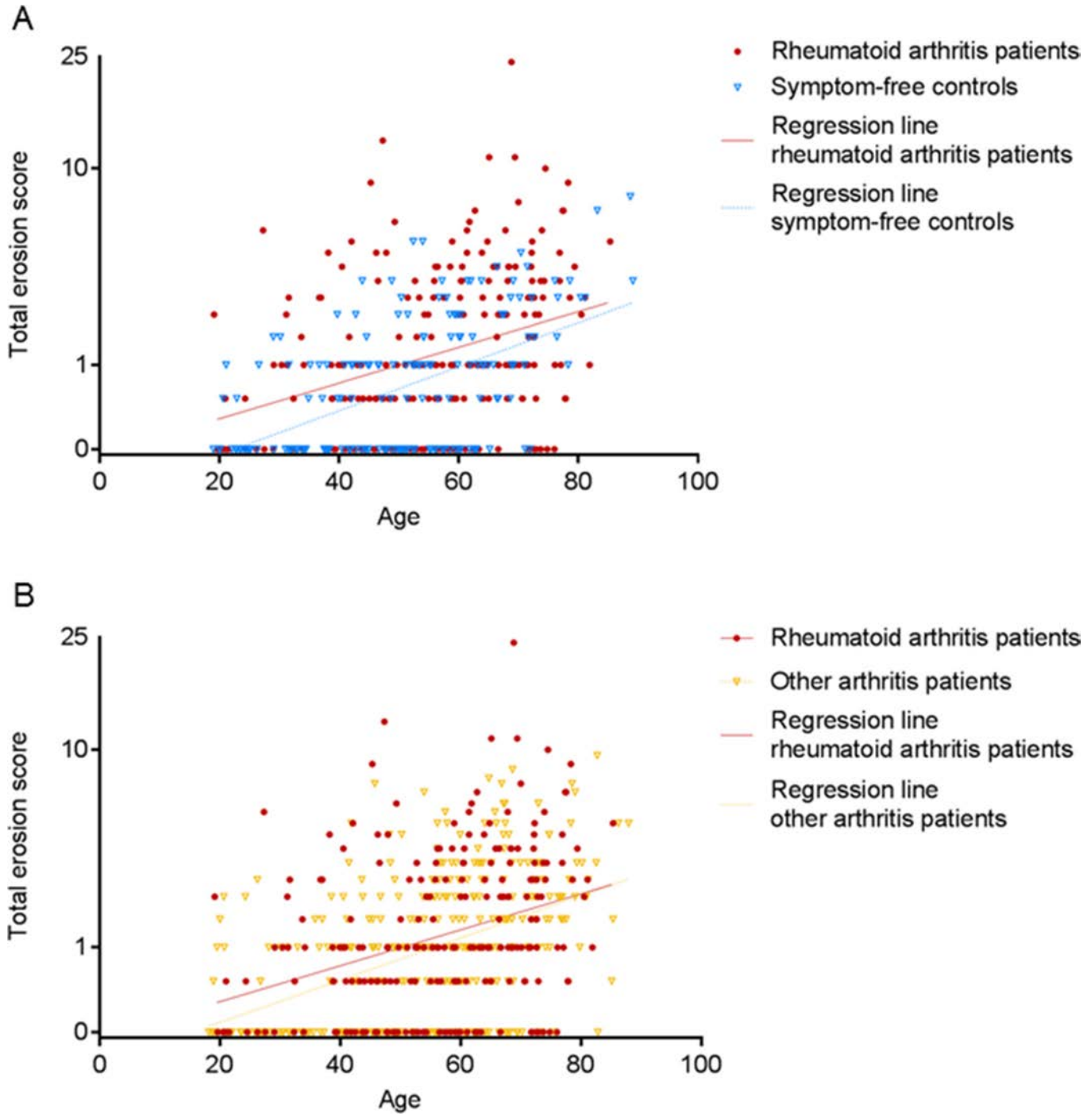


Figure 1 MRI-detected erosions in metacarpophalangeal (MCP) and metatarsophalangeal (MTP) joints in relation to age in patients with rheumatoid arthritis (RA) and in controls (A) and in patients with other arthritides (B); both figures show an overlap at the individual level. Linear regression analyses were performed with the mean total erosion score as detected by MRI as outcome and group (patients with RA and healthy controls (A) or other arthritides (B)), age and gender as independent variables. Y-axis is log transformed.

was 1.0 (IQR 0–2.0) for patients with RA and 0 (IQR 0–1.0) for symptom-free controls (Mann-Whitney U test: P=0.001). Within the different age groups there were no significant differences in the two oldest groups. In the group <40 years, patients with RA had more erosions than controls (median 0 (IQR 0–1.0) vs 0 (IQR 0–0), P=0.007) though differences were too small to identify a number of MRI-detected erosions that were specific for RA.

To determine whether grade ≥2 erosions were RA specific, the frequency of grade ≥2 erosions was considered per joint location (online supplementary table 2). This revealed that grade ≥2 erosions were almost exclusively present in RA (specificity 98%–100% for different age groups, table 2). However, within all age groups they were only sporadically observed in RA at disease presentation (sensitivity 5%–10%). Evaluation on person level showed that 8% of the patients with RA had at least one grade ≥2 erosion in an MCP and/or MTP joint, while in controls this was only 1% (table 3). Thus, the presence of grade ≥2 erosions was highly specific for RA, but also infrequent in RA at disease presentation.

Table 2 Test characteristics of grade ≥2 erosions (A), an erosion in MTP5 (B), an erosion in MTP1 (C) and erosions in combination with inflammation (D) for RA

	Patients with RA		Patients with RA versus healthy controls	Patients with RA versus patients with other arthritides
	Sensitivity (95% CI)		Specificity (95% CI)	Specificity (95% CI)
A	Test characteristics of grade ≥2 erosion			
	<40 years	9% (3 to 24)	100% (93 to 100)	100% (95 to 100)
	40–59 years	5% (2 to 12)	99% (94 to 100)	96% (91 to 98)
	≥60 years	10% (6 to 17)	98% (90 to 100)	96% (91 to 98)
B	Test characteristics of erosion in MTP5			
	<40 years	24% (13 to 41)	98% (90 to 100)	100% (95 to 100)
	40–59 years	9% (5 to 17)	90% (82 to 95)	89% (83 to 93)
	≥60 years	16% (10 to 24)	92% (82 to 97)	90% (84 to 94)
C	Test characteristics of erosion in MTP1			
	<40 years	18% (9 to 34)	98% (90 to 100)	93% (84 to 97)
	40–59 years	19% (12 to 28)	86% (77 to 91)	77% (69 to 83)
	≥60 years	36% (27 to 45)	63% (50 to 75)	66% (57 to 73)
D	Test characteristics of erosion in combination with inflammation			
	<40 years	33% (20 to 50)	100% (93 to 100)	85% (75 to 92)
	40–59 years	24% (17 to 33)	91% (83 to 95)	69% (61 to 76)
	≥60 years	56% (47 to 65)	71% (58 to 82)	49% (41 to 57)

MTP, metatarsophalangeal joint; RA, rheumatoid arthritis.

MTP5 and MTP1 are more often affected in patients with RA than in symptom-free controls

Then the location (the affected MCP or MTP joint) was assessed (table 4). In both patients with RA and controls, most erosions were located in the proximal part of the MCP and MTP joints: in patients with RA, 82%–95% of the erosions were located proximal in the joint, and in controls this was 81%–100% for the different age groups. As presented in table 4, overall the MCP and MTP bones that were frequently affected in patients with RA were also frequently affected in healthy controls. For instance, MCP2 and MCP3 were predilection sites for MRI-detected erosions in RA, but also in controls. However there were also some differences: erosions in MTP5 were more frequently present in patients with RA than in controls in most age groups (specificity 90%–98% for different age groups, table 2). In addition, erosions in MTP1 in the age group <40 almost exclusively occurred in RA (specificity 98%); the specificity was lower in older age groups (specificity 86% if aged 40–59 and 63% if aged ≥60). Examples of MRI-detected erosions are shown in figure 2.

Erosions with the simultaneous presence of BME and/or synovitis are more frequent in patients with RA than in symptom-free controls

Then we questioned whether the combined presence of erosions with surrounding inflammation was specific for RA. At bone level, in patients with RA, 33% (95/285) of the total number of MCP and MTP bones with erosions only had erosions without synovitis and/or BME while in controls this was 77% (105/136, table 5). Similarly, when analysed on person level, 16% of the patients with RA only had erosions without inflammation and 40% had at least one erosion with inflammation in that same joint while in controls this was 30% and 12%, respectively (table 3). When analysing the different age groups, it appeared that within the age group <40 years, the simultaneous presence of erosions with inflammation was exclusively observed in patients with RA (specificity 100%). In the age group 40–59 years, the specificity was 91% and it was lower in persons aged ≥60 (specificity 71%), since in this age group erosions with inflammation were also observed in healthy controls (table 2). Thus, the presence of erosions with inflammation was specific for RA, but only if aged <60.

Altogether, the presence of grade ≥2 erosions and MTP5 erosions was specific for RA in all age groups, erosions with inflammation were specific for RA if aged <60 and MTP1 erosions if aged <40. Although these erosion characteristics were highly specific for RA, only 29% of all patients with RA had ≥1 erosion(s) with ≥1 of these characteristics.

Erosions in MTP5 and grade ≥2 erosions in all age groups and erosions in MTP1 if aged <40 remain specific for RA when compared with patients with other arthritides

Thus far, different erosion characteristics were compared between patients with RA and controls revealing some RA-specific characteristics. The next question is whether these characteristics are truly RA specific or are also present in other arthritides. Therefore, all analyses were repeated with patients with other arthritides as reference group. The total erosion scores of both patient groups were not significantly different (beta 0.92; 95% CI 0.84 to 1.01, figure 1B). Comparison of the different erosion characteristics showed that the presence of grade ≥2 erosions was RA specific in all age groups (specificity 100% if aged <40% and

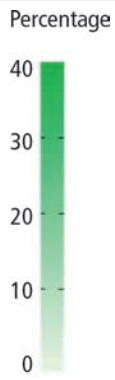
Table 3 Frequencies of patients with RA and controls with grade ≥2 erosions and with erosions with the simultaneous presence of local inflammation in an MCP and/or MTP joint; analyses on person level

			Grade ≥2 erosions		Erosions with inflammation	
		Number of persons with erosions	No grade ≥2 erosions	Grade ≥2 erosions	Erosion+ Inflammation−	Erosion+ Inflammation+
RA	<40 years (n=33)	14	11 (79%)	3 (21%)	3 (21%)	11 (79%)
	40–59 years (n=96)	39	34 (87%)	5 (13%)	16 (41%)	23 (59%)
	≥60 years (n=109)	79	68 (86%)	11 (14%)	18 (23%)	61 (77%)
		238				
Control	<40 years (n=51)	9	9 (100%)	0 (0%)	9 (100%)	0 (0%)
	40–59 years (n=90)	36	35 (97%)	1 (3%)	28 (78%)	8 (22%)
	≥60 years (n=52)	36	35 (97%)	1 (3%)	21 (58%)	15 (42%)
		193				

The presence of grade ≥2 erosions and erosions with inflammation (BME and/or synovitis) was evaluated per MCP and MTP bones according to the Rheumatoid Arthritis MRI Scoring System. Grade ≥2 erosions indicate that >10% of the bone is eroded. The presence of BME and/or synovitis was defined as a score of ≥1.
BME, bone marrow oedema; MCP, metacarpophalangeal joint; MTP, metatarsophalangeal joint; RA, rheumatoid arthritis.

Table 4 Location of erosions in bones of the MCP and MTP joints of patients with RA and symptom-free controls, depicted per age category (18–39, 40–59, ≥60 years)

		<40 years		40–59 years		≥60 years	
Erosions		RA n=33	Control n=51	RA n=96	Control n=90	RA n=109	Control n=52
MCP2	Proximal	6	6	7	11	23	23
	Distal	0	0	4	2	9	10
MCP3	Proximal	9	8	15	12	30	23
	Distal	0	0	0	1	5	4
MCP4	Proximal	3	0	3	2	9	8
	Distal	0	0	0	0	0	0
MCP5	Proximal	0	2	5	6	20	13
	Distal	0	0	0	0	1	6
MTP1	Proximal	18	2	19	14	36	37
	Distal	0	0	2	0	11	6
MTP2	Proximal	0	0	2	0	4	0
	Distal	3	0	0	1	4	0
MTP3	Proximal	0	0	2	0	6	0
	Distal	0	0	0	0	2	0
MTP4	Proximal	3	0	3	0	1	0
	Distal	0	0	0	0	0	0
MTP5	Proximal	24	2	9	10	16	8
	Distal	0	0	2	0	1	2



Values are the percentages of persons with an erosion of all persons in that age category. The presence of an erosion is defined as an erosion score of at least 1 in that bone.
MCP, metacarpophalangeal joint; MTP, metatarsophalangeal joint; RA, rheumatoid arthritis.

96% if aged 40–59 and ≥60, table 2, online supplementary table 3). Also, MTP5 erosions were highly specific for RA in all age groups (specificity 100% if aged <40, 89% if aged 40–59, and 90% if aged ≥60, table 2, online supplementary table 4). The specificity of MTP1 erosions was 93% in patients aged <40, but at higher age specificity decreased to 66%. Erosions with inflammation were less specific for RA (specificity 49%–85% within different age groups) as these were also present in other arthritides. Thus, erosions with inflammation were not RA specific, but MTP5 erosions and grade ≥2 erosions were specific in all age groups and MTP1 erosions in patients aged <40. Twenty-one per cent of patients with RA had ≥1 erosion(s) with these characteristics (sensitivity 21%). Additionally, of all patients with erosions with one of these three finally identified features, 53% fulfilled the criteria for RA (positive predictive value (PPV) 53%), and of all patients without such erosions criteria were not fulfilled in 62% (negative predictive value (NPV) 62%).

MRI-detected erosions do not contribute to the identification of patients with UA that will progress to RA

Finally, the value of MRI-detected erosions was evaluated within patients with UA. Of the patients with UA, 15% (28/192) fulfilled the criteria for RA after 1 year. Of these patients, 11% had an RA-specific erosion at baseline, whereas 9% of the non-convertors had an RA-specific erosion (OR 1.3; 95% CI 0.3 to 4.8).

DISCUSSION

Radiographic erosions specific for RA have been defined as the presence of ≥3 radiographic erosions on MCP, PIP, wrist or MTP joints and their presence is considered sufficiently specific to classify RA.[11] MRI is a sensitive imaging modality that depicts cortical defects and therefore is suitable to detect erosive damage. Thus far, it was unknown which MRI-detected erosions on hand and foot joints are specific for RA. This large cross-sectional MRI study showed that on the group level, patients with RA had higher MRI-detected erosion scores in MCP and MTP joints than controls, but also that there was a large overlap on the individual level. Several erosion characteristics were studied in detail; this was done within three age strata as the total MRI erosion score was associated with age. Compared with controls from the general population, four characteristics were identified as RA specific: grade ≥2 erosions, MTP5 erosions, MTP1 erosions if aged <40 and erosions with local inflammation if aged <60. At least one of these characteristics is present in 29% of patients with RA.

Subsequently, patients with RA were compared with patients with early arthritis with other diagnoses, because studies comparing established cases and healthy controls will reveal the maximal contrast. Differences are often smaller when more clinically relevant patient groups are studied.[21 22] Indeed, we observed that some erosion characteristics that were specific for RA when compared with controls were not specific when RA was compared with other arthritides. This was most prominent for the combined presence of erosions with inflammation. Nonetheless, some characteristics (grade ≥2 erosions, MTP5 erosions, MTP1 erosions in persons aged <40) were RA specific in both settings. Twenty-one per cent of patients with RA had ≥1 erosion(s) with ≥1 of these characteristics.

Although some erosion characteristics were identified as RA specific, an important overlap between patients with early RA and controls was observed. It has been recommended that novel imaging modalities, such as MRI, can be used to detect erosions early.[1] The present data show that if all MRI-detected erosions (according to RAMRIS) would be considered as characteristic for RA or disease, this would yield many false-positive results.

We used the RAMRIS definition of erosions that basically evaluated the volume of the erosion in relation to the assessed bone. Others showed that small lesions on high-resolution peripheral quantitative CT were not entirely specific for RA and suggested

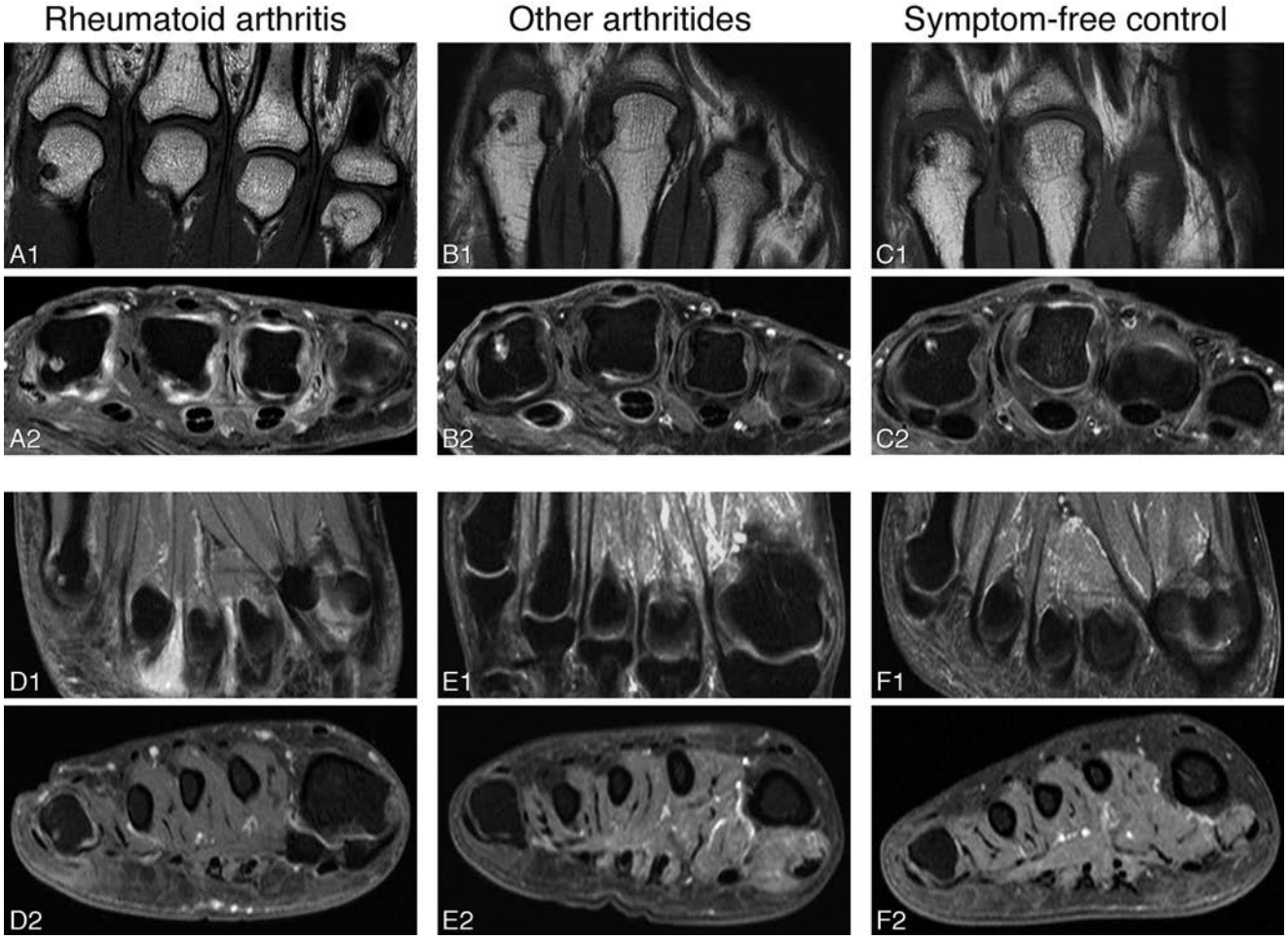


Figure 2 Examples of erosions in patients with rheumatoid arthritis (RA), patients with other arthritides and symptom-free controls. MR images of patients with RA (A, D), patients with other arthritides (B, E) and symptom-free controls (C, F). Examples of erosions in MCP2 (A–C) and of a small erosion in MTP5 are shown (D). Erosions in MCP2 were observed in all different groups (A–C) while erosions in MTP5 were mainly observed in patients with RA (D). Patient B was diagnosed with polyarticular gout. Person C was aged 48 years. Coronal (A1, B1, C1, D1, E1, F1) and axial (A2, B2, C2, D2, E2, F2) images are shown. MRI sequences included coronal T1-weighted fast spin echo (FSE) sequences and axial T1-weighted FSE sequences with fat suppression after contrast enhancement. MCP, metacarpophalangeal joint; MTP, metatarsophalangeal joint.

that lesions >1.9 mm in diameter were highly specific.[23 24] It remains to be determined if a phenotypic definition of MRI erosions, for instance, one that includes a description of the size of the cortical break, will be more discriminative; this is subject of further studies and is also considered within an ongoing EULAR task force.

Some of the findings on MRI-detected erosions are in line with previous findings on radiographic erosions. Radiographic

Table 5 Frequencies of erosions in combination with inflammation in MCP and MTP bones of symptom-free controls and patients with RA; analysis on bone level

			Number of MCP and MTP bones with erosions				
		Total number of MCP and MTP bones evaluated	Erosion+ BME− Synovitis−	Erosion+ BME+ Synovitis−	Erosion+ BME− Synovitis+	Erosion+ BME+ Synovitis+	Number of MCP and MTP bones without erosions
RA	<40 years	594	7 (32%)	7 (32%)	4 (18%)	4 (18%)	572
	40–59 years	1728	28 (39%)	10 (14%)	12 (17%)	21 (30%)	1657
	≥60 years	1962	60 (31%)	12 (6%)	65 (34%)	55 (29%)	1770
		4284					3999
Control	<40 years	918	10 (100%)	0 (0%)	0 (0%)	0 (0%)	908
	40–59 years	1620	45 (83%)	2 (4%)	5 (9%)	2 (4%)	1566
	≥60 years	936	50 (69%)	7 (10%)	9 (13%)	6 (8%)	864
		3474					3338

Values are the number of MCP and MTP bones with erosions and without erosions. MCP and MTP bones with erosions are divided in subgroups of erosions without BME and synovitis and with BME and/or synovitis. Erosions, BME and synovitis were defined as a score of ≥1 according to the Rheumatoid Arthritis MRI Scoring System.
BME, bone marrow oedema; MCP, metacarpophalangeal joint; MTP, metatarsophalangeal joint; RA, rheumatoid arthritis.

erosions have been shown to occur more frequently at disease onset with higher age.[25–31] MTP5 has been shown as a predilection site for RA-related erosions as well.[32–34] We observed that the large majority of erosions (both in RA and in the other groups studied) were located in the proximal bone of the joint which is completely in line with previous findings.[23 33 35]

Erosive lesions in symptom-free controls have also been reported in other studies.[12] The nature of these lesions is unclear. Because of the association with age, degenerative subchondral bone cysts may be one of the explanations. In addition, a very recent study, evaluating bone microstructure of MCP joints using high-resolution tomography and micro-CT, demonstrated that the number of so-called cortical microchannels (linking the synovial and bone marrow compartments) was higher in patients with RA than in healthy controls and was associated with erosions and age.[36] It is intriguing to speculate that these channels have a causal role in erosion development, both in RA and controls. Another possibility is that mechanical strains are involved in erosion development, since erosions were frequently located in the foot (49% of the erosions in patients with RA and 38% in symptom-free controls). However, a pathophysiological explanation for the findings done in symptom-free persons is beyond the scope of this study.

The location of erosions within the bone was not studied here. This information could not be discerned using RAMRIS as this method evaluates the volume of the erosive lesion per bone. However, previous studies have shown that the majority of erosive MRI lesions in MCP joints occurred adjacent to the radial collateral ligaments, both in patients with RA and in healthy controls.[37 38] Similar observations were done in a study in patients with RA and healthy controls on the location of erosions as detected on CT.[23] The location of erosions in the symptom-free controls that were studied here has been reported previously,[13] and showed that also in these persons erosions were present adjacent to the collateral ligaments and were not situated centrally in the bone. Because of these previous reports, showing no difference in location of erosions within the bone between patients with RA and controls, we anticipated that this characteristic will not result in further discrimination of RA-specific erosions from other erosions.

Cross-sectional analyses revealed that of all patients with an erosion that was identified as characteristic for RA 53% actually had RA (PPV). Likewise, 62% of all patients without such erosions did not have RA (NPV), whereas 38% did fulfil the criteria for RA. These data illustrate that the absence or presence of RA-specific erosions at disease presentation is of moderate value to identify patients who fulfil the criteria for RA at the same point in time.

Longitudinal analysis within patients with UA suggested that the presence of RA-specific erosions was also not predictive for the development of RA. However, this analysis was of limited power. Additionally, other outcomes, such as the start of disease-modifying antirheumatic drugs (DMARD), should be studied, since DMARD treatment might hamper progression to RA. Finally, it was not possible to study the different RA-specific erosion features separately due to the limited number of patients. Further studies are warranted.

We studied an early RA population. Thirty-six per cent of the patients were rheumatoid factor negative and 48% were anti-citrullinated protein antibody negative which is comparable to other early RA cohorts.[39 40] Our population is somewhat different from patients with RA included in clinical trials where generally a selection of patients with RA is included.

A limitation of this study is that it was cross-sectional in nature and that imaging follow-up was not studied. Sensitivity of readers could have been a problem that could be equally present in the three groups. The presence of serial MRI data facilitates the differentiation of erosions from vascular channels and entheseal attachments, as these should not change during follow-up. Erosions in contrast could progress over time, although this progression may also have been hampered by up-to-date treatment strategies. Serial MRIs were not made but would have been beneficial to evaluate if some erosions were falsely identified as such. However, if MRI will be used for early identification of patients in clinical practice, single MRI measurements will be made.

In conclusion, MRI-detected erosions (according to the RAMRIS definition) in MCP and MTP joints are not confined to RA, but also present in other arthritides and in symptom-free persons from the general population. On the individual level, there was a large overlap. Some erosion characteristics were identified as specific for RA (grade ≥2 erosions, MTP5 erosions and MTP1 erosions if aged <40), though these occurred in a minority (21%) of the patients. Longitudinal MRI may improve specificity; however, this was not tested in this study. The present data imply that if single measurements with novel imaging modalities such as MRI are used for the early detection of structural damage in clinical practice, the risk of false-positive findings should be considered.

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EXTENDED REPORT

Risk of preterm delivery and small-for-gestational-age births in women with autoimmune disease using biologics before or during pregnancy: a population-based cohort study

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ABSTRACT

Objectives To assess the risk of preterm delivery and small-for-gestational-age (SGA) births in women with autoimmune diseases using biologics before or during pregnancy.

Methods Using population-based administrative data in British Columbia, Canada, women with one or more autoimmune diseases who had pregnancies between 1 January 2002 and 31 December 2012 were included. Exposure to biologics was defined as having at least one biologic prescription 3 months before or during pregnancy. Each exposed pregnancy was matched with five unexposed pregnancies using high-dimensional propensity scores (HDPS). Logistic regression modelling was used to evaluate the association between biologics use and preterm delivery and SGA.

Results There were 6218 women with 8607 pregnancies who had an autoimmune disease diagnosis; of which 109 women with 120 pregnancies were exposed to biologics 3 months before or during pregnancy. In unadjusted analyses, the ORs for the association of biologics exposure with preterm deliveries were 1.64 (95% CI 1.02 to 2.63) and 1.34 (95% CI 0.72 to 2.51) for SGA. After HDPS matching with 600 unexposed pregnancies, the ORs for the association of biologics exposure and preterm deliveries were 1.13 (95% CI 0.67 to 1.90) and 0.91 (95% CI 0.46 to 1.78) for SGA. Sensitivity analyses using HDPS deciles, continuous HDPS covariate or longer exposure window did not result in marked changes in point estimates and CIs.

Conclusions These population-based data suggest that the use of biologics before and during pregnancy is not associated with an increased risk of preterm delivery or SGA births.

INTRODUCTION

Pregnancy is a unique state of coexistence of genetically different individuals, which is possible due to dramatic shifts in maternal immune function during pregnancy, protecting the fetus from immunological attack.[1] In women with chronic inflammatory diseases, this interaction between autoimmunity and pregnancy becomes complex. The pathology underscoring autoimmune diseases including rheumatoid arthritis (RA), ankylosing spondylitis (AS), psoriatic arthritis (PsA), psoriasis (Ps) and inflammatory bowel disease (IBD) are perpetuated mainly by the dysfunction of cytokines and chemokines regulating immune system activity, with tumour necrosis factor (TNF)-alpha being a key cytokine in this abnormal immune response.[2–5]

In pregnancy, TNF-alpha controls cyclo-oxygenases that affect blastocyst implantation, endometrial permeability and decidualisation,[6] and contributes to the process of labour.[7] Abnormally high levels of TNF-alpha and other cytokines have been implicated in pregnancy complications including preterm delivery, fetal growth retardation, early and unexplained spontaneous abortions, and miscarriages.[7–10] As such, evidence suggests that higher autoimmune disease activity at the time of conception and during pregnancy is correlated with increased risks of adverse maternal and neonatal outcomes.[11 12]

Biologics work to treat autoimmune diseases by modulating the immune system by targeting key inflammatory cytokines including TNF-alpha, interleukin (IL)-1, IL-6 or receptors of these cytokines.[13] With these medications available only within the last 15 years, their use by women during pregnancy has been growing and becoming more clinically acceptable.[14] However, prior studies on this topic included only a small number of women enrolled in registries, and with comparison groups often selected from external sources; furthermore, majority of the studies have not implemented any methods to adjust for differences in baseline characteristics between groups.[15–19] The aim of this study was to assess the risk of preterm delivery and small-for-gestational-age (SGA) births—two related outcomes that remain as leading causes of infant morbidity and mortality[20]—in women with autoimmune diseases exposed to biologics, compared with those who were not exposed to biologics before or during pregnancy.

METHODS

Data sources

Population Data British Columbia (Population Data BC) is an extensive data repository that holds individual-level, de-identified, longitudinal data on all health services covering the entire population of BC (estimated 4.6 million residents, December 2016[21]). These include all provincially funded physician visits, laboratory tests and diagnostic procedures (X-rays, ultrasounds and so on) from the Medical

Services Plan (MSP) database,[22] hospitalisations from the Discharge Abstract Database (DAD)[23] and demographics and vital statistics since 1985.[24–26] Population Data BC also includes the comprehensive prescription drug database, PharmaNet, which captures all prescriptions dispensed in community pharmacies regardless of payment source, since 1996.[27] These data were linked to the BC Perinatal Database Registry (BCPDR),[28] which contains validated information on the date of conception, antenatal, intrapartum and postpartum maternal and infant data abstracted from medical records for 99% of births in BC. Altogether, linkage of these data sources created a source population comprising women (n=305 351) in BC who had one or more pregnancies (n=449 098) ending in a live or stillbirth between 1 January 2002 and 31 December 2012 and were continuously covered by BC's provincial health plan for at least 12 months prior to the start of pregnancy and in the 12 months following delivery. Details of these data sources are described in previous work.[14]

Study cohort

We created a cohort of women who had a recorded diagnosis of one or more autoimmune diseases that could be treated with a biologic, which included RA, IBD (Crohn's disease and ulcerative colitis), Ps/PsA, AS, juvenile idiopathic arthritis and systemic autoimmune rheumatic diseases—including systemic lupus erythematosus and other connective tissue diseases. These were defined as having the same ICD-9/10 code for a specific autoimmune disease from two separate physician visits that were at least 60 days apart and within 2 years, any time prior to the date of conception; or, having at least one hospitalisation with an ICD-9/10 code for an autoimmune disease any time prior to the date of conception. Given that the unit of analysis was individual pregnancy, each pregnancy had to satisfy the above criteria in order to be included in the analyses.

Exposure ascertainment

Using dispensation dates and Canadian Drug Identity Codes for biologics in PharmaNet, we identified pregnancies in women in the autoimmune disease cohort who had at least one prescription for a biologic at any point during the drug exposure period of interest for each study outcome. For preterm delivery, this period was defined as 3 months prior to the date of conception (referred to as the preconception period) until the date of delivery or 36 weeks+6 days of gestation, which ever came first, for each pregnancy. This was to avoid classifying pregnancies as exposed if they were exposed to a biologic on or after 37 completed weeks of gestation in which by definition they would not be susceptible to the outcome occurring. For SGA, the exposure period was defined as 3 months prior to the date of conception, until the date of delivery. Disease-matched women with pregnancies who were not exposed to biologics during the drug exposure periods of interest comprised the unexposed groups. All biologics available in BC for the treatment of autoimmune diseases of interest during the study period are listed in the online supplementary table 1.

Outcomes

The outcomes of interest were preterm delivery and SGA births. We had access to exact date of birth for all babies born to the women in our cohort from the BCPDR, as well as valid gestational age estimates based on information from early gestational ultrasounds or from the date of last menstrual period if an early gestational ultrasound was not performed. If neither field was recorded, gestational age was estimated from newborn clinical exam and/or chart documentation. Preterm delivery was defined as a binary outcome of delivery occurring before 37 completed weeks of gestation, regardless of the reason. We also included infants with ICD-9/10 codes for preterm births from the MSP database or DAD. SGA was defined as a newborn weighing less than the 10th percentile of gestational age-specific and sex-specific weights for neonates in BC[29] using birth weights recorded in the BCPDR.

Statistical analysis

To minimise bias due to confounding by indication, we used a high-dimensional propensity score (HDPS) algorithm that incorporated investigator-specified covariates and additional factors that acted as proxy variables for unmeasured confounders.[30] The HDPS was generated using logistic regression models to identify candidate covariates derived from four dimensions of data comprising aforementioned data sources: (1) MSP database; (2) DAD; (3) PharmaNet and (4) BCPDR. Within the MSP database, DAD and PharmaNet, only claims or codes that occurred during the 12 months prior to the date of conception for each pregnancy were assessed as candidate covariates to be included in the HDPS. We specified the HDPS algorithm to prioritise covariates across data dimensions by their potential for controlling confounding based on the bias term estimator proposed by Bross,[31] meaning that the covariates must both be associated with the exposure and the outcome to mitigate the potential for including variables that were only associated with the exposure, which may actually introduce bias into estimates.[32] The top 50 empirically derived covariates for each outcome were included along with investigator specified confounders for propensity score estimation (see the online supplementary tables 2 and 3). For each outcome, biologics-exposed pregnancies were matched with unexposed pregnancies using HDPS in a ratio of 1:5 without replacement. Match performance was evaluated using standardised mean differences in baseline characteristics of matched and unmatched cohorts.

Using logistic regression models we analysed each study outcome among biologics-exposed and unexposed women in the HDPS-matched cohort (model 1). Because the length of pregnancies does not affect the risk of exposure, exposures were not modelled as time-dependent. As sensitivity analyses for each outcome, we conducted multivariable logistic regression models with deciles of HDPS included as indicator terms (model 2) and with continuous HDPS as a covariate (model 3). As sensitivity analysis for the exposure, we defined the exposure window beginning at 12 months prior to conception for both outcomes, and used HDPS matching (model 4). Using robust variance estimators to account for correlation between multiple pregnancies within the same woman did not appreciably change CIs in the outcome models, as such, all correlation structures were omitted. All analyses were conducted using SAS statistical software V.9.3.

RESULTS

From a source population of 305 351 women in BC who have had one or more pregnancies over the study period, approximately 2% had a diagnosis of one of the autoimmune diseases of interest resulting in 6218 women with 8607 pregnancies in the study cohort. Table 1 shows baseline characteristics for the unmatched cohorts as well as HDPS-matched cohorts for analyses of respective study outcomes. Marked imbalances between exposure groups in the distribution of autoimmune

Table 1 Baseline characteristics in unmatched and matched samples of biologic-exposed and unexposed pregnancies

	Unmatched sample overall			HDPS matched for preterm delivery analysis			HDPS matched for SGA analysis		
	Biologic exposed	Biologic unexposed	SMD	Biologic exposed	Biologic unexposed	SMD	Biologic exposed	Biologic unexposed	SMD
Current pregnancy									
Maternal age at delivery, years (mean (SD))	31.1	31.2	0.002	31.2	31.3	0.023	31.2	31.2	0.004
Multiparous	62 (43%)	4980 (59%)	0.309	52 (43%)	262 (44%)	0.007	52 (43%)	255 (43%)	0.017
Antenatal visits (mean (SD))	9.0	9.0	0.003	9.0	9.1	0.013	9.0	9.1	0.024
Obstetrical history									
Prior premature delivery	8 (6%)	495 (6%)	0.007	5 (4%)	28 (5%)	0.024	5 (4%)	30 (5%)	0.040
Prior spontaneous abortion	40 (28%)	2130 (25%)	0.069	37 (31%)	156 (26%)	0.107	37 (31%)	161 (27%)	0.088
Prior delivery with neonatal death	<5	52 (0.6%)	0.012	<5	7 (1%)	0.034	<5	6 (1%)	0.017
Prior stillbirth	<5	103 (1.2%)	0.072	<5	16 (3%)	0.011	<5	19 (3%)	0.040
Prior low birth weight	8 (6%)	240 (3%)	0.143	5 (4%)	24 (4%)	0.008	5 (4%)	27 (5%)	0.016
Prior anomalies	–	74 (0.9%)	0.133	<5	<5	–	<5	<5	–
Autoimmune disease type*									
Rheumatoid arthritis	67 (47%)	1733 (21%)	0.587	55 (46%)	297 (50%)	0.073	55 (46%)	272 (45%)	0.010
Inflammatory bowel disease	66 (46%)	2455 (29%)	0.335	57 (48%)	276 (46%)	0.030	57 (48%)	286 (48%)	0.003
Psoriasis/psoriatic arthritis	24 (17%)	3433 (41%)	0.535	20 (17%)	82 (14%)	0.084	20 (17%)	100 (17%)	0.000
Juvenile idiopathic arthritis	12 (8%)	89 (1%)	0.357	9 (8%)	42 (7%)	0.019	9 (8%)	33 (6%)	0.081
Systemic autoimmune rheumatic diseases	9 (6%)	1059 (13%)	0.209	7 (6%)	34 (6%)	0.007	7 (6%)	37 (6%)	0.014
Ankylosing spondylitis	8 (6%)	414 (5%)	0.037	5 (4%)	24 (4%)	0.008	5 (4%)	24 (4%)	0.008
Biologics†‡									
Infliximab	58 (37%)			47 (39%)			47 (39%)		
Etanercept	48 (31%)			36 (30%)			36 (30%)		
Adalimumab	40 (26%)			30 (25%)			30 (25%)		
Certolizumab	<5			<5			<5		
Ustekinumab	<5			<5			<5		
Rituximab	<5			<5			<5		
Golimumab	<5			<5			<5		
Alefacept	<5			<5			<5		
Concomitant medications									
DMARDs	81 (56%)	1693 (20%)	0.791	62 (52%)	311 (52%)	0.003	62 (52%)	314 (52%)	0.013
Glucocorticoids	66 (46%)	880 (10%)	0.854	56 (47%)	251 (42%)	0.097	56 (47%)	254 (42%)	0.087
Traditional NSAIDs	47 (33%)	2218 (26%)	0.131	35 (29%)	193 (32%)	0.065	35 (29%)	185 (31%)	0.036
Antidepressants	30 (21%)	1280 (15%)	0.146	26 (22%)	120 (20%)	0.041	26 (22%)	123 (21%)	0.029
Anxiolytics	15 (10%)	638 (8%)	0.087	12 (10%)	53 (9%)	0.040	12 (10%)	60 (10%)	0.000
COX2 NSAIDs	7 (5%)	281 (3%)	0.084	6 (5%)	31 (5%)	0.008	6 (5%)	30 (5%)	0.000
Comorbidities									
Anxiety	18 (13%)	814 (10%)	0.081	16 (13%)	76 (13%)	0.020	16 (13%)	80 (13%)	0.000
Mood disorders	12 (8%)	430 (5%)	0.139	10 (8%)	45 (8%)	0.031	10 (8%)	45 (8%)	0.031
Hospitalisation at baseline	49 (34%)	2062 (24%)	0.219	40 (33%)	168 (28%)	0.116	40 (33%)	187 (31%)	0.046

All cell sizes <5 are suppressed due to privacy restrictions of data sharing agreements.
*Sum of percentages exceed 100% due to some individuals having more than one diagnosis.
†Anytime from 3 months prior to date of conception until date of delivery for unmatched and SGA analyses, or until 36+6 weeks gestational age for preterm deliveries.
‡Sum of percentages exceed 100% due to some pregnancies being exposed to more than one drug.
COX, cyclooxygenase; DMARD, disease-modifying antirheumatic drugs; HDPS, high-dimensional propensity score; NSAID, non-steroidal anti-inflammatory drugs; SGA, small-for-gestational-age; SMD, standardised mean difference.

disease types and concomitant medication use, as seen with large standardised mean differences in the unmatched cohort, were mitigated in the HDPS-matched cohorts.

Preterm delivery

The HDPS-matched cohort for analysis of preterm delivery outcomes comprised 109 women and 120 babies exposed to biologics during 3 months preconception to the date of delivery, and 584 women and 600 babies unexposed to biologics during that time (table 1). Most of the women had a diagnosis of RA or IBD (49% and 46%, respectively) and filled prescriptions for one of three commonly prescribed TNF-alpha inhibitors (infliximab 39%, etanercept 30% or adalimumab 25%) (table 1). In the HDPS-matched cohort, 21 of the 120 babies (18%) exposed to biologics preconception or during pregnancy and 95/600 (16%) babies unexposed to biologics were born preterm. Table 2 shows the results of crude analyses of the association between biologic exposure and preterm delivery with an unadjusted OR of 1.64 (95% CI 1.02 to 2.63). In primary analyses, the OR for the association between biologic exposure and preterm delivery was 1.13 (95% CI 0.67 to 1.90) (table 2, model 1). Sensitivity

Table 2 Proportion of pregnancies ending in preterm delivery or SGA births based on exposure group and timing of biologic exposure

	Preterm delivery		SGA	
	Biologic exposed	Biologic unexposed	Biologic exposed	Biologic unexposed
Overall	21/120 (18%)	95/600 (16%)	11/120 (9%)	60/600 (10%)
Preconception*	20/114 (18%)	96/606 (16%)	11/114 (10%)	60/606 (10%)
First trimester	18/96 (19%)	98/624 (16%)	9/96 (9.4%)	62/624 (10%)
Second trimester	12/55 (22%)	104/665 (16%)	5/55 (9.1%)	66/665 (10%)
Third trimester	12/57 (21%)	104/663 (16%)	5/57 (8.8%)	66/663 (10%)
Unadjusted OR (95% CI)	1.64 (1.02 to 2.63)		1.34 (0.72 to 2.51)	
Model 1 OR (95% CI)†	1.13 (0.67 to 1.90)		0.91 (0.46 to 1.78)	
Model 2 OR (95% CI)‡	1.21 (0.74 to 2.00)		1.00 (0.53 to 1.92)	
Model 3 OR (95% CI)§	0.96 (0.56 to 1.64)		1.03 (0.53 to 2.01)	
Model 4 OR (95% CI)¶	0.94 (0.56 to 1.55)		1.03 (0.56 to 1.90)	

*Defined as 3 months prior to the date of conception.
†Logistic regression in matched cohort.
‡Multivariable logistic regression with HDPS deciles.
§Multivariable logistic regression with continuous HDPS as covariate.
¶Exposure window starting from 12 months preconception, logistic regression in HDPS matched cohort.
HDPS, high-dimensional propensity score; SGA, small-for-gestational-age.

analyses involving multivariable logistic regression based on the unmatched cohort adjusting for HDPS deciles (model 2) and continuous HDPS (model 3) and extending the exposure window to 12 months preconception (model 4) did not appreciably change the results. Finally, examination of the birth data showed that the mean gestational age at delivery was 38 weeks (range 27–43 weeks) among women exposed to biologics and 38 weeks (range 19–43 weeks) among those unexposed (figure 1).

SGA births

The HDPS-matched cohort for analysis of SGA comprised 109 women and 120 babies exposed to biologics during 3 months preconception to the date of delivery, and 585 women and 600 babies unexposed to biologics during that time. RA and IBD remained the most common disease types (45% and 48%, respectively), and infliximab, etanercept and adalimumab were the most commonly prescribed biologics (table 1). In the HDPS-matched cohort, SGA births occurred in 11/120 (9%) pregnancies in the biologics-exposed group and in 60/600 (10%) pregnancies that were in the biologics unexposed group. Table 2 shows the results of crude analyses of the association between biologic exposure and SGA with an unadjusted OR of 1.34 (95% CI 0.72 to 2.51). In primary analyses, the OR for the association between biologic exposure and SGA was 0.91 (95% CI 0.46 to 1.78) (table 2, model 1). Sensitivity analyses (models 2, 3 and 4) again showed similar results. Furthermore, examination of the Apgar scores of SGA newborns showed inappreciable differences; those exposed to biologics had mean Apgar scores of 8.1 (SD 1.5) at 1 minute, and 9.0 (SD 1.0) at 5 minutes, and those

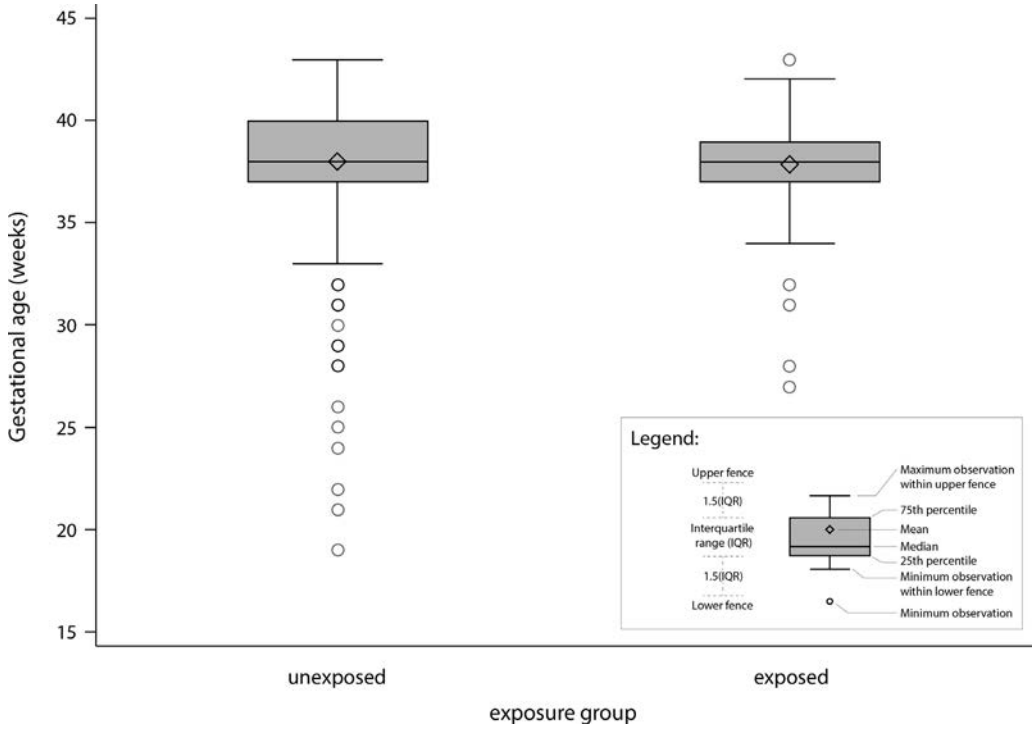

Figure 1 Distribution of gestational age by exposure groups.

unexposed had Apgar scores of 7.7 (SD 2.2) at 1 minute and 8.7 (SD 1.7) at 5 minutes.

DISCUSSION

Our objective was to use population-based administrative health data with valid information on estimated date of conception and complete information on all dispensed prescriptions in BC to evaluate the association between biologic exposure preconception, or during pregnancy, and preterm delivery or SGA births in women with autoimmune diseases. We applied HDPS methods—primarily matching—to account for differences in baseline characteristics between women exposed and unexposed to biologics. Prior to restricting the population using HDPS matching, we found that differences in baseline characteristics in the unmatched sample led to suggestion of an association between biologics use and the risk of preterm deliveries. However, after successful implementation of HDPS to control for confounding by indication and proxies of unmeasured confounders, we did not find an association between biologics exposure and the outcomes of interest, in primary and various sensitivity analyses. While we examined all biologics used in the cohort, TNF-alpha inhibitor biologics (94%) were the most common, and as such, our results mostly apply to these biologics and less so to those that are not TNF-alpha inhibitors.

Indeed the population-based setting of this study lends more generalisability to the results, and the implementation of HDPS-based methods allows for better control of confounding compared with traditional modelling methods, thus contributing to better understanding of the use of biologics in the pregnant population. With respect to the outcome of preterm delivery, several single-centre studies using maternal medical records have reported risks of preterm delivery in those exposed to biologics during pregnancy compared with those who were not exposed, ranging from OR 2.00 (95% CI 0.19 to 20.51) to 2.71 (0.44 to 16.52).[18 19 33] Registry-based studies from the British Society for Rheumatology Biologics Register in patients with RA, and the German Registry Rheumatoide Arthritis: Beobachtung der Biologika-Therapie in patients with IBD reported risk estimates of 1.42 (95% CI 0.25 to 7.73) and 2.14 (0.10 to 44.28), respectively, of preterm deliveries in women who were using biologics before or during pregnancy.[15 34] These studies have relatively small sample sizes (50–80 individuals), and have not implemented methods to adjust for the effects of the underlying disease severity or effects from measured and unmeasured confounders, as such these estimates have lower generalisability and high uncertainty, as evidenced by the wide confidence intervals. At the time of this publication, only two studies have reported adjusted risk estimates, one abstract by Chambers *et al*[35] and one publication by Burmester *et al*,[36] with data from the Organisation of Teratology Information Services registry and the Adalimumab Pregnancy Exposure Registry. Chambers (total N of 722) using propensity score methods found that the adjusted HR for preterm delivery was 0.82 (95% CI 0.50 to 3.84) in pregnancies exposed to adalimumab compared with those unexposed; and Burmester (total N of 373) reported an adjusted HR for preterm delivery of 1.08 (95% CI 0.41 to 2.83) in patients with RA using adalimumab during pregnancy, compared with patients with RA not using adalimumab.

With respect to SGA outcome, there are fewer studies—only two to date—with conflicting findings. Using medical records from a university hospital, Schnitzler *et al* reported 6% of pregnancies exposed to infliximab ending in a very SGA birth (<5th percentile) compared with 11% of unexposed pregnancies; in our study, there were no occurrences of very SGA births. Martinez *et al*, using medical records, reported that among women with IBD exposed to a biologic during pregnancy, 12.5% resulted in SGA births compared with 9% among unexposed pregnant women with IBD.[18 19] These rates appear similar to our results, however, again neither of these studies accounted for baseline differences between exposure groups. Thus, with respect to SGA outcome among women with autoimmune disease taking biologics, our study is the first to use population-based data to conduct analyses adjusted for measured and unmeasured confounders using HDPS.

Strengths and limitations of our study bear discussion. High-quality, high coverage, population-based databases from Population Data BC, and the linkage with the perinatal registry (BCPDR) and the prescription dispensations database (PharmaNet) provided the ability to accurately determine the timing of all medication dispensations with respect to milestone pregnancy dates, for each pregnancy in the cohort, thus minimising potential biases caused by problems such as misclassification, patient recall bias and selection bias. The comprehensive BCPDR data also allowed for the ascertainment of SGA using babies' gestational age and birth weights, whereas currently available research focus mainly on the outcome of low birth weight, which is itself confounded by gestational age whereby about two-thirds of low-birthweight infants are preterm.[37] As such, SGA is not only a more useful outcome measure, but also allowed the investigation of the impact of biologics on SGA and preterm delivery outcomes independently. Using HDPS matching is another strength which lends this study high internal validity, as it allows for better adjustment of confounding by indication and adjustment of proxies of unmeasured confounders.[30] Indeed addressing confounding by indication is of utmost importance in the population of women with autoimmune disease given the association between disease activity and adverse pregnancy outcomes,[11 12] and the fact that those with higher disease activity are also more likely to be on biologics given the current treatment pathways. The main limitation of our study remains the relatively small sample size in the matched cohorts; however, the use of HDPS matching inherently prioritises validity over precision of estimates, of which the latter can only be overcome by accumulation of further evidence or pooling of multiple databases.

Altogether, we found no association between biologics use before or during pregnancy and preterm delivery or SGA births in women with autoimmune diseases, compared with those who had comparable propensity to receive biologics during that time but did not. As such, our findings suggest that biologics may be a safe treatment option for women with certain autoimmune diseases who, as previous research suggest, are at higher risk of adverse pregnancy outcomes due to their disease. Given that exposures and outcomes in biologics use during pregnancy remain fairly rare, relatively small samples are a continual challenge, as such our study represents an important contribution to the accumulation of evidence on the safety of the use of biologics in pregnant women, which may lead to increased prescriber comfort and patient acceptance, decreased uncertainty and improved maternal and neonatal outcomes in this population.

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Competing interests NWT is a PhD Candidate and Canadian Institutes of Health Research Fellowship holder. ECS is a biostatistician with Arthritis Research Canada. GH is an Assistant Professor, Canadian Institutes of Health Research New Investigator, and a recipient of Canadian Cancer Society Research Institute Capacity Development Award. MS is an Assistant Professor, Canadian Institutes of Health Research New Investigator, and a Michael Smith Foundation for Health Research Scholar. LDL is a Professor, and Director of Collaboration for Outcomes Research and Evaluation. CAM is a Professor and Dean at the Otago School of Pharmacy, New Zealand. MADV is an Assistant Professor, Canada Research Chair in Medication Adherence, Utilization, and Outcomes, The Arthritis Society Network Scholar, and Michael Smith Foundation for Health Research Scholar. LDL has received honoraria from Boehringer Ingelheim and Pfizer Canada for consulting services unrelated to this study.

Patient consent Detail has been removed from this case description/these case descriptions to ensure anonymity. The editors and reviewers have seen the detailed information available and are satisfied that the information backs up the case the authors are making.

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EXTENDED REPORT

Intramuscular glucocorticoid injection versus placebo injection in hip osteoarthritis: a 12-week blinded randomised controlled trial

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ABSTRACT

Objectives Guidelines recommend intra-articular glucocorticoid injection in patients with painful hip osteoarthritis. However, intra-articular hip injection is an invasive procedure. The efficacy of systemic glucocorticoid treatment for pain reduction in hip osteoarthritis is unknown. This randomised, double-blind, trial assessed effectiveness in hip pain reduction of an intramuscular glucocorticoid injection compared with a placebo injection in patients with hip osteoarthritis.

Methods Patients with painful hip osteoarthritis were randomised to either 40 mg triamcinolone acetate or placebo with an intramuscular injection into the gluteus muscle. The primary outcomes were severity of hip pain at rest, during walking (0–10) and WOMAC pain at 2-week postinjection. We used linear mixed models for repeated measurements at 2, 4, 6 and 12 weeks for the intention-to-treat data analysis.

Results Of the 107 patients randomised, 106 could be analysed (52 in the glucocorticoid group, 54 in the placebo group). At 2-week follow-up, compared with placebo injection, the intramuscular glucocorticoid injection showed a significant and clinically relevant difference in hip pain reduction at rest (difference −1.3, 95% CI −2.3 to −0.3). This effect persisted for the entire 12-week follow-up. For hip pain during walking, the effect was present at 4-week, 6-week and 12-week follow-ups, and for WOMAC pain the effect was present at 6-week and 12-week follow-up.

Conclusions An intramuscular glucocorticoid injection showed effectiveness in patients with hip osteoarthritis on one of the three primary outcomes at 2-week postinjection. All primary outcomes showed effectiveness from 4 to 6 weeks, up to a 12-week follow-up.

Trial registration number NTR2966.

INTRODUCTION

Several international guidelines recommend intra-articular (IA) glucocorticoid injections for patients with hip osteoarthritis (OA) experiencing moderate to severe pain and not responding to oral analgesics.[1–3] A systematic review on the efficacy of intra-articular steroids in moderate/severe hip OA included five randomised controlled trials (RCT) and the assessed quality of the studies was high.[4] The treatment effect was large at 1 week post-injection, but declined afterwards. At 8 weeks, there were two trials that reported a reduction in pain with a moderate effect size.[4]

However, injection into the hip joint is challenging because the joint cannot be palpated and is adjacent to important neurovascular structures. An IA hip injection is best performed under fluoroscopic or ultrasound guidance.

A serious side effect of an IA injection is a septic arthritis. The incidence of this side effect is very low, and scarce in the available literature. A systematic review and meta-analysis of IA injection in knee OA comparing effectiveness of pharmacological interventions included 29 studies (3152 patients, 9500 IA glucocorticoid injections) and reported only 1 septic arthritis (in the IA placebo group).[5]

A systemic effect of glucocorticoids on joint pain has been indicated in patients with subacromial impingement shoulder pain. A double-blinded RCT showed no important differences in effectiveness on pain of ultrasound-guided subacromial glucocorticoid injection compared with gluteal injection.[6] A systemic effect of glucocorticoids was also suggested in an RCT reporting the effect of local glucocorticoid injection for greater trochanteric pain syndrome: patients with concurrent hip OA or chronic low back pain had an equal or even more pronounced decrease in pain.[7 8]

If an intramuscular (IM) glucocorticoid injection is shown to have a clinically relevant effect on pain, this would offer a less complex alternative treatment for episodes of increased pain in hip OA. Therefore, this study assessed the efficacy of an IM glucocorticoid injection compared with an IM placebo injection on hip pain severity in patients with hip OA who were not responding to oral analgesics.

METHODS

Trial design

This was a multicentre, double-blinded, randomised controlled superiority trial with two parallel groups and a follow-up period of 12 weeks: details of the study protocol were published earlier.[9 10] The Medical Ethics Committee of the Erasmus University Medical Centre (EMC; Rotterdam) approved the study protocol (MEC2011-115) and all included patients provided written informed consent.

Patients

Patients with hip OA were invited to participate in the trial by general practitioners and orthopaedic surgeons located in the south-west of the Netherlands. Patients (aged >40 years) were eligible for inclusion if they met the American College for

Rheumatology (ACR) clinical criteria for hip OA during clinical screening and radiological evidence of hip OA was present (Kellgren & Lawrence 'score' (KL) ≥2).[11 12] Patients were included if they had symptomatic disease for ≥6 months, and had moderate to severe hip pain score ≥3 (scale 0–10; 0=no pain) despite the use of oral analgesics at time of inclusion.

Radiological hip OA was scored on an anterior–posterior pelvic radiograph of (at most) 6-month old. The radiological grade of hip OA was scored by two researchers (DD, PKB) independently and the interobserver reliability was κ=0.7 for KL <2 vs KL ≥2. In case of disagreement, a consensus was formed during a consensus meeting. If a patient had bilateral hip OA, the more painful hip was selected as the study hip.

Patients were excluded if they had diabetes mellitus, were using oral glucocorticoids, had local/systemic infection, had presence of inflammatory rheumatic diseases (eg, rheumatoid arthritis, psoriatic arthritis and spondylarthropathies), coagulopathy, used coumarins, had a gastric ulcer, allergy to glucocorticoids, radiological signs of osteonecrosis, had an IA injection in the hip in the previous 6 months, were on the waiting list for total hip replacement (THR) surgery or were unable to complete questionnaires in Dutch.

Interventions

Patients received either 40 mg triamcinolone acetate (1 mL) or 1 mL normal saline (placebo) with an IM injection. At the research centre, the trial nurse administered the allocated injection in the upper lateral quadrant of the gluteal musculature on the ipsilateral side of the study hip.

Randomisation

An independent pharmacy assistant allocated each included patient based on a computerised randomisation list using random blocks of 2 and 4 to either placebo (saline) injection or triamcinolone acetate 40 mg injection. Randomisation was stratified for setting (general practice and orthopaedic outpatient clinic). After randomisation, the vials for the injections were prepared, packed and sealed in an identical way for both groups by the pharmacy of the EMC. The randomisation list was available only to the pharmacy assistant.

Blinding

In this trial, the outcome assessors, patients, treating physicians, researchers (including the statistical analyses) and research assistants involved in data collection were blinded to the content of the injections. Since normal saline is transparent and triamcinolone acetate is a white solution, the independent nurse who prepared and injected the injection was non-blinded. To assure blinding of the others, the independent trial nurse prepared and administered the injection out of sight of the patient, assessors, treating physicians and the researchers. This trial nurse was not involved with follow-up measurements. After preparation and before injection, the syringe was covered with an opaque foil to assure blinding of the patient.

Outcomes

The primary outcomes were severity of hip pain at 2-week post-injection measured on an 11-point numerical rating scale (NRS: 0–10, 0=no pain) at rest and during walking, and measured with the Western Ontario and McMaster University Osteoarthritis Index pain subscale (WOMAC pain: 0–100, 0=no symptoms).[13 14]

Secondary outcomes were the primary outcomes at 4-week, 6-week and 12-week follow-ups. Additional secondary outcome measures were WOMAC function and stiffness, WOMAC total score, Hip disability and Osteoarthritis Outcome Score for pain (HOOS pain), quality of life (EQ-5D), Intermittent and Constant Osteoarthritis Pain (ICOAP) and patients' perceived recovery assessed on a 7-point Likert scale.[15–17] At all time points, the WOMAC and ICOAP scales are presented as normalised scores (0–100, 0=no symptoms). The HOOS subscale is presented as normalised score (0–100, 100=no symptoms). Also recorded was patients' medical consumption, including analgesic use and adverse reactions at all time points. Patients were allowed to use escape pain medication as needed.

Another secondary outcome was the percentage of responders as defined by the OMERACT-OARSI criteria (improvement in at least two of the three following domains: ≥20% improvement in WOMAC pain, ≥20% improvement in WOMAC function and markedly improved on patients' global assessment).[16] For patients' global assessment the 7-point Likert scale for patients' perceived recovery was dichotomised in 'improved' (scores: completely recovered, almost completely recovered and slightly recovered) and 'not improved' (scores: no change, slightly worse, significantly worsened and worse than ever).

At baseline and at a 12-week follow-up, patients visited the research centre to undergo a physical examination of hips, spine and knees. At baseline, blood samples were collected to measure the erythrocyte sedimentation rate and C reactive protein to gain insight in the inflammatory processes.[14]

Sample size

Power calculations were based on a study with similar inclusion criteria.[18] A 10-point difference (SD 20) on hip pain at rest and during walking (visual analogue scale: 0–100) was assumed to be the minimal clinically important difference between both groups.[19] The accompanying effect size of 0.5 is from the clinical standpoint a moderate effect and in general considered clinically relevant.[20] For our study, this implies one point on the NRS pain scale. With a power of 80% and an alpha of 5%, 64 patients per group were required (including 5% loss to follow-up=67 patients per group). The same sample size was needed when an 8-point difference (SD 16) on the standardised WOMAC total score and pain subscale score (0–100) was assumed as a clinically relevant difference between the groups.

Statistical analysis

The intention-to-treat principle was used. Descriptive statistics were used to describe patients' characteristics at baseline, items of physical examination and the severity of radiologic hip OA. Linear mixed models with repeated measures were used for continuous outcomes. When patients underwent a THR, data of these patients were included up to the date of surgery. To model the covariance of repeated measures by patients, the option for data structure in the analyses was set on 'unstructured', because this yielded the lowest Akaike's information criterion. Fixed effects were time and time by treatment. Analyses were adjusted for baseline variables and for the baseline value of the outcome of interest.[9] Therefore, at a 2-week follow-up presented are the outcomes at 2 weeks, corrected for baseline scores. At 4 weeks, presented are the results at a 4-week follow-up time point, corrected for baseline and 2 weeks scores and so on.

Generalised estimating equations (GEE) analyses with repeated measures were performed for the dichotomous outcomes perceived improvement, and the OMERACT-OARSI responder.

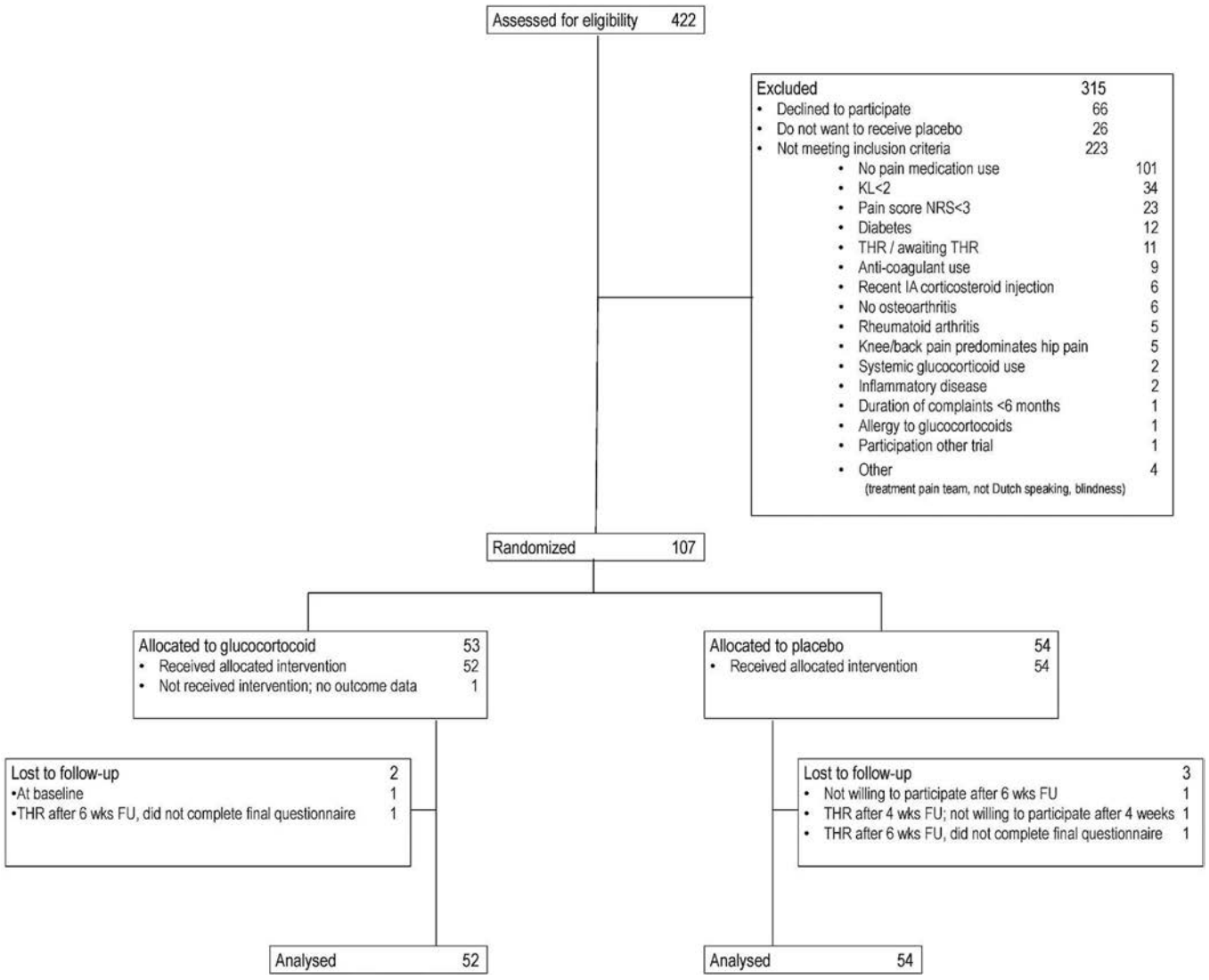

Figure 1 Flow diagram of the study participants. NRS, Numerical Rating Scale (0–10; 0=no pain); FU, follow -up; IA, intra-articular; KL, Kellgren & Lawrence 'score' for hip osteoarthritis; THR, total hip replacement; wks, weeks.

Before GEE analyses, multiple imputations were performed for missing values, creating 100 imputed datasets.

The Pearson χ^2 test was used to analyse differences between groups concerning medical consumption, analgesic use and adverse events. An explorative, predefined, subgroup analysis was performed assessing the interaction effects between injections and setting on the primary outcomes.[9]

All analyses were performed using SPSS V.24.

RESULTS

Patient flow

A total of 422 invited patients contacted the research centre and were screened for eligibility; of these, 92 refused to participate and 223 did not meet the inclusion criteria (figure 1).

Finally, 107 patients provided informed consent: 53 were randomised to the glucocorticoid injection and 54 to the placebo injection. One patient in the glucocorticoid group withdrew his consent just before the appointment for baseline physical examination and subsequent injection, because his pain had resolved spontaneously. Because this patient did not receive the allocated treatment and was not willing to send us the completed baseline questionnaire or any follow-up questionnaires, he was not included in the analyses.

Recruitment

Recruitment of patients took place between September 2011 and October 2014 and follow-up measurements were done until January 2015. Of the 107 included patients, general practitioners referred 81 patients.

Lost to follow-up

At a 6-week follow-up, one patient in the glucocorticoid group reported being scheduled for a THR; in the placebo group two patients (at 4-week and 6-week follow-up, respectively) reported being scheduled for a THR. One patient in the placebo group was not willing to participate after 6 weeks due to logistical problems.

Patient population

Of all patients, 52 received the allocated glucocorticoid injection and 54 the allocated placebo injection, and were included in the analyses. Baseline characteristics of both patient groups are presented in table 1. Of the 106 patients, 73 (68%) were women; the mean age was 64 (SD 11) years, and the duration of hip OA symptoms was ≥1 year for 74 (70%) patients.

Table 1 Patients' characteristics at baseline

Characteristic		Glucocorticoid (n=52)	Placebo (n=54)
Age, years, mean±SD		66±11	63±10
Body mass index, kg/m^2, mean±SD		27±3.7	28±6.4
Women, n(%)		40 (77)	33 (61)
Duration of symptoms, n(%)	≥1 year	40 (77)	34 (63)
Referral to study by, n(%)	General practitioner	39 (75)	41 (76)
Kellgren & Lawrence score hip OA, n(%)	KL 2	42 (81)	38 (70)
Ethnicity Dutch, n(%)		51 (98)	47 (87)
Employment, n(%)		17 (33)	23 (43)
Comorbidities			
Osteoarthritis of knee(s), n(%)		20 (39)	15 (28)
Osteoarthritis of hand(s), n(%)		12 (23)	14 (26)
Low back pain, n(%)		33 (64)	30 (56)
Signs and symptoms			
Stiffness of the hip, n(%)	Morning stiffness	40 (77)	35 (65)
Severity of hip pain last week NRS 0–10, mean±SD	Pain at rest	4.3±2.4	4.2±2.5
	Pain at walking	5.4±2.1	5.1±2.3
WOMAC 0–100, mean±SD	Total score	46±19	47±18
	Pain	43±17	43±17
	Function	47±20	48±19
	Stiffness	52±21	48±24
HOOS 0–100, mean±SD	Pain	52±17	52±16
ICOAP 0–100, mean±SD	Total score	38±18	39±17
	Intermittent pain	41±21	41±17
	Continuous pain	34±21	36±19
EQ-5D score, mean±SD		0.66±0.23	0.68±0.26
Treatment			
Frequent pain medication use*, n(%)	Acetaminophen	25 (48)	26 (48)
	NSAID	14 (27)	14 (26)
	Opiates	8 (15)	6 (11)
Visited healthcare giver for hip OA in the previous 3 months, n(%)	General practitioner n of visits (median, IQR)	24 (46) 1 (1)	22 (41) 1 (0)
	Physiotherapist n of visits (median, IQR)	14 (27) 6 (11)	22 (41) 6 (5)
	Medical specialist n of visits (median, IQR)	16 (31) 1 (0)	18 (33) 1 (0.3)
Patient's expected effect of injection, n(%)	Much or very much	36 (69)	29 (54)
Laboratory outcomes			
CRP, median (IQR)		2 (3.1)	1.5 (3.4)
ESR, median (IQR)		9.5 (13)	10 (12)

*Frequent pain medication use=3–5 times/week or daily use in the past 3 weeks.
ADL, function in daily living; CRP, C reactive protein; EQ-5D, Euroqol; ESR, erythrocyte sedimentation rate; HOOS, Hip Disability and Osteoarthritis Outcome Score (0=extreme problems); ICOAP, Intermittent and Constant Osteoarthritis Pain (0=no pain); KL, Kellgren & Lawrence grading of radiologic hip OA; NRS, Numeric Rating Scale (0=no pain); NSAID, non-steroidal anti-inflammatory drug; OA, osteoarthritis; WOMAC, Western Ontario and McMaster University Osteoarthritis Index (0=no pain).

The KL score of hip OA at baseline, ethnicity, morning hip stiffness and patients' expected effect of the injection changed the estimates for the primary outcome ≥10%. We adjusted for these baseline variables in the statistical analyses.

Primary outcomes

At a 2-week follow-up, compared with the placebo injection, the glucocorticoid injection showed a significant association with hip pain reduction at rest (between group difference −1.3, 95% CI −2.3 to −0.3) (table 2 and figure 2). Also, at a 2-week follow-up, there were no significant associations between glucocorticoid injection and hip pain during walking (difference −0.9, 95% CI −1.9 to 0.1) and WOMAC pain (difference −6.1, 95% CI −13.4 to 1.2). The correlation between the primary endpoints ranged from r 0.64 to r 0.84. The results of the unadjusted linear mixed model analysis were similar (online supplementary table S1).

Secondary outcomes

At 4-week, 6-week and 12-week follow-ups, the glucocorticoid injection was associated with a significant hip pain reduction at rest and during walking (table 2 and figure 2). Moreover, at almost all follow-up measurements, the estimates showed significant differences in favour of the glucocorticoid injection on WOMAC pain, function, stiffness and total; HOOS pain; and ICOAP total, intermittent and constant. No significant differences between groups were found for quality of life (table 2). At a 2-week follow-up, perceived improvement and

Table 2 Results of the multivariable linear mixed model analysis with repeated measurements regarding primary outcomes between the glucocorticoid and placebo group

	2 weeks					4 weeks					6 weeks					12 weeks				
	Difference *					Difference *					Difference *					Difference *				
Follow-up	CCS	Placebo	Δ	95% CI	P value	CCS	Placebo	Δ	95% CI	P value	CCS	Placebo	Δ	95% CI	P value	CCS	Placebo	Δ	95% CI	P value
Primary outcomes																				
NRS pain (rest)	2.6 (2.3)	3.9 (2.5)	−1.3	−2.3 to −0.3	0.01	2.8 (2.1)	3.9 (2.5)	−1.2	−2.1 to −0.2	0.01	2.6 (2.3)	4.0 (2.6)	−1.4	−2.4 to −0.5	0.005	3.2 (2.4)	4.2 (2.8)	−1.2	−2.3 to −0.2	0.02
NRS pain (walking)	3.5 (2.4)	4.2 (2.5)	−0.9	−1.9 to 0.1	0.07	3.5 (2.2)	4.5 (2.5)	−1.1	−2.0 to −0.2	0.01	3.4 (2.2)	4.6 (2.5)	−1.4	−2.3 to −0.4	0.004	4.0 (2.5)	5.0 (2.7)	−1.3	−2.2 to −0.3	0.01
WOMAC pain	35 (18)	39 (17)	−6.1	−13.4 to 1.2	0.10	34 (19)	39 (18)	−7.0	−14.4 to 0.4	0.06	32 (18)	40 (20)	−9.9	−17.7 to −2.2	0.01	33 (18)	40 (23)	−9.6	−18.0 to −1.2	0.03
Secondary outcomes																				
WOMAC function	36 (20)	43 (19)	−7.6	−15.6 to 0.4	0.06	36 (19)	44 (21)	−9.3	−17.2 to −1.4	0.02	36 (20)	43 (21)	−8.2	−16.5 to 0.1	0.05	37 (19)	44 (24)	−8.9	−17.6 to −0.1	0.05
WOMAC stiffness	39 (21)	47 (21)	−9.4	−17.2 to −1.6	0.02	39 (23)	48 (23)	−11.6	−20.1 to −3.2	0.008	38 (23)	46 (25)	−10.9	−20.1 to −1.7	0.02	39 (25)	48 (26)	−12.2	−21.7 to −2.8	0.01
WOMAC total	36 (19)	42 (18)	−7.5	−15.0 to −0.1	0.05	36 (18)	43 (20)	−8.9	−16.4 to −1.4	0.02	35 (19)	43 (20)	−9.0	−17.0 to −1.0	0.03	37 (19)	44 (24)	−9.4	−17.8 to −0.9	0.03
HOOS pain	59 (19)	55 (17)	6.7	−0.7 to 14.1	0.08	60 (18)	56 (17)	6.4	−0.7 to 13.5	0.08	61 (18)	54 (19)	9.0	1.6 to 16.4	0.02	60 (18)	54 (22)	8.7	0.8 to 16.6	0.03
ICOAP intermittent	30 (19)	37 (20)	−8.0	−16.0 to 0.1	0.05	31 (19)	40 (21)	−10.0	−18.0 to −1.9	0.02	28 (20)	40 (22)	−13.1	−21.4 to −4.7	0.002	30 (20)	40 (23)	−11.7	−20.4 to −2.9	0.009
ICOAP constant	24 (20)	32 (21)	−9.8	−18.2 to −1.4	0.02	25 (20)	34 (23)	−10.4	−19.0 to −1.8	0.02	23 (21)	33 (23)	−11.8	−20.5 to −3.1	0.008	25 (17)	36 (25)	−12.2	−20.7 to −3.8	0.005
ICOAP total	27 (18)	35 (20)	−8.8	−16.3 to −1.3	0.02	28 (18)	37 (22)	−10.2	−18.1 to −2.3	0.01	26 (18)	37 (22)	−12.5	−20.5 to −4.4	0.003	28 (17)	38 (23)	−11.9	−20.1 to −3.8	0.004
Quality of life EQ-5D	0.77 (0.14)	0.71(0.21)	0.05	−0.02 to 0.13	0.14	0.74 (0.20)	0.71 (0.24)	0.03	−0.06 to 0.12	0.51	0.78 (0.17)	0.71 (0.20)	0.06	−0.01 to 0.14	0.10	0.76 (0.18)	0.69 (0.26)	0.08	−0.01 to 0.17	0.09

*Placebo group is reference group.
Values in mean (SD); NRS presented as 0–10 score; WOMAC and ICOAP presented as 0–100 score; model adjusted for KL-score at baseline, ethnicity, morning stiffness and patients' expected effect of injection and for the baseline value of the outcome of interest; glucocorticoid group n=52; placebo group n=54.
Range missing values: n = 0 at baseline and 2-week follow-up to n = 4 at 12-week follow-up.
Δ, between group difference; CCS, glucocorticoid; EQ-5D, EuroQol five dimension scale; HOOS, Hipdisability and Osteoarthritis Outcome Score (0=extreme problems); ICOAP, Intermittent and Constant Osteoarthritis Pain; NRS, Numerical Rating Scale (0=no pain); WOMAC, Western Ontario and McMaster Universities Index (0 = no pain).

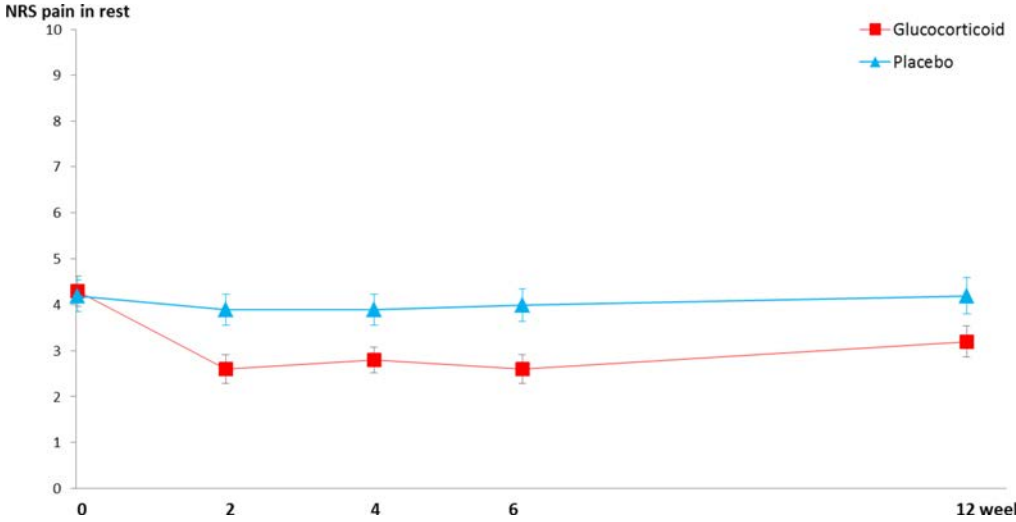

Figure 2 Pain score in rest during the follow-up of glucocorticoid and placebo group. Data shown as mean; SE of the mean (see the online supplementary table S3 for data). NRS, Numerical Rating Scale.

the OMERACT-OARSI responders showed a significant effect in favour of glucocorticoid injection: RR 1.7 (95% CI 1.1 to 2.7) and 2.0 (95% CI 1.1 to 3.6), respectively (table 3).

Adverse events and medical consumption

In the glucocorticoid group, 19 patients reported 27 adverse events. In the placebo group 13 patients reported 18 adverse events. All adverse events were classified as non-serious (table 4). Hot flushes, headache and itching were reported most frequently in the glucocorticoid group. There were no significant differences in medical consumption between the two groups (online supplementary table S2).

Ancillary analyses

In the explorative subgroup analyses, the results of the interaction of setting on injections showed no significant differences between the two groups (NRS at rest 1.5, 95% CI −0.6 to 3.7).

DISCUSSION

We found that an IM glucocorticoid injection showed effectiveness in patients with hip OA on one of the three primary outcomes at a 2-weeks postinjection and that the effect is probably clinically relevant. All primary outcomes and almost all secondary pain and function outcomes showed effectiveness from 4 to 6 weeks that lasted for the entire 12- weeks follow-up. The highest effects were seen at 4–12 weeks follow-up instead of at the expected 2-weeks follow-up.

In this study, three primary endpoints were used. This increases the risk of a type 1 error. However, with a bonferroni correction for the three primary outcomes ($p<0.017$) pain at rest was still significantly associated with pain reduction at a 2-week follow-up.

It was surprising that hip pain reduction after IM glucocorticoid injection was still present at a similar degree at 12-week follow-up. Previous studies on IA glucocorticoid injections in hip OA studies mostly showed a peak effect after 1–3 weeks, but still showed significant pain reduction at 8–12 weeks follow-up.[18 21–23] In a recent Cochrane review on IA glucocorticoid injections in knee OA, the effects were moderate at 1–2 weeks after treatment (effect size 0.48), small to moderate at 4–6 weeks (effect size 0.41) and small at 13 weeks after treatment (effect size 0.22).[24] Therefore, our findings should be replicated in future research.

Also, a surprising finding was that, for patients' perceived improvement and the OMERACT-OARSI responders, there was a significant association in favour of glucocorticoid injection only at a 2-week follow-up. There are two possible explanations for this. First, for patients' perceived improvement we dichotomised the 7-point Likert scale, which resulted in less power. Second, the answer options we provided in the questionnaire were not clearly formulated. For example, answer options were 'completely recovered', 'almost completely recovered' and 'slightly recovered', resulting in a large step between 'almost completely recovered' and 'slightly recovered'. A better delineation would have been: 'completely improved', 'markedly improved' and 'slightly improved'.

In our RCT, we gave a single IM glucocorticoid injection. In clinical practice, patients are sometimes offered multiple IA injections per year. A recent trial in patients with knee OA showed that three monthly injection with glucocorticoid in the knee joint during 2 years resulted in a significantly greater cartilage volume loss than did saline injection.[25] However, there are concerns that even one IA glucocorticoid injection may cause toxicity to chondrocytes, possibly resulting in progression of OA. This has been confirmed in in vitro and in vivo animal studies and needs further study in humans.[26 27] It is unknown whether a single IM glucocorticoid injection has a negative effect on chondrocytes.

The effect of an IA glucocorticoid injection for pain reduction in hip OA has been reported in several RCTs.[18 21–23] The present study shows that systemic treatment with an IM glucocorticoid injection is effective compared with placebo on pain reduction in patients with hip OA. The administration of an IM injection is much easier than an IA hip joint injection without the need for ultrasound/radiologic guidance and can, therefore, be performed in both secondary and primary care. However, the comparative effectiveness of an IM injection compared with an IA injection is unknown.

Strengths and limitations

An important strength of our placebo RCT is that it was blinded for outcome assessors, patients, treating physicians, and researchers (including the statistical analyses). Second, we had

Table 3 Results of the GEE analyses with repeated measurements regarding recovery and treatment responders between the glucocorticoid and placebo group

Follow-up	2 weeks					4 weeks					6 weeks					12 weeks				
Outcome	CCS	Placebo	RR	95% CI	P value	CCS	Placebo	RR	95% CI	P value	CCS	Placebo	RR	95% CI	P value	CCS	Placebo	RR	95% CI	P value
Perceived improvement*	30 (58)	17 (32)	1.7	1.1 to 2.7	0.02	25 (48)	15 (28)	1.6	1.0 to 2.8	0.06	23 (44)	17 (32)	1.4	0.8 to 2.2	0.23	22 (42)	16 (30)	1.3	0.8 to 2.1	0.37
Responder†	24 (46)	12 (22)	2.0	1.1 to 3.6	0.03	21 (40)	15 (28)	1.5	0.8 to 2.5	0.23	23 (44)	16 (30)	1.5	0.9 to 2.5	0.15	22 (42)	17 (32)	1.2	0.7 to 2.7	0.47

Values are n (%); analyses adjusted for KL-score at baseline, ethnicity, morning stiffness and patients expected effect of injection and for the baseline value of the outcome of interest; corticosteroid group n=52; placebo group n=54.
*Perceived improvement indicates scores completely improved, significantly improved and slightly improved.
†According to the OMERACT-OARSI criteria.
CCS, glucocorticoid; GEE, generalised estimating equations; RR, relativerisk. Range missing values: n=0 at baseline and a 2-week follow-up to n=4 at a 12-week follow-up.

Table 4 Adverse events in the two study groups at a 2-week follow-up

Adverse events by organ system*	Glucocorticoid (n=52)	Placebo (n=54)
Reproductive system and breast disorders		
Hot flushes	8 (15)	4 (7)
Irregular menstruation	0	0
Immune system disorders		
Itching	4 (8)	1 (2)
Urticaria	1 (2)	0
Respiratory, thoracic and mediastinal disorders		
Dyspnoea	0	1 (2)
Epistaxis	1 (2)	0
Nervous system disorders		
Headache	5 (10)	4 (7)
Cramp	2 (4)	0
Paresthesia	0	1 (2)
Sweating	2 (4)	0
Musculoskeletal and connective tissue disorder		
Pain in extremity	2 (4)	2 (4)
Gastrointestinal disorders		
Bowel complaints	0	1 (2)
Nausea	0	1 (2)
General disorders and administration site condition		
Pain	2 (4)	0
Fatigue	0	1 (2)
Psychiatric disorders		
Agitation	0	1 (2)
Nervous	0	1 (2)

Values in n (%).
*Classified according to Common Terminology Criteria for Adverse Events Version 4.0, National Institutes of Health National Cancer Institute.

a high follow-up rate, that is, 100% at 2 weeks in both groups, which was the primary outcome time point. At a 12-week follow-up, the follow-up rate was 98% in the glucocorticoid injection group and 94% in the placebo group.

A limitation was not including 128 participants. Nevertheless, we were still able to significantly detect a 10-point difference on a 0–100 scale that was beforehand assumed to be the minimal clinically important difference.

A total of 92 patients (22%) declined to participate, because they did not want to risk receiving a placebo. Similarly, Lambert *et al* found that almost 50% of their patients refused to participate to avoid the risk of placebo treatment.[23] Second, although our patients reported moderate to severe pain (NRS ≥3), the main exclusion reason was that they had not used any analgesics during the past 3 weeks. It would be interesting to establish why patients with moderate to severe pain do not take analgesics. For hip OA little is known about patients' preference and perceptions on treatment. In knee OA, although about 75% of patients use over-the-counter oral analgesics, they do not perceive this treatment as being the most effective; instead, patients perceived viscosupplementation (74.1%), narcotics (67.8%) and steroid injection (67.6%) as being the most effective.[28]

To exclude patients with other painful hip diseases, we set strict criteria for the presence of radiological hip OA (KL ≥2). A final point was the exclusion of patients with diabetes mellitus, a frequently occurring comorbidity in this patient population. It is well known that glucocorticoids can give

rise to hyperglycaemia in diabetic patients.[29] For reasons of patient safety, our medical ethics committee stipulated that we excluded diabetic patients from the present trial; this means that we cannot extrapolate our results to patients with diabetes and hip OA.

Based on the present results, we conclude that an IM glucocorticoid injection showed effectiveness in patients with hip OA in only one of the three primary outcomes at 2-weeks post-injection, but that all primary outcomes and almost all secondary pain and function outcomes showed effectiveness from 4 to 6 weeks that lasted for the entire 12-week follow-up.

Contributors DMJD: trial coordination, data-acquisition, data-analysis and data interpretation, writing article. PAJL: study design, participation in trial coordination, data interpretation and extensive review of article. MR, MK, JANV and PJEB: study design and review of article. PKB: study design, radiological assessment, data interpretation and review of article. SMAB-Z: conceived the study, study design, data interpretation and extensive review of article. All authors read and approved the final manuscript.

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Patient consent Obtained.

Ethics approval Medical Ethics Committee of the Erasmus University Medical Center.

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EXTENDED REPORT

Phase III trial results with blisibimod, a selective inhibitor of B-cell activating factor, in subjects with systemic lupus erythematosus (SLE): results from a randomised, double-blind, placebo-controlled trial

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ABSTRACT

Background Targeted inhibitors of B-cell activating factor (BAFF) have been evaluated in phase III trials in over 4000 patients with systemic lupus erythematosus (SLE). Post hoc analyses of these studies identify greater treatment effect in patients entering with higher disease activity, greater corticosteroid doses, anti double-stranded DNA (dsDNA) and low complement C3 or C4.

Objectives To evaluate the efficacy and safety of blisibimod, a BAFF inhibitor, in a population of patients with SLE enriched for high disease activity.

Methods 442 patients with SLE with antinuclear antibodies or anti-dsDNA and Safety of Estrogen in Lupus Erythematosus National Assessment – Systemic Lupus Erythematosus Disease Activity Index (SELENA-SLEDAI) score ≥10 on standard-of-care medications were randomised to receive weekly subcutaneous blisibimod (200 mg) or placebo. Corticosteroid taper was encouraged from week 8. The primary end point was the week 52 SLE Responder Index-6 (SRI-6).

Results The SRI-6 primary end point was not met. There was a statistically significant steroid-sparing effect, and significantly more blisibimod-treated subjects achieved corticosteroid taper. Increased blisibimod treatment effect on SRI-6 was observed in subjects who achieved a concomitant decrease in corticosteroid dose from baseline. In subjects with baseline urinary protein:creatinine ratio (UPCR) ≥56.5 mg/mmol, significantly higher proportions of blisibimod subjects achieved >50% reduction in UPCR and/or UPCR <56.5 mg/mmol. Reductions in SLE autoantibodies and B cells, and increases in complement C3 and C4 were observed with blisibimod. Blisibimod was well-tolerated. The most common adverse events were upper respiratory tract infection, urinary tract infection, injection site erythema/reaction and diarrhoea.

Conclusions Although the SRI-6 end point was not met, blisibimod was associated with successful steroid reduction, decreased proteinuria and biomarker responses.

Trial registration number NCT01395745.

INTRODUCTION

Targeted, biologic inhibitors of B-cell activating factor (BAFF), belimumab and tabalumab, have been evaluated in four large phase III clinical trials in nearly 4000 patients with systemic lupus erythematosus (SLE).[1–4] Data from these studies indicate that the greatest treatment effect is discernible using more stringent end points and focusing on patients who enter with higher disease activity scores, higher corticosteroid doses, demonstrable antidouble-stranded DNA (dsDNA) and low complement C3 or C4.[5 6]

These observations provide important guidance to clinical trial design, especially in light of the too-common failures of large clinical trials in SLE. Moreover, the data provide important guidance to treatment practice where use of high-dose corticosteroids, immunosuppressive agents and cytotoxic agents remain the standard-of-care for moderate and severe SLE despite their toxicities.[7 8]

Blisibimod (A-623, AMG 623) is a potent and selective BAFF inhibitor composed of a tetrameric BAFF binding domain fused to a human IgG1 Fc region.[9] Although the phase II Placebo-controlled Evaluation of A-623 Response in Lupus (PEARL-SC) trial with blisibimod in SLE failed to meet the primary efficacy end point, SLE Responder Index-5 (SRI-5), it corroborated the observations from larger Phase III trials in that better treatment effect was observed in subjects with higher disease activity, taking corticosteroids, who had abnormalities in anti-dsDNA and/or complement.[10] Furthermore, in keeping with the belimumab observations,[11] favourable effects on proteinuria were observed.[10] In addition, blisibimod treatment was associated with significant decreases in anti-dsDNA, immunoglobulins, total B-cell counts and fatigue[12] and significant increases in complement C3 and C4. Accordingly, the phase III Clinical and Health Assessments with BLISibimod SC, Study 1 (CHABLIS-SC1) trial prospectively evaluated a population of subjects with SLE enriched for the traits that, in earlier studies, were associated with greater treatment effect relative to background standard-of-care treatments.

METHODS

Patient population and trial design

The CHABLIS-SC1 study (NCT01395745) was conducted in Belarus, Brazil, Colombia, Georgia, Guatemala, Hong Kong, Korea, Singapore, Malaysia, Mexico, Russia, Sri Lanka, Taiwan, Thailand and the Philippines. The study enrolled 442 adults aged ≥18 years who were seropositive for anti-nuclear antibody (ANA) (≥1:80 in

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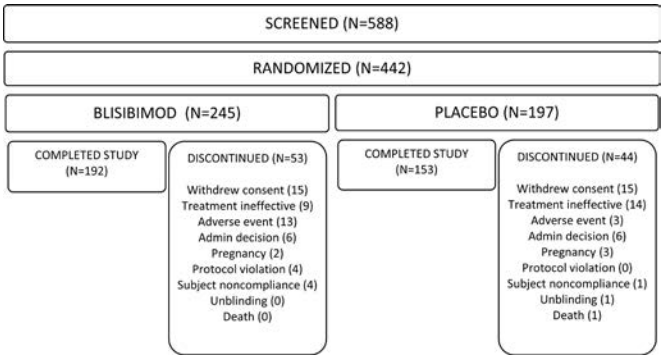

Figure 1 Disposition of subjects in the CHABLIS-SC1 trial, including randomisation (5:4) into the treatment arms, numbers of subjects who completed study and reasons for discontinuation (and numbers of subjects).

immunofluorescence assay) or anti-dsDNA (≥30 IU/mL), met at least four of the SLE Classification Criteria defined by the American College of Rheumatology (ACR),[13] and had a baseline disease activity score of ≥10 on the SELENA-SLEDAI scale despite concomitant and stable use of systemic corticosteroids at the lesser of 0.5 mg/kg or 40 mg/day of prednisone or equivalent. Other standard-of-care medications in stable doses were allowed (antimalarials, methotrexate, mycophenalate, azathioprine, leflunomide, non-steroidal anti-inflammatory drugs). Subjects were excluded from study if they had active vasculitis, central nervous system lupus or severe active lupus nephritis, proteinuria >6 g/24 hours (or equivalent) or a history of malignancy in the last 5 years, HIV infection, hepatitis B or C infection or tuberculosis or recent use of a B-cell targeted drug, investigational agents, cyclophosphamide or other alkylators, transfusion, intravenous immunoglobulin, plasmapheresis, plasma exchange, high-dose corticosteroid, antitumour necrosis factor-α or other biologics, ciclosporin or live vaccines.

Eligible subjects were randomly assigned using an interactive web-based randomisation system in a 5:4 ratio to receive subcutaneous blisibimod 200 mg once weekly or corresponding volume and frequency-matched placebo using a double-blind interactive web response system. The dose of blisibimod in this trial was based on the observations from a prior Phase II trial in SLE.[10] Randomisation was stratified by race (African vs non-African race), baseline SELENA-SLEDAI score (≤12 vs >12) and proteinuria (<2 vs ≥2 g/24 hours equivalent). Screening was initiated in March 2013. The sponsor, all investigators, study personnel and subjects remained blind to treatment allocation until after the effects on SRI-6 at week 52 were analysed in November 2016.

Subjects were required to continue background SLE medication at the stable, prestudy doses throughout the study except for corticosteroids. Corticosteroids could be increased during the first 8 weeks of the trial but were not allowed not to exceed 5 mg or 25% above baseline (whichever was lower) by week 8, after which time a subject at higher dose would be considered a treatment failure. Steroid taper was encouraged from week 8 onwards with a goal of achieving ≤7.5 mg prednisone/day or equivalent. Subjects received study drug for 53 weeks.

The study was conducted in accordance with the provisions of the Declaration of Helsinki, and governmental, state and local laws. Informed consent was obtained from each patient prior to study screening. The trial started in April 2013 and the last patient completed the last study visit September 2016.

Table 1 Demographics and baseline disease characteristics

	Blisibimod (n=245)	Placebo (n=197)	Overall (n=442)
Age, mean (SD) (years)	36.7 (10.98)	35.6 (10.78)	36.2 (10.89)
Gender (female:male, %)	92.7:7.3	94.9:5.1	93.7:6.3
Race, n (%)			
Black or African-American	10 (4.1)	4 (2.0)	14 (3.2)
White	167 (68.2)	132 (67.3)	299 (67.8)
Asian	67 (27.3)	60 (30.6)	127 (28.8)
Native Hawaiian or other Pacific Islander	1 (0.4)	0	1 (0.2)
Region, n (%)			
Asia Pacific	67 (27.3)	60 (30.5)	127 (28.7)
Central and South America	89 (36.3)	58 (29.4)	147 (33.3)
Europe	89 (36.3)	79 (40.1)	168 (38.0)
Weight, mean (SD) (kg)	65.1 (15.46)	64.8 (15.31)	65.0 (15.38)
BMI, mean (SD) (kg/m^2)	25.1 (5.33)	25.0 (4.96)	25.0 (5.17)
SELENA-SLEDAI mean score (SD)	13.4 (4.31)	13.5 (4.01)	13.5 (4.18)
SELENA-SLEDAI activity, n (%)			
Total score ≤12	133 (54.3)	107 (54.3)	240 (54.3)
Total score >12	112 (45.7)	89 (45.2)	201 (45.5)
Dermal	234 (95.5)	188 (95.9)	422 (95.7)
Immunologic	222 (90.6)	178 (90.8)	400 (90.7)
Musculoskeletal	193 (78.8)	159 (81.1)	352 (79.8)
Renal	78 (31.8)	54 (27.6)	132 (29.9)
BILAG A, B or C domain score, n (%)			
Mucocutaneous	236 (96.3)	190 (96.9)	426 (96.6)
Musculoskeletal	209 (85.3)	169 (86.2)	378 (85.7)
Haematological	121 (49.4)	101 (51.5)	222 (50.3)
Renal	96 (39.2)	64 (32.7)	160 (36.3)
Constitutional	35 (14.3)	28 (14.3)	63 (14.3)
PGA score, mean (SD)	1.59 (0.475)	1.64 (0.475)	1.61 (0.475)
PGA score (range)	0.1–2.7	0.4–2.8	0.1–2.8
Corticosteroid dose (prednisone dose equivalent, mg (SD))	15.6 (8.58)	15.6 (9.75)	15.6 (9.11)
<Median prednisone dose, n (%)	108 (44.1)	103 (52.3)	211 (47.7)
≥Median prednisone dose, n (%)	137 (55.9)	94 (47.7)	231 (52.3)
Systemic SLE medications, n (%)			
Any (including corticosteroid)	245 (100.0)	195 (99.5)	440 (99.8)
Antimalarial	150 (61.2)	122 (62.2)	272 (61.7)
Any immunosuppressive agent	104 (42.4)	82 (41.8)	186 (42.2)
Azathioprine	60 (24.5)	47 (24.0)	107 (24.3)
Mycophenolate (any derivative)	35 (14.3)	22 (11.7)	58 (13.1)
Methotrexate	14 (5.7)	15 (7.7)	29 (6.6)
SLE markers, n (%)			
Anti-dsDNA positive	239 (97.6)	194 (98.5)	433 (98.0)
Low C3 (<0.9 g/L)	132 (53.9)	105 (53.6)	237 (53.7)
Low C4 (<0.18 g/L)	138 (56.3)	100 (51.0)	238 (54.0)
Low C3/C4 and anti-dsDNA	153 (62.4)	121 (61.7)	274 (62.1)
UPCR, n (%)			
≥56.5 mg/mmol	80 (32.7)	55 (27.9)	135 (30.5)
≥113 mg/mmol	47 (19.2)	35 (17.8)	82 (18.6)
≥226 mg/mmol	23 (9.4)	15 (7.7)	38 (8.6)

BMI, body mass index; dsDNA, double-stranded DNA; SLE, systemic lupus erythematosus; UPCR, urinary protein:creatinine ratio.

Statistical analyses and sample size

Efficacy analyses were conducted in the modified intent-to-treat (mITT) population, defined as all subjects who received at least one dose of study drug. The primary end point was the

proportion of responders to a composite SRI at week 52, the SRI-6, defined as: a ≥6 point reduction in SELENA-SLEDAI score compared with baseline; no new British Isles Lupus Assessment Group (BILAG) A organ domain scores or no more than 1 new BILAG B organ domain score at time of assessment compared with baseline and no worsening in Physician's Global Assessment (PGA) score (<0.3 point increase from baseline on a 3-point scale). Subjects who required increases in background SLE medication, including increases in corticosteroid by >25% or 5 mg prednisone (or equivalent), new or increased SLE medications or withdrew from study for any reason were defined as treatment failures for all SRI variables. The primary end point compared SRI-6 response in the blisibimod arm to placebo using a two-sided test at the 5% level of significance at week 52. The primary end point was analysed using a logistic regression model with treatment group, region, race, baseline SELENA-SLEDAI score (≤12 vs >12), prednisone dose, immunosuppressant use and proteinuria category fitted as explanatory variables.

Based on observations at week 24 in a phase II study with blisibimod[10] and abstract presentations of emerging week 52 data with the BAFF-targeted monoclonal antibody, tabalumab,[3 4] SRI-6 responder rates at week 52 with drug and placebo were modelled to be 39% and 25%, respectively. Assuming these responder rates and a two-sided test for significance at an alpha level of 0.05, the power to detect a treatment difference for SRI-6 at week 52 for 400 subjects is 84%.

Secondary end points were analysed using the following methods: time-to-event variables were analysed using Cox proportional hazards regression models, proportion end points were analysed using logistic regression models, quantitative end points were analysed using analysis of covariance (ANCOVA) models and flare rate was analysed using a negative binomial regression model. All models included the same factors as used for the analysis of the primary efficacy end point. The ANCOVA models additionally included the baseline value of the corresponding parameter as a covariate. Secondary end points were to be tested in a prespecified hierarchical order, each at the 0.05 significance level. However, since the study null hypothesis was rejected due to failure of the primary efficacy analysis, all secondary analyses are considered to be exploratory and are reported here as such, without adjustment for multiplicity.

An interim futility analysis using the SRI-6 response at week 24 was conducted by an independent statistician after approximately 100 subjects completed 24 weeks of treatment or had withdrawn from study.

RESULTS

Of the 588 subjects who were screened for this study, 442 were randomised, one of whom did not receive study drug (in the placebo arm) and was excluded from the mITT analyses (figure 1). The majority of subjects were female (93.7%) and of childbearing potential (average age 36.2 years), and the most common SLE organ manifestations based on SELENA-SELDAI score were dermal (95.7%), immunologic (90.7%) and musculoskeletal (79.8%). Approximately 29.9% of subjects had evidence of renal disease at enrolment, defined as UPCR ≥56.5 mg/mmol. The mean SELENA-SLEDAI score and corticosteroid dose were 13.5±4.2 and 15.6±9.1 mg, respectively. Demographics, baseline

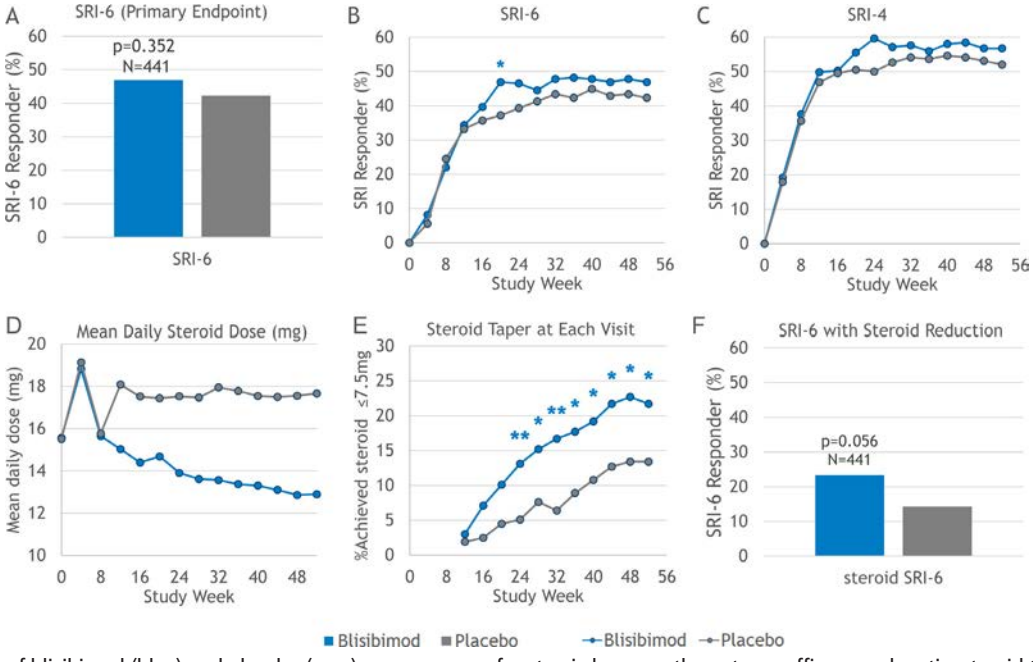

Figure 2 Effects of blisibimod (blue) and placebo (grey) on measures of systemic lupus erythematosus efficacy and corticosteroid taper in the modified intent-to-treat (mITT) population. SLE Responder Index -6 (SRI-6) data for week 52 (A) and at all visits (B), are plotted along with SRI-4 at all visits (C). The mean daily prednisone (or equivalent) dose is shown in (D), along with the proportion of subjects who achieved corticosteroid taper to prednisone ≤7.5 mg/day or equivalent at each visits after week 8 (the time after which steroid taper was encouraged) (E). In the evaluation of SRI-6 with steroid reduction (F), responders met all of the SRI-6 criteria week 52 while also having an oral corticosteroid dose from week 40 through week 52 that was lower than the dose on day 1. SRI analyses were conducted using a non-responder imputation method, wherein subjects were considered to be non-responders if they received new or increased corticosteroid or immunosuppressive or antimalarial medication relative to baseline, or used prohibited medication, or withdrew from study for any reason from the event date onwards. In all graphs, the proportion of subjects meeting the relevant responder criteria are reported relative to the number of subjects randomised to that treatment arm in the mITT or the relevant evaluable population. Statistical outcomes are annotated as *P<0.05 and **P<0.01.

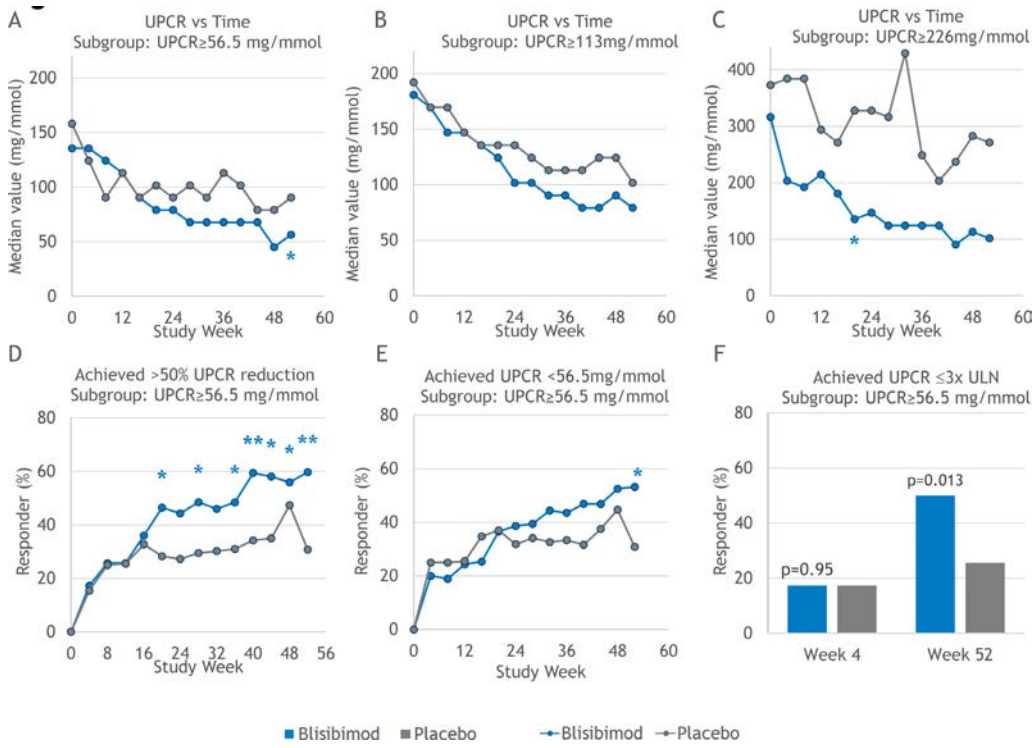

Figure 3 Effects of blisibimod (blue) and placebo (grey) on urinary protein:creatinine ratio (UPCR) in UPCR subgroups. Median UPCR were computed for both treatment arms in subgroups based on baseline UPCR: UPCR ≥56.5 mg/mmol (n=135, A), ≥113 mg/mmol (n=82, B) and ≥226 mg/mmol (n=35, C). Additional analyses in the subgroup of subjects with baseline UPCR ≥56.5 mg/mmol evaluated the proportion of subject who achieved >50% reduction in UPCR from baseline (D), and/or UPCR <56.5 mg/mmol (E), and/or UPCR ≤3 times the upper limit of normal (ULN, defined as 0.15 g/24 hours equivalent (F). For graphs A, B and C, data are shown as median value for all available observations from subjects in the relevant subgroup. Responder analyses in graphs D, E and F summarise the proportion of responder relative to number of subjects in the subgroup of subjects with baseline UPCR ≥56.5 mg/mmol. Statistical outcomes are annotated as *P<0.05 and **P<0.01.

disease characteristics and SLE medications were approximately balanced between the two treatment arms (table 1). In general, the use of background SLE mediations in this study was similar in recent global clinical trials in SLE.[1–4]

Effects on SRI, SELENA-SLEDAI and corticosteroid taper

The study did not meet the primary efficacy end point of SRI-6 at week 52 (46.9% with blisibimod, 42.3% in the standard-of-care control group, figure 2A). However, consistent trends from week 16 through week 52 were observed with blisibimod for both the SRI-6 and SRI-4 end points (figure 2B, C).

The mean steroid dose after week 8 remained below baseline in the blisibimod group and above baseline in the placebo group, with the difference between groups slowly widening from ~3 mg/day prednisone at week 12 to ~5 mg/day at week 52 (figure 2D). Consistent with this observation, significantly more subjects in the blisibimod arm with a baseline prednisone dose of >7.5 mg/day or equivalent achieved corticosteroid taper to ≤7.5 mg/day at each visit from week 24 through week 52 (figure 2E), and more subjects on blisibimod achieved sustained corticosteroid taper over weeks 40 through 52 compared with placebo (17.2% vs 8.9%, P=0.019). A secondary analysis of the SRI-6 response was undertaken to examine whether the SRI-6 primary end point may have been confounded by imbalances in corticosteroid taper. In this end point, adapted from a recently published trial result,[14] response was defined as meeting the week 52 SRI-6 criteria while also having an oral corticosteroid dose from week 40 through week 52 that was lower than the dose on day 1. With this analysis, 23.3% of blisibimod-treated subjects met the end point vs 14.3% of the control arm (P=0.056, figure 2F).

Effects on UPCR

Treatment effects on UPCR were evaluated in subgroups of subjects defined by baseline UPCR ≥56.5 mg/mmol (n=135), ≥113 mg/mmol (n=82) and ≥226 mg/mmol (n=35). Within each subgroup, baseline UPCR levels were approximately balanced between treatment arms. Greater decreases in UPCR were observed among subjects randomised to blisibimod at all three ranges of proteinuria (figure 3A, B, C). The modest decreases from baseline in UPCR observed in subjects in the placebo arms of each of these subgroups may reflect the monthly clinical assessments and better adherence to therapy of subjects in trials compared with pretrial status. In the subgroup of subjects with baseline UPCR ≥56.5 mg/mmol, significantly more of the subjects who received blisibimod achieved >50% reduction in UPCR from baseline (figure 3D), and/or the threshold of UPCR <56.5 mg/mmol (figure 3E), and/or the threshold of UPCR ≤3 times the upper limit of normal (16.95 mg/mmol) at week 52 (figure 3F).

Effects on blood and serum markers of B-cell activity or SLE disease activity

A trend towards decreased anti-dsDNA was observed with blisibimod (figure 4A), which was not statistically significant. Significant increases in complement C3 and C4 were observed with blisibimod compared with the standard-of-care control group (figure 4B, C) with the following changes in peripheral B-cell counts (detected by flow cytometry gated on side scatter and CD45, and expressed relative to concurrent lymphocyte counts): decreases in total B cells (CD20+CD19+), naïve B cells (CD20+CD19+IgD+CD27−), activated B cells

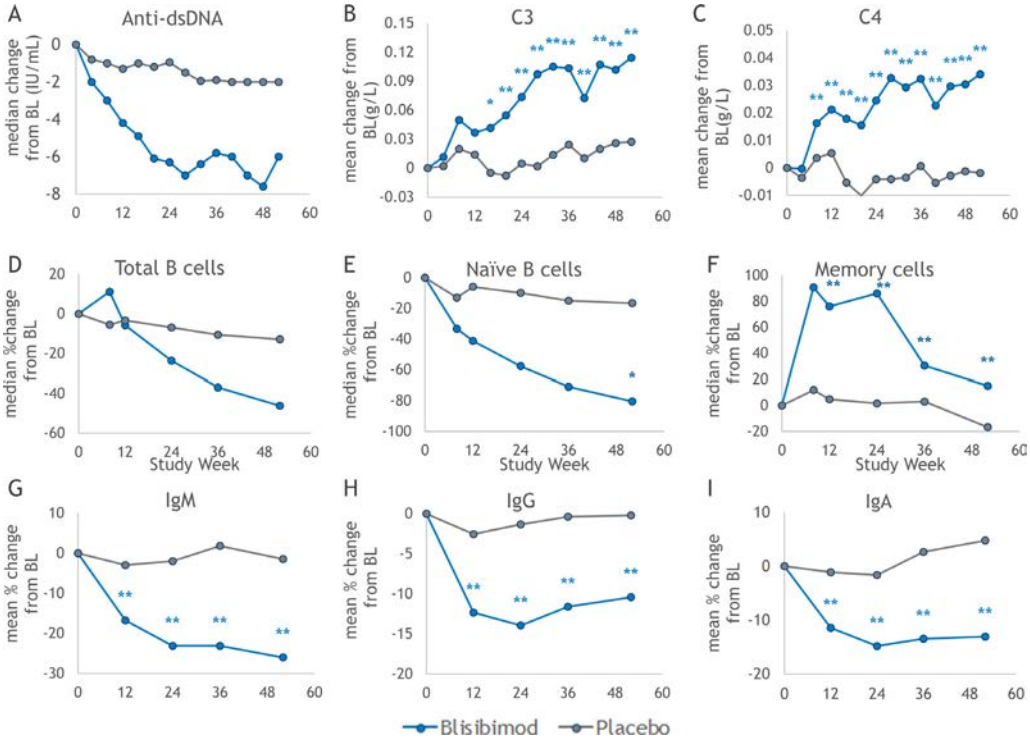

Figure 4 Treatment effects on SLE biomarkers, B cells and quantitative immunoglobulins. (A) Median change from baseline (BL) in serum concentrations of anti-dsDNA antibodies (mean at baseline 200.6±763.7 and 175.4±576.7 IU/mL for blisibimod and placebo), (B) mean change from BL in complement C3 (mean at baseline 0.881 and 0.885 g/L), (C) mean change from BL in C4 (mean baseline 0.174 and 0.175 g/L) are plotted for blisibimod (blue) and placebo (grey). Similarly, data are plotted for mean % changes from BL in B-cell counts determined by flow cytometry are reported as median per cent change from baseline for: (D) total B cells, (E) naïve B cells and (F) memory B cells (I), and mean % change from BL in serum concentrations of (D) immunoglobulin IgM (mean at baseline 1.15 and 1.07 g/L), (E) IgG (mean at baseline 14.53 and 14.94 g/L) and (F) IgA (mean at baseline 3.024 and 3.188 g/L). For all graphs, data are plotted using all available observations from subjects in the modified intent- to- treat population (*P<0.05, **P<0.01).

(CD19+CD38+CD138+) (data not shown) and transient increases in the memory B cells (CD19+CD27+, figure 4D, E, F). Finally, significant decreases in mean concentrations of serum immunoglobulins IgM, IgG and IgA ranging from 10% to 26% (figure 4G, H, I), and anticardiolipin-directed IgG and IgM antibodies were observed with blisibimod compared with placebo. Five of the 245 (2.0%) subjects randomised to blisibimod and 3/197 (1.5%) subjects randomised to placebo were observed to have reduction in IgG to <4 g/L or lower. In all but two subjects, one in each treatment arm, the low IgG value was transient, recovering by the next observation. No infections were reported as serious adverse events in any subject with such reductions in IgG.

Effects on quality of life

At 3-month intervals throughout the study, subjects were asked to conduct a self-evaluation of their quality of life using the Lupus Quality of Life Questionnaire (Lupus QoL).[15] To minimise bias, these evaluations were required to be conducted at study visit prior to any assessments of disease activity by the investigator or site staff. Modest improvements in the Lupus QoL total score were observed with blisibimod compared with control from week 12 onwards (P=ns).

Safety

Blisibimod was well tolerated, with similar numbers of subjects reporting treatment-emergent adverse events (TEAEs 69.8% vs 64.8%) and treatment-emergent serious adverse events (13.1% vs 17.3%) in the blisibimod and control arms, respectively. The only imbalances in reported TEAEs related to reactions at the injection site (erythema, reaction, rash, pain), which were reported more frequently with blisibimod than placebo (table 2); none was severe or serious. The latter imbalance also accounts in large part for the higher proportion TEAEs considered due to study drug (35.9% vs 23.5%), and TEAEs leading to discontinuation (5.3% vs 1.5%) on blisibimod.

DISCUSSION

Post hoc analyses of the combined datasets from two Phase III trials with belimumab, BLISS-52 and BLISS-76 demonstrated that better treatment benefit was detected among subjects with higher disease activity, seropositivity defined by anti-dsDNA high or complement C3/C4 low and ongoing corticosteroid use.[5] These observations, corroborated by subsequent observations from the Phase III trials with tabalumab,[4] provided the rationale for the design for the phase III CHABLIS-SC1 study, which prospectively evaluated a population of subjects with SLE enriched for the traits that, in earlier studies, were associated with greater treatment effect relative to standard-of-care alone.

The SRI-6 response rate in the control subjects in this study (42.3%) was very high compared with prior SLE trials conducted in subjects with baseline SELENA-SLEDAI scores of ≥6 (20.4%,[1] 29.2%,[3] 27.0%[4]) or ≥8 (33.7%[16]). Analysis of SRI-6 was based on a logistic regression model with the following covariates fitted as explanatory variables: region, SELENA-SLEDAI at baseline (<=12, >12), UPCR at screening (<226, ≥226 mg/mmol), prednisone dose at baseline (<median dose, ≥median dose) and immunosuppressant use at baseline (yes, no). None of these

Table 2 Treatment-emergent adverse events

	Blisibimod (n=245)	Placebo (n=196)	Overall (n=441)
Subjects with any TEAE, n (%)			
TEAE	171 (69.8)	127 (64.8)	298 (67.6)
Serious TEAE	32 (13.1)	34 (17.3)	66 (15.0)
Mild TEAE	151 (61.6)	110 (56.1)	261 (59.2)
Moderate TEAE	71 (29.0)	63 (32.1)	134 (30.4)
Severe TEAE	16 (6.5)	14 (7.1)	30 (6.8)
TEAE related to study drug	88 (35.9)	46 (23.5)	134 (30.4)
TEAE leading to discontinue	13 (5.3)	3 (1.5)	16 (3.6)
TEAE leading to death	1 (0.4)	2 (1.0)	3 (0.7)
TEAEs in>3% of subjects (overall), n (%)			
Subjects with any TEAE	171 (69.8)	127 (64.8)	298 (67.6)
Infections and infestations	107 (43.7)	86 (43.9)	193 (43.8)
Upper respiratory tract infection	26 (10.6)	28 (14.3)	54 (12.2)
Urinary tract infection	17 (6.9)	21 (10.7)	38 (8.6)
Influenza	9 (3.7)	9 (4.6)	18 (4.1)
Herpes zoster	9 (3.7)	7 (3.6)	16 (3.6)
Nasopharyngitis	13 (5.3)	3 (1.5)	16 (3.6)
Viral infection	3 (1.2)	10 (5.1)	13 (2.9)
General disorders and administration site conditions	60 (24.5)	26 (13.3)	86 (19.5)
Injection site erythema	19 (7.8)	4 (2.0)	23 (5.2)
Injection site reaction	18 (7.3)	5 (2.6)	23 (5.2)
Gastrointestinal disorders	44 (18.0)	31 (15.8)	74 (17.0)
Diarrhoea	18 (7.3)	5 (2.6)	23 (5.2)
Musculoskeletal and connective tissue disorders	34 (13.9)	34 (17.3)	68 (15.4)
Back pain	9 (3.7)	5 (2.6)	14 (3.2)
Nervous system disorders	33 (13.5)	35 (17.9)	68 (15.4)
Headache	15 (6.1)	16 (8.2)	31 (7.0)
Dizziness	6 (2.4)	6 (3.1)	12 (2.7)
Investigations	12 (4.9)	20 (10.2)	32 (7.3)
Transaminases increased	4 (1.6)	6 (3.1)	10 (2.3)
Blood and lymphatic system diseases	11 (4.5)	20 (10.2)	31 (7.0)
Anaemia	3 (1.2)	8 (4.1)	11 (2.5)
Vascular disorders	12 (4.9)	13 (6.6)	25 (5.7)
Hypertension	7 (2.9)	13 (6.6)	20 (4.5)
Treatment-emergent serious adverse events in>1 subject, n (%)			
Infections and infestations	15 (6.1)	17 (8.7)	32 (7.3)
Pneumonia	2 (0.8)	6 (3.1)	8 (1.8)
Urinary tract infection	2 (0.8)	2 (1.0)	4 (0.9)
Appendicitis	3 (1.2)	0	3 (0.7)
Sepsis	1 (0.4)	2 (1.0)	3 (0.7)
Cellulitis	1 (0.4)	1 (0.5)	2 (0.5)
Gastroenteritis	0	2 (1.0)	2 (0.5)
Herpes zoster	2 (0.8)	0	2 (0.5)
Pyelonephritis	1 (0.4)	1 (0.5)	2 (0.5)
Musculoskeletal and connective tissue disorders	5 (2.0)	5 (2.6)	10 (2.3)
SLE	1 (0.4)	3 (1.5)	4 (0.9)
Blood and lymphatic system disorders	3 (1.2)	4 (2.0)	7 (1.6)
Anaemia	1 (0.4)	1 (0.5)	2 (0.5)
Gastrointestinal disorders	2 (0.8)	4 (2.0)	6 (1.4)
Upper gastrointestinal haemorrhage	1 (0.4)	1 (0.5)	2 (0.5)
Vascular disorders	3 (1.2)	1 (0.5)	4 (0.9)
Deep vein thrombosis	2 (0.8)	0	2 (0.5)
Pregnancy, puerperium and perinatal conditions	1 (0.4)	2 (1.0)	3 (0.7)
Abortion spontaneous	1 (0.4)	2 (1.0)	3 (0.7)

SLE, systemic lupus erythematosus; TEAE, treatment-emergent adverse event.

was found to significantly influence SRI-6 outcome (range of covariate P values: 0.26–0.81).

Secondary analyses of SRI-6 response provide possible explanations for the high response rates of control subjects. Specifically, when steroid reduction is included in the end point, analogous to the phase II SLE trial with anifrolumab,[14] lower responder rates were observed and greater treatment effect with blisibimod. The secondary analyses of the effects of blisibimod on corticosteroid taper suggest that background corticosteroid use may have confounded the primary efficacy end point, since many of the control subjects received corticosteroid doses that were higher than baseline throughout the trial, averaging 3–5 mg/day higher than the blisibimod group, in which the mean dose was below baseline from week 12 onwards. Perhaps alternative methods of normalising corticosteroid use and taper between treatment arms might be considered in future SLE trials. Incorporation of steroid reduction within outcomes has also been found informative.[14]

The contribution of each organ system to improvements in the SELENA-SLEDAI was examined. The primary contributions to disease activity at baseline were, not surprisingly, from the musculoskeletal and mucocutaneous domains, but immunologic and renal manifestations were also substantial. Rapid improvements in both mucocutaneous and musculoskeletal disease activity were observed in both treatments arms at week 4 (>10%), week 8 (>25%), week 12 (>40%) and beyond. Responses in these domains can be sensitive to small changes in corticosteroids, and the higher steroid dosing in the placebo arm may have contributed to the relatively high response rates in that group.

In an analysis of subjects with baseline UPCR ≥56.5, ≥113 and/or ≥226 mg/mmol, reductions in proteinuria (UPCR) were observed with blisibimod. These observations, generated in subgroups of fewer than one-third of the subjects enrolled, support the hypothesis that blisibimod has therapeutic potential in subjects with renal lupus. Interpretation of these data is limited by the lack of renal biopsy data in most subjects, and by the exclusion of subjects at entry to the trial whose renal disease was deemed to require immediate initiation of new or increased immunosuppressive therapy. However, these findings are consistent with the observed treatment effects of blisibimod on anti-dsDNA autoantibodies, complement C3 and C4, serum immunoglobulins and B-cell lineages, which were similar to those reported previously for blisibimod[9 10] and other BAFF inhibitors.[1 2] These observations additionally confirm that subjects in the blisibimod arm achieved pharmacological inhibition of BAFF.

In summary, the CHABLIS-SC1 (see online supplementary file 1) trial prospectively evaluated an SLE population enriched for disease characteristics that had been previously associated with greater treatment effect, but failed to meet its primary end point of SRI-6. However, key secondary analyses of corticosteroid use and UPCR suggest that blisibimod might have important clinical benefits.

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Contributors All authors provided substantial input to the design, conduct and/or analysis of this trial. In addition, all authors participated in the drafting of this manuscript and the responses to reviewers and approve this version for publication.

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Competing interests JTM, MS, KCK, DW are current or prior consultants for the sponsor, Anthera Pharmaceuticals. MS was a clinical investigator in this trial. RSM and WRS are employees and shareholders of Anthera Pharmaceuticals.

Patient consent Detail has been removed from this case description/these case descriptions to ensure anonymity. The editors and reviewers have seen the detailed information available and are satisfied that the information backs up the case the authors are making.

Ethics approval Multiple EC/IRB bodies reviewed and approved this trial (in Belarus, Brazil, Colombia, Georgia, Guatemala, Hong Kong, Korea, Singapore, Malaysia, Mexico, Russia, Sri Lanka, Taiwan, Thailand and the Philippines).

Provenance and peer review Not commissioned; externally peer reviewed.

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EXTENDED REPORT

Secukinumab improves active psoriatic arthritis symptoms and inhibits radiographic progression: primary results from the randomised, double-blind, phase III FUTURE 5 study

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ABSTRACT

Objectives To evaluate the effect of subcutaneous (s.c.) secukinumab, an interleukin-17A inhibitor, on clinical signs and symptoms and radiographic progression in patients with psoriatic arthritis (PsA).

Methods Adults (n=996) with active PsA were randomised 2:2:2:3 to s.c. secukinumab 300 mg or 150 mg with loading dose (LD), 150 mg without LD or placebo. All groups received secukinumab or placebo at baseline, weeks 1, 2 and 3 and then every 4 weeks from week 4. The primary endpoint was the proportion of patients achieving an American College of Rheumatology 20 (ACR20) response at week 16.

Results Significantly more patients achieved an ACR20 response at week 16 with secukinumab 300 mg with LD (62.6%), 150 mg with LD (55.5%) or 150 mg without LD (59.5%) than placebo (27.4%) (p<0.0001 for all; non-responder imputation). Radiographic progression, as measured by van der Heijde-modified total Sharp score, was significantly inhibited at week 24 in all secukinumab arms versus placebo (p<0.01 for 300 mg with LD and 150 mg without LD and p<0.05 for 150 mg with LD; linear extrapolation). Adverse event rates at week 24 were similar across treatment arms: 63.1% (300 mg with LD), 62.7% (150 mg with LD), 61.1% (150 mg without LD) and 62.0% (placebo). No deaths or new safety signals were reported.

Conclusion S.c. secukinumab 300 mg and 150 mg with and without LD significantly improved clinical signs and symptoms and inhibited radiographic structural progression versus placebo at week 24 in patients with PsA.

Trial registration number NCT02404350; Results.

INTRODUCTION

Psoriatic arthritis (PsA) is a chronic, inflammatory disease characterised by peripheral arthritis, axial disease, dactylitis, enthesitis and skin and nail psoriasis.[1 2] It can have a substantial impact on quality of life and work productivity,[3] with many patients experiencing irreversible joint damage and disability.[1 4] Indeed, bone erosions occur in approximately half of patients within 2 years.[5]

Enhanced understanding of the pathophysiology of PsA has aided the development of targeted therapies to manage its signs and symptoms. The proinflammatory cytokine interleukin (IL)-17A mediates multiple biological functions that result in joint and entheseal inflammation, damage and tissue remodelling, which are characteristic of PsA.[6–8] Recommendations from the European League Against Rheumatism (EULAR)[9] and the Group for Research and Assessment of Psoriasis and Psoriatic Arthritis (GRAPPA)[10] recognise targeting IL-17A as a therapeutic strategy to manage all the main clinical domains of PsA.

Secukinumab, a fully human monoclonal antibody that selectively neutralises IL-17A,[11] is approved in many countries for the treatment of PsA, psoriasis and ankylosing spondylitis. There is now an extensive body of evidence involving more than 2700 patients demonstrating the efficacy of secukinumab in PsA.[12–18] Data from the phase III studies, FUTURE 1 and FUTURE 2, have shown that secukinumab provides rapid and significant improvements in the signs and symptoms of PsA that are sustained for up to 3 years of therapy.[12–18] These clinical benefits have been observed in patients naïve to biological therapy and in those with an intolerance or inadequate response to agents targeting tumour necrosis factor (TNF).[9 10 13–18] Data from FUTURE 1 have shown that secukinumab significantly inhibits joint structural damage through 24 weeks,[13] with benefits maintained out to 2 years.[17] FUTURE 1 used an intravenous loading followed by subcutaneous (s.c.) dosing regimen for secukinumab and did not evaluate a dose higher than 150 mg.[13] FUTURE 2 used s.c. loading and maintenance dosing of secukinumab 300, 150 and 75 mg[14] (the 300 and 150 mg dosing regimens were subsequently adopted as the approved regimens in PsA), but it did not examine radiographic progression and, until now, these data have been lacking. Here, we report primary results from the ongoing FUTURE 5 study, the largest randomised, controlled study to date of a biologic in PsA. The study was designed to evaluate the impact of s.c. secukinumab 300 and 150 mg on clinical signs and symptoms and radiographic progression as well as evaluating the short-term benefit of the loading regimen. This trial is ongoing and will provide long-term data out to 2 years.

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METHODS

Study design and participants

FUTURE 5 (NCT02404350) is a randomised, double-blind, placebo-controlled, parallel-group phase III trial. The study design is shown in online supplementary figure 1. The study was conducted in accordance with the principles of the Declaration of Helsinki.[19] Patients provided written informed consent before study-related procedures were undertaken.

Patients were aged ≥18 years and met the ClASsification criteria for Psoriatic ARthritis[2] at screening, with symptoms of moderate-to-severe PsA for at least 6 months: ≥3 tender joints and ≥3 swollen joints despite ≥4 weeks of treatment with non-steroidal anti-inflammatory drugs (NSAIDs), or an intolerance to them, and active or documented history of plaque psoriasis or psoriatic nail changes. Concomitant corticosteroids (≤10 mg/day prednisone or equivalent), NSAIDs and methotrexate (≤25 mg/week) were allowed, provided the dose was stable and remained so for the first 24 weeks of the study. Patients who had previously used anti-TNF agents could enrol if they had experienced an inadequate response or stopped treatment due to safety or intolerance (anti-TNF-IR). Patients taking anti-TNF therapy and/or a disease-modifying antirheumatic drug other than methotrexate required a washout ranging from 4 to 10 weeks before randomisation, depending on the prohibited treatment used. Key exclusion criteria included active/history of ongoing infection, prior use of a biologic other than an anti-TNF agent, use of ≥3 anti-TNF agents and active inflammatory disease other than PsA.

Treatment and randomisation

Following a screening period of up to 10 weeks, Interactive Response Technology was used to randomly assign eligible patients in a 2:2:2:3 ratio to one of four treatment groups: secukinumab 300 mg with loading dose (LD), secukinumab 150 mg with LD, secukinumab 150 mg without LD or placebo, all administered s.c. (online supplementary figure 1). Patients self-administered their own treatment using prefilled syringes at baseline, weeks 1, 2 and 3 followed by treatment every 4 weeks from week 4. Patients in the secukinumab 150 mg without LD arm were administered placebo at weeks 1, 2 and 3 to conceal treatment allocation. At week 16, patients in the placebo arm with <20% improvement from baseline in tender and swollen joint counts (SJCs) were switched in a double-blind manner to receive s.c. secukinumab 300 mg or 150 mg, preassigned at original randomisation. Remaining patients in the placebo arm were switched to blinded secukinumab 300 mg or 150 mg at week 24.

Randomisation was stratified according to previous anti-TNF therapy use, with patients being anti-TNF-naïve (planned enrolment about 70%) or anti-TNF-IR. Patients, investigators and assessors remain masked to the treatment assignment until all patients reach week 52.

Outcomes

The primary efficacy endpoint was the proportion of patients with an American College of Rheumatology 20 (ACR20) response at week 16. The key secondary hierarchical endpoint was radiographic structural progression at week 24, as measured by change from baseline in van der Heijde-modified total Sharp score (vdH-mTSS), which ranges from 0 to 528 (higher scores indicating more articular damage)[20] based on independent assessments of hand/wrist/foot radiographs obtained at baseline, week 16 (non-responders) and week 24 by two central blinded readers (plus an adjudicator if required). Other hierarchical secondary endpoints were assessed at week 16 and included: proportion of patients achieving a 75% or 90% improvement from baseline in the Psoriatic Area and Severity Index (PASI75 and PASI90, respectively);[21] proportion of patients with an ACR50 response; change from baseline on the Health Assessment Questionnaire-Disability Index (HAQ-DI scores range from 0 to 3 with higher scores indicate greater disability);[22] change from baseline in the 28-joint Disease Activity Score using C reactive protein (DAS28-CRP, with higher scores indicating more active disease);[23] and resolution of enthesitis and dactylitis. Definitions of the ACR20 response and secondary points used in the study are provided in the online supplementary methods.

Prespecified exploratory endpoints of ACR70 response, the proportion of patients with no structural progression (change from baseline in vdH-mTSS ≤0.5) at week 24 and both the primary endpoint (ACR20 at week 16), change from baseline vdH-mTSS at week 24 by prior use of anti-TNF therapy and the proportion of patients achieving minimal disease activity (MDA) are also reported. MDA is assessed as five of the seven following: ≤1 tender joint count, ≤1 SJC, PASI≤1 or body surface area (BSA)≤3%, patient pain visual analogue score (VAS)≤15, patient global assessment of disease activity VAS≤20, HAQ-DI≤0.5, tender entheseal points≤1.[24] Disease Activity index for PSoriatic Arthritis (DAPSA) was analysed posthoc. DAPSA is a continuous index, calculated by summing of individual scores for: (1) tender joint count (TJC), (2) SJC, (3) patient global assessment, (4) patient assessment of pain and (5) CRP level, using 66/68 joint counts.[25] DAPSA ranges from 0 to 164; DAPSA remission (REM; range 0–4) and low disease activity (LDA; range 5–14) were assessed. Overall safety and tolerability of secukinumab over the 24 weeks was assessed by monitoring adverse events (AEs), serious AEs (SAEs), laboratory assessments and vital signs.

Statistical analyses

Sample sizes were calculated based on an overall two-sided 5% type I error rate. As three secukinumab regimens were tested versus placebo for ACR20, the type I error was separated into a 1.67% two-sided for each comparison. Based on ACR20 response rates of previous studies,[14] a sample size of 220 patients in each secukinumab group, and 330 in the placebo group was estimated to provide around 99% power (for all three dose regimens) to detect a treatment difference in the primary endpoint (ACR20 at week 16) based on Fisher's exact test.

For the key secondary endpoint, radiographic structural progression (vdH-mTSS), a treatment difference of 0.52 and SD of 1.13 with active treatment and 2.44 with placebo were observed in historical data.[13] Based on these assumptions, there is 83% power to detect a treatment difference using Satterthwaite t-test.

A sequential hierarchical testing method (online supplementary figure 2) was used to maintain the familywise type I error rate at 5% across the primary and ranked secondary specified endpoints. P values were calculated as 2-sided. Patients were analysed according to randomised treatment.

Statistical analyses were based on logistic regression for binary efficacy variables (eg, ACR20/50/70 and so on), non-parametric analysis of covariance for radiographic data (if baseline and ≥1 postbaseline radiographic assessments were available) and mixed-effects models for repeated measures (MMRM) for continuous variables (eg, DAS28-CRP, HAQ-DI). All models fitted included anti-TNF status, weight and the corresponding baseline value as a covariate as well as treatment as a factor (time,

treatment by time and baseline by time interaction were also used for MMRM models). Missing values and placebo patients rescued at week 16 were imputed as non-responders for binary endpoints (rescue penalty), linear extrapolation was applied for radiographic data (if baseline and week 16 values were available) and the missing at random assumption of the MMRM analysis was applied for continuous endpoints.

Safety endpoints were assessed for all patients who received ≥1 dose of study drug and were summarised descriptively. Patients were evaluated according to the treatment they received.

RESULTS

Patients

A total of 996 patients were randomly assigned to receive secukinumab 300 mg with LD (n=222), secukinumab 150 mg with LD (n=220), secukinumab 150 mg without LD (n=222) or placebo (n=332). At week 24, a total of 66 (6.9%) patients had discontinued, with the greatest number (n=37) coming from the placebo group. The most common reason for discontinuation was patient/guardian decision (3.2% overall; 5.7% placebo, 3.2% 150 mg without LD and 1.4% for both 150 mg and 300 mg with LD) followed by AEs (1.6% overall; 2.7% placebo, 0.9% 150 mg each with and without LD, and 1.4% 300 mg with LD). Patient disposition is shown in online supplementary figure 3.

Demographics and baseline characteristics were balanced between treatment arms (table 1). Overall, the mean age was 48.8 years, 49.8% were female and the mean time since PsA diagnosis was 6.6 years. At baseline, 70.4% of patients were anti-TNF-naïve and 50.1% were receiving concomitant methotrexate. Approximately half (51.6%) of the patients had psoriasis affecting ≥3% of their BSA. Enthesitis was present in 60.4% and dactylitis in 39.1% of patients. Mean swollen and tender joint counts were 11.5 and 21.0, respectively and mean HAQ-DI score was 1.3.

Efficacy

The primary endpoint was met with all secukinumab doses. ACR20 response rates at week 16 were significantly higher with secukinumab 300 mg with LD (62.6%), 150 mg with LD (55.5%) or 150 mg without LD (59.5%) than placebo (27.4%; p<0.0001 for all doses vs placebo; figure 1, online supplementary table 1 and table 2). ACR50/70 response rates at week 16 were also significantly higher with all secukinumab doses versus placebo (figure 1 and online supplementary table 1). ACR20/50/70 response rates at week 16 were numerically higher

Table 1 Demographics and baseline characteristics for the randomised set

Characteristic	Secukinumab 300 mg with LD (n=222)	Secukinumab 150 mg with LD (n=220)	Secukinumab 150 mg without LD (n=222)	Placebo (n=332)	Total (n=996)
Age (years), mean (SD)	48.9 (12.8)	48.4 (12.9)	48.8 (11.8)	49.0 (12.1)	48.8 (12.4)
Female, n (%)	114 (51.4)	109 (49.5)	102 (45.9)	171 (51.5)	496 (49.8)
Weight (kg)	81.9 (16.9)	83.3 (19.6)	84.1 (20.5)	84.1 (19.6)	83.4 (19.2)
Race, n (%)					
White	184 (82.9)	178 (80.9)	180 (81.1)	274 (82.5)	816 (81.9)
Asian	24 (10.8)	29 (13.2)	27 (12.2)	33 (9.9)	113 (11.3)
American Indian or Alaska Native	1 (0.5)	1 (0.5)	6 (2.7)	2 (0.6)	10 (1.0)
Black or African American	1 (0.5)	0 (0.0)	0 (0.0)	5 (1.5)	6 (0.6)
Unknown	0 (0.0)	0 (0.0)	2 (0.9)	2 (0.6)	4 (0.4)
Other	12 (5.4)	12 (5.5)	7 (3.2)	16 (4.8)	47 (4.7)
Time since first diagnosis of psoriatic disease (years), mean (SD)	6.7 (8.3)	6.7 (7.1)	6.2 (6.1)	6.6 (7.6)	6.6 (7.3)
Number of prior anti-TNF therapies, n (%)					
0	154 (69.4)	155 (70.5)	158 (71.2)	234 (70.5)	701 (70.4)
1	45 (20.3)	43 (19.5)	44 (19.8)	65 (19.6)	197 (19.8)
≥2	23 (10.4)	22 (10.0)	20 (9.0)	33 (9.9)	98 (9.8)
Methotrexate use at randomisation, n (%)	112 (50.5)	108 (49.1)	120 (54.1)	159 (47.9)	499 (50.1)
Systemic glucocorticoid at randomisation, n (%)	34 (15.3)	44 (20.0)	37 (16.7)	53 (16.0)	168 (16.9)
Patients with specific disease characteristics, n (%)					
Psoriasis affecting≥3% of BSA	110 (49.5)	125 (56.8)	117 (52.7)	162 (48.8)	514 (51.6)
Presence of enthesitis	140 (63.1)	141 (64.1)	129 (58.1)	192 (57.8)	602 (60.4)
Presence of dactylitis	82 (36.9)	80 (36.4)	103 (46.4)	124 (37.3)	389 (39.1)
Disease and quality of life scores, mean (SD)					
Tender joint count (78 joints)	19.8 (15.1)	21.2 (15.9)	21.8 (16.0)	21.2 (16.2)	21.0 (15.8)
Swollen joint count (76 joints)	10.0 (8.0)	12.1 (10.5)	11.9 (10.3)	11.7 (10.8)	11.5 (10.1)
DAS28-CRP score	4.5 (1.0)	4.7 (1.0)	4.6 (1.1)	4.6 (1.1)	4.6 (1.1)
HAQ-DI score	1.2 (0.6)	1.3 (0.6)	1.3 (0.7)	1.3 (0.6)	1.3 (0.6)
vdH-mTSS	12.9 (23.7)	13.6 (25.9)	15.3 (37.5)	15 (38.2)	–
PsA pain, VAS 0–100 mm	52.8 (24.8)	56.5 (22.8)	54.5 (22.9)	53.6 (24.5)	54.3 (23.9)
Patients' global assessment of disease activity, VAS 0–100 mm	55.0 (22.8)	53.9 (22.6)	54.6 (23.5)	52.5 (22.2)	53.9 (22.7)
Physician's global assessment of disease activity, VAS 0–100 mm	55.4 (18.3)	57.7 (18.6)	57.3 (19.2)	54.3 (20.3)	55.9 (19.3)

BSA, body surface area; DAS28-CRP, 28-joint Disease Activity Score using C reactive protein; HAQ-DI, Health Assessment Questionnaire-Disability Index; LD, loading dose; PsA, psoriatic arthritis; TNF, tumour necrosis factor; VAS, visual analogue scale; vdH-mTSS, van der Heijde-modified total Sharp score.

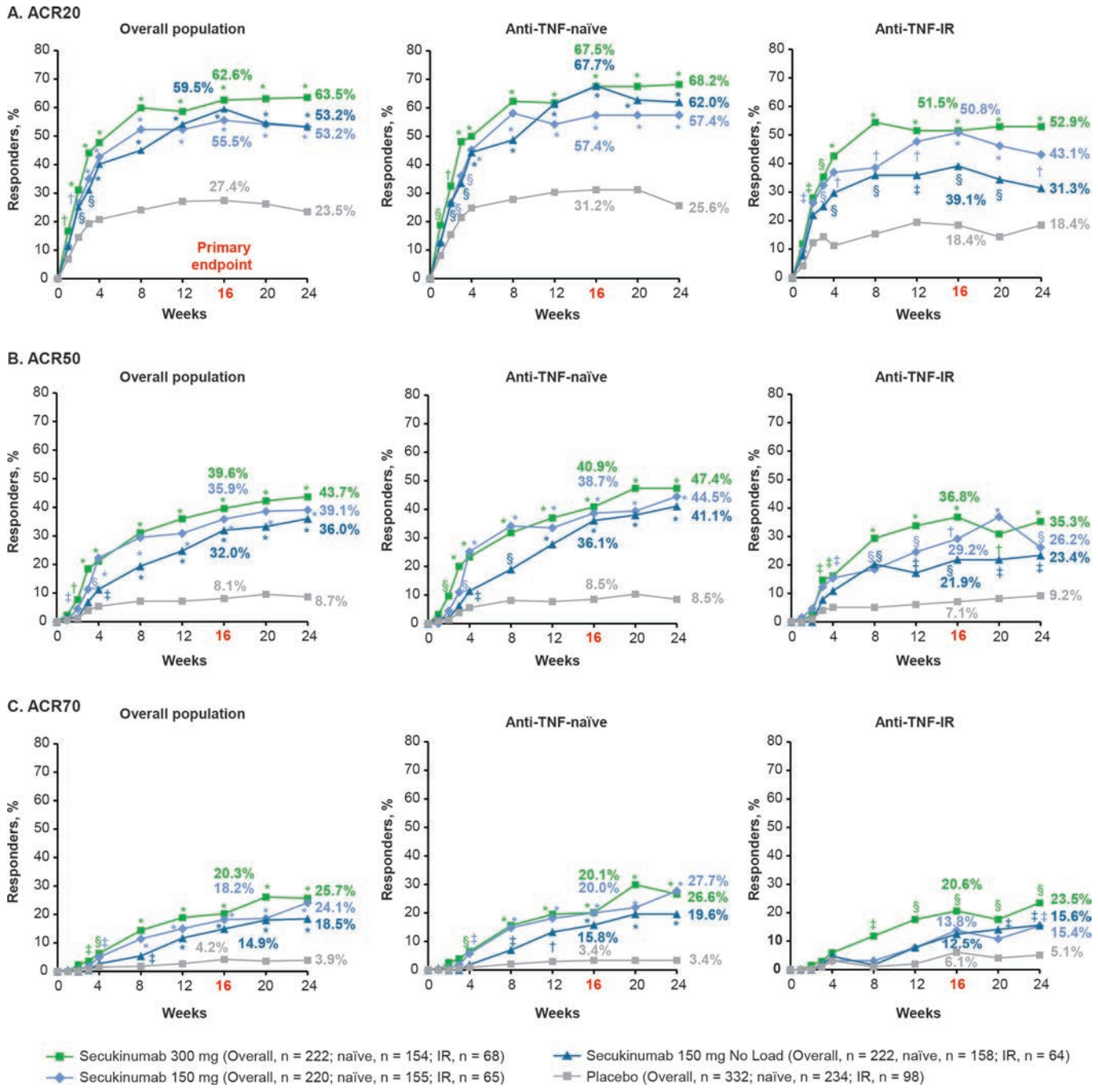

Figure 1 (A) ACR20, (B) ACR50 and (C) ACR70 response rates from baseline up to week 24[a] in the overall population and by anti-TNF status. *P<0.0001; †p<0.001; §p<0.01; ‡p<0.05 unadjusted p values versus placebo (Statistical analysis was based on logistic regression. Missing values and placebo patients rescued at week 16 were imputed as non-responders.) [a]The primary endpoint was ACR20 response in the overall population at week 16. ACR20/50/70, ≥20/50/70% improvement from baseline in American College of Rheumatology response criteria; anti-TNF-IR, intolerance or inadequate response to antitumour necrosis factor therapy.

in anti-TNF-naive than anti-TNF-IR patients for all secukinumab doses. All secondary hierarchical endpoints were significant for secukinumab versus placebo at week 16, except for enthesitis and dactylitis resolution for the 150 mg without LD group (table 2, figure 2 and online supplementary table 2).

Mean changes from baseline in vdH-mTSS demonstrated significant inhibition of radiographic structural progression at week 24 in all secukinumab groups versus placebo: 0.08 (300 mg with LD; p<0.01), 0.17 (150 mg with LD; P<0.05), –0.09 (150 mg without LD; p<0.05) versus 0.50 (placebo; figure 3, online supplementary table 3 and table 2). The proportion of patients with no radiographic structural progression at week 24, defined as ≤0.5 change from baseline in vdH-mTSS, was higher across all secukinumab dose regimens than placebo: 191/217 (88.0%) patients in the secukinumab 300 mg with LD group, 170/213 (79.8%) in the 150 mg with LD group and 176/210 (83.8%) in the 150 mg without LD group versus 218/296 (73.6%) in the placebo group (online supplementary figure 4).

The 300 mg secukinumab dose provided numerically better efficacy in all hierarchical endpoints versus the 150 mg regimens, with the strongest treatment difference observed for psoriasis improvement. PASI 75 response rates at week 16 were

Table 2 Comparison of secukinumab versus placebo at week 16 for prespecified hierarchical endpoints

	Secukinumab 300 mg with LD (n=222)	Secukinumab 150 mg with LD (n=220)	Secukinumab 150 mg without LD (n=222)	Placebo (n=332)
Primary endpoint				
ACR20 response (%)	62.6***	55.5***	59.5***	27.4
Prespecified secondary endpoints				
vdH-mTSS structural progression (mean change from BL)†	0.08**	0.17*	−0.09*	0.50
PASI 75 response (%)‡	70.0*	60.0*	58.1*	12.3
PASI 90 response (%)‡	53.6*	36.8*	31.6*	9.3
ACR50 response (%)	39.6*	35.9*	32.0*	8.1
HAQ-DI score (LS mean change from BL)	−0.55*	−0.44*	−0.45*	−0.21
DAS28-CRP score (LS mean change from BL)	−1.49*	−1.29*	−1.29*	−0.63
Enthesitis resolution (%)§	55.7*	54.6*	41.9	35.4
Dactylitis resolution (%)¶	65.9*	57.5*	56.3	32.3

*P<0.05; **p<0.01; ***p<0.0001 unadjusted p values versus placebo are shown for endpoints that were significant in the hierarchical testing.
†Week 24 data.
‡Data from patients with baseline psoriasis affecting ≥3 BSA.
§Data from patients with enthesitis at baseline.
¶Data from patients with dactylitis at baseline.
ACR20, American College of Rheumatology 20; BL, baseline; BSA, body surface area; DAS28-CRP, 28-joint Disease Activity Score using C reactive protein; HAQ-DI, Health Assessment Questionnaire-Disability Index; LD, loading dose; LS, least squares; PASI, Psoriasis Area and Severity Index; vdH-mTSS, van der Heijde-modified total Sharp score.

70.0% with 300 mg with LD, 60.0% with 150 mg with LD, 58.1% with 150 mg without LD and 12.3% with placebo; corresponding PASI 90 responses rates were 53.6%, 36.8%, 31.6% and 9.3%, respectively. Treatment responses were greater in anti-TNF-naïve patients than in anti-TNF-IR patients (Figures 1,3). In the 150 mg groups, patients receiving LD generally had an earlier onset of response and numerically greater efficacy versus patients without LD (figure 1).

The proportion of patients achieving MDA at week 16 were 33.0% with secukinumab 300 mg, 28.2% with 150 mg with LD, 23.0% with 150 mg without LD and 8.4% with placebo (p<0.001 for all secukinumab doses versus placebo; online supplementary figure 5). The proportion of patients achieving DAPSA REM/LDA states at week 16 were 15.2%/37.4% with secukinumab 300 mg, 13.4%/27.8% with 150 mg with LD, 9.0%/34.8% with 150 mg without LD and 2.3%/18.9% with placebo (online supplementary figure 5).

Safety

Exposure and safety are reported in table 3. The duration of exposure over the 24 weeks was higher for secukinumab (329.7 patient-years) than placebo (122.7 patient-years) due to 158 patients in the placebo group switching to secukinumab at week 16. The most commonly reported AEs represented upper respiratory tract infections; other common AEs are reported in table 3. No deaths or major adverse cardiac events were reported. Non-fatal SAE rates were low overall and similar for secukinumab (3.0%) and placebo (3.6%). Most SAEs represented single events with no discernible pattern.

Selected SAEs of interest included: one report of suicidal thoughts in a patient with a history of anxiety who continued in the study without further episodes; one anaphylactic reaction after the second secukinumab dose, which resulted in patient discontinuation; one new diagnosis of ulcerative colitis in a secukinumab patient with no prior gastrointestinal

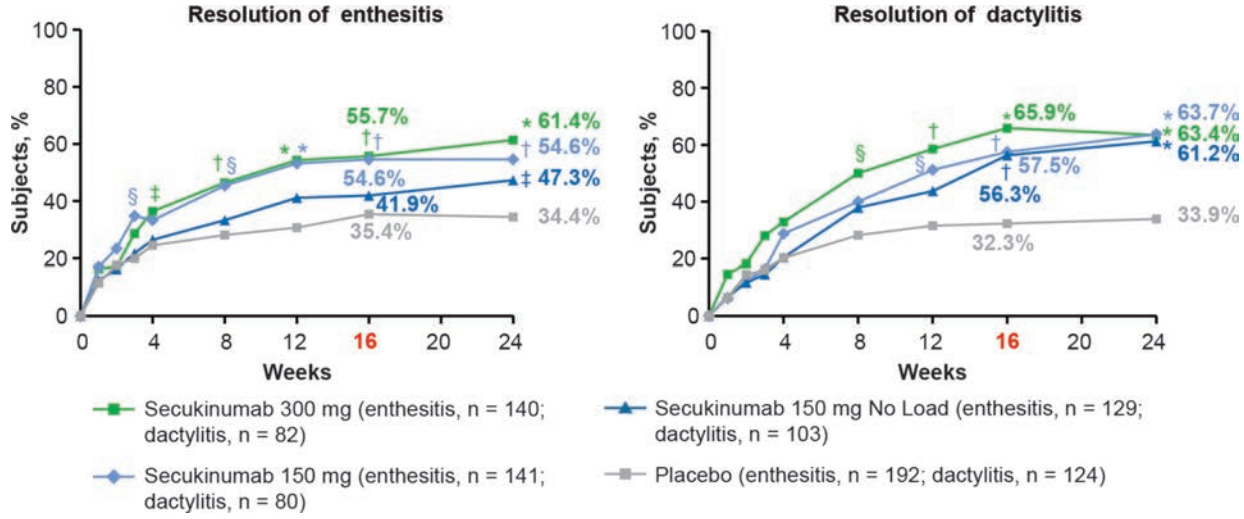

Figure 2 Resolution of enthesitis and dactylitis in the overall population from baseline up to week 24[a].*P<0.0001; †p<0.001; §p<0.01; ‡p<0.05 unadjusted p values versus placebo. (Statistical analysis was based on logistic regression. Missing values and placebo patients rescued at week 16 were imputed as non-responders.) [a]Resolution of dactylitis and enthesitis were not significant for secukinumab 150 mg without load in hierarchical testing.

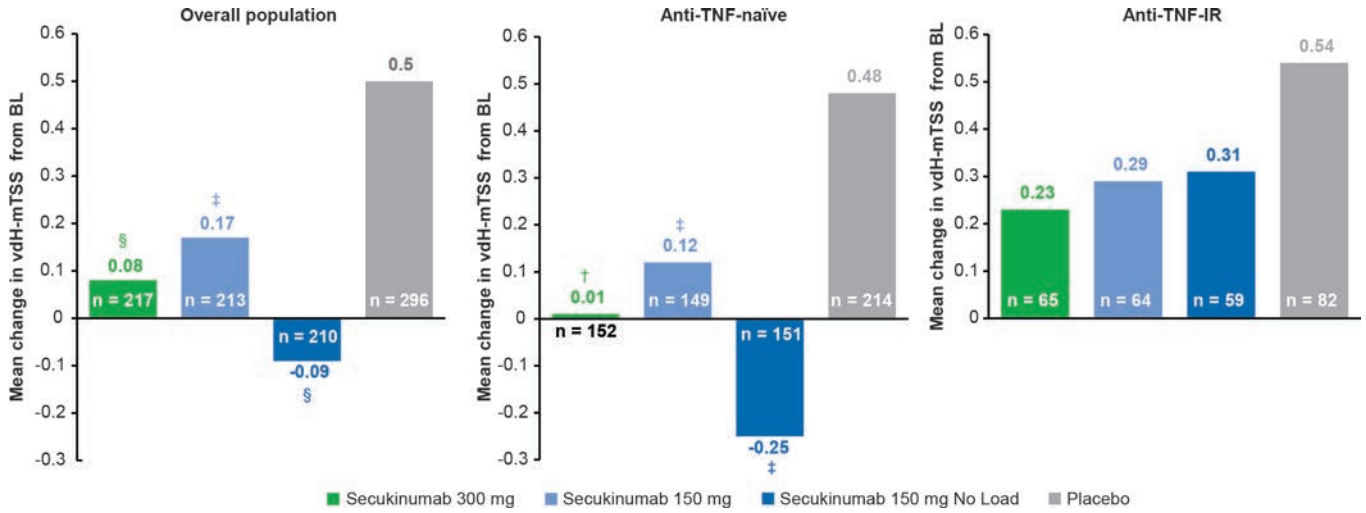

Figure 3 Change in vdH-mTSS from baseline at week 24 (non-parametric ANCOVA-linear extrapolation in the overall population and by anti-TNF status. †P<0.001; §p<0.01; ‡p<0.05 unadjusted p values versus placebo (Statistical analysis was based on a non-parametric ANCOVA. Linear extrapolation was applied if a baseline and week 16 value were available). ANCOVA, analysis of covariance; anti-TNF-IR, intolerance or inadequate response to antitumour necrosis factor therapy; LD, loading dose; vdH-mTSS, van der Heijde-modified total Sharp score.

Table 3 Exposure and rates of deaths, discontinuations, AEs, SAEs and selected AEs and SAEs of interest up to week 24†

Variable	Secukinumab 300 mg with LD (n=222)	Secukinumab 150 mg with LD (n=220)	Secukinumab 150 mg without LD (n=222)	Any secukinumab (n=822)	Placebo (n=332)
Exposure					
Patient-years	102.0	101.8	101.2	329.7	122.9
Days (mean)	167.8	169.0	166.5	146.5	135.0
Death and AEs					
Death, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Discontinuation due to AE, n (%)	3 (1.4)	4 (1.8)	3 (1.4)	11 (1.3)	7 (2.1)
Non-fatal SAE, n (%)	7 (3.2)	9 (4.1)	6 (2.7)	25 (3.0)	12 (3.6)
Number of patients with any AE, n (%)	140 (63.1)	138 (62.7)	136 (61.3)	463 (56.3)	206 (62.0)
Most common AEs, n (%)‡					
Viral upper respiratory tract infection	14 (6.3)	15 (6.8)	13 (5.9)	44 (5.4)	29 (8.7)
Upper respiratory tract infection	7 (3.2)	17 (7.7)	14 (6.3)	38 (4.6)	11 (3.3)
Dyslipidaemia	8 (3.6)	4 (1.8)	8 (3.6)	23 (2.8)	11 (3.3)
Headache	5 (2.3)	9 (4.1)	8 (3.6)	23 (2.8)	13 (3.9)
Hypertension	8 (3.6)	5 (2.3)	9 (4.1)	22 (2.7)	10 (3.0)
Diarrhoea	9 (4.1)	4 (1.8)	7 (3.2)	21 (2.6)	22 (6.6)
Hypercholesterolaemia	3 (1.4)	9 (4.1)	8 (3.6)	20 (2.4)	2 (0.6)
Urinary tract infection	6 (2.7)	8 (3.6)	6 (2.7)	20 (2.4)	8 (2.4)
Selected AEs of interest, n (%)					
Candida infection	1 (0.5)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)
Oral candidiasis	2 (0.9)	1 (0.5)	0 (0.0)	3 (0.4)	1 (0.3)
Vulvovaginal candidiasis	1 (0.5)	2 (0.9)	0 (0.0)	3 (0.4)	1 (0.3)
Injections site reactions	6 (2.7)	5 (2.3)	3 (1.4)	15 (1.8)	4 (1.2)
Selected SAEs of interest, n (%)					
Crohn's disease	0 (0.0)	0 (0.0)	1 (0.5)	1 (0.1)	0 (0.0)
Ulcerative colitis	0 (0.0)	1 (0.5)	0 (0.0)	1 (0.1)	0 (0.0)
Neoplasms benign, malignant and unspecified	0 (0.0)	0 (0.0)	1 (0.5)§	2 (0.2)¶	0 (0.0)

†Up to the data cut-off point for interim analysis.
‡AEs that occurred at an incidence rate of >2% in the 'any secukinumab' group. Any secukinumab group represents each originally randomised secukinumab patient *plus* patients who switched to active treatment at week 16 due to non-response.
§Bladder neoplasm reported as an non-serious AE (day 34).
¶Includes one case of melanoma (day 139) in a placebo patient switched to secukinumab (day 113).
AE, adverse event; LD, loading dose; SAE, serious adverse event.

medical history and who remained in the study and one case of Crohn's disease in a patient with a history of colitis. There was one mild, non-serious exacerbation of Crohn's disease in a secukinumab-treated patient who continued on study treatment and remained in the study; the event was resolved at the time of reporting.

Reports of *Candida* infections included: one case of oral thrush (300 mg with LD), four cases of oral candidiasis (one in 150 mg with LD; two in 300 mg with LD; one in placebo) and four cases of vulvovaginal candidiasis (two in 150 mg with LD; one in 300 mg; one in placebo). These were of mild severity, except one moderately severe vulvovaginal candida infection; all resolved with standard therapy. No systemic fungal infections or newly diagnosed tuberculosis infections were reported, and incidences of injection site reactions were low across all groups.

DISCUSSION

FUTURE 5 is the largest randomised phase III trial to date of a biologic in PsA. In this study, s.c. administration of secukinumab 300 mg and 150 mg provided rapid and significant improvement versus placebo in most clinical domains of PsA and inhibited radiographic progression at week 24.

The primary endpoint, ACR20 response at week 16, was met for all secukinumab regimens, and secondary endpoints were significant for all secukinumab doses except for enthesitis and dactylitis resolution in the 150 mg without LD group. These results confirm and extend previous findings relating to the efficacy of secukinumab in PsA.[12–18] In addition, clinical response rates (ACR20/50/70) at week 16 were higher in anti-TNF-naive patients than in those who were anti-TNF-IR for all secukinumab doses.

These data provide the first evidence that s.c. secukinumab loading and maintenance dosing regimens and the higher dose of 300 mg significantly inhibit joint structural damage in PsA. More patients who received secukinumab versus those who received placebo had no radiographic progression through week 24. Inhibition of radiographic progression was observed in both anti-TNF-naïve and anti-TNF-IR patients, although statistical significance was not reached in the anti-TNF-IR population. The lack of significance could be affected by a number of factors, including the relatively small number of anti-TNF-IR patients and the heterogeneity of this subpopulation, which comprised patients who previously failed anti-TNF treatment for any one of several reasons, including lack of primary or secondary efficacy, intolerance or safety concerns.[26] This study was limited in that it was not designed to identify a difference between doses or to assess differences in response according to previous anti-TNF use.

Patients enrolled in this study had a long duration of disease (mean around 6.5 years) and very active disease as evidenced by the relatively high tender and swollen joint scores and the large proportion of patients with enthesitis and dactylitis at baseline. Additionally, around 30% of patients had previously received one or more anti-TNF treatments before entering this trial. While anti-TNF agents have been shown to improve outcomes in PsA,[27–31] many patients experience inadequate disease control, treatment intolerance or loss of response over time.[1 32] In agreement with previous studies,[9 10 13–18] secukinumab treatment in FUTURE 5 was shown to be efficacious in both anti-TNF-naïve and anti-TNF-IR patients, with clinical responses generally being higher in anti-TNF-naïve patients. These results confirm that secukinumab is a suitable treatment option for biologic-naïve patients and those who have previously failed anti-TNF therapy.

The secukinumab 300 mg dose consistently provided numerically better responses versus the 150 mg dose, with or without LD, in clinical endpoints such as ACR20/50/70, resolution of enthesitis and dactylitis, HAQ-DI, DAS28-CRP, PASI 75 and PASI 90, particularly in anti-TNF-IR patients. Data from FUTURE 5 also demonstrate the benefit of a loading regimen in terms of providing an earlier onset of action, especially on higher efficacy endpoints such as ACR50/70, dactylitis and in the treatment of psoriasis symptoms, as indicated by PASI 75 and PASI 90, and in the proportion of patients achieving MDA and DAPSA-REM.

The safety profile was consistent with that previously reported for PsA[12–18] and psoriasis.[32] The types and incidences of most AEs with secukinumab were similar to those for placebo for the entire 24-week treatment period, without evidence of dose-dependency. The rate of *Candida* infections was higher with secukinumab treatment than with placebo; this is consistent with previous reports[13 14] and considered to be related to the role of IL-17 in mucocutaneous defence against *Candida* infections.[33] No systemic fungal infections were reported. All cases of *Candida* infection resolved with standard oral therapy and patients continued in the study.

This study demonstrated that s.c. secukinumab 300 mg and 150 mg provided significant inhibition of radiographic progression, and has demonstrated the benefit of a LD regimen (regardless of the dose), particularly when aiming for higher levels of response and faster outcomes in joint and skin endpoints. The safety profile of secukinumab was consistent with previous reports, with no new safety signals observed.[12–18] FUTURE 5 confirms and extends the results of previous data demonstrating the efficacy of s.c. administration of secukinumab in achieving comprehensive treatment goals in PsA.

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EXTENDED REPORT

Impact of temporary methotrexate discontinuation for 2 weeks on immunogenicity of seasonal influenza vaccination in patients with rheumatoid arthritis: a randomised clinical trial

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ABSTRACT

Objective To determine whether a 2-week methotrexate (MTX) discontinuation after vaccination improves the efficacy of seasonal influenza vaccination in patients with rheumatoid arthritis (RA).

Methods In this prospective randomised parallel-group multicentre study, patients with RA on stable dose of MTX were randomly assigned at a ratio of 1:1 to continue MTX or to hold MTX for 2 weeks after 2016–2017 quadrivalent seasonal influenza vaccine containing H1N1, H3N2, B-Yamagata and B-Victoria. The primary outcome was frequency of satisfactory vaccine response, defined as greater than or equal to fourfold increase of haemagglutination inhibition (HI) antibody titre at 4 weeks after vaccination against ≥2 of four vaccine strains. Secondary endpoints included seroprotection (ie, HI titre ≥1:40) rate, fold change in antibody titres.

Results The modified intention-to-treat population included 156 patients in the MTX-continue group and 160 patients in the MTX-hold group. More patients in MTX-hold group achieved satisfactory vaccine response than the MTX-continue group (75.5% vs 54.5%, p<0.001). Seroprotection rate was higher in the MTX-hold group than the MTX-continue group for all four antigens (H1N1: difference 10.7%, 95% CI 2.0% to 19.3%; H3N2: difference 15.9%, 95% CI 5.9% to 26.0%; B-Yamagata: difference13.7%, 95% CI 5.2% to 22.4%; B-Victoria: difference 14.7%, 95% CI 4.5% to 25.0%). The MTX-hold group showed higher fold increase in their antibody titres against all four influenza antigens (all p<0.05). Change in disease activity was similar between groups.

Conclusions A temporary MTX discontinuation for 2 weeks after vaccination improves the immunogenicity of seasonal influenza vaccination in patients with RA without increasing RA disease activity.

Trial registration NCT02897011.

INTRODUCTION

Rheumatoid arthritis (RA) is a common chronic systemic inflammatory diseases, affecting 1% of general population.[1] It requires long-term treatment with disease-modifying antirheumatic drugs (DMARDs), which as immune-suppressive agents inhibit both cellular immunity and humoral immunity. Since the underlying immune dysfunction and the treatment-associated immune suppression render patients with RA more susceptible to infections,[2] vaccines are strongly recommended against preventable diseases in patients with RA.[3–5] This is of particular importance when patients are confronted with new epidemics such as pandemic influenza infection.[6]

Methotrexate (MTX) is the most commonly prescribed DMARD for the treatment of RA due to its high efficacy and favourable safety profile. Even in the era of biologic DMARDs, MTX remains as the anchor drug because of its synergistic effect with biologic DMARDs.[7] However, MTX significantly decreases vaccine response to pneumococcal and seasonal influenza vaccines, particularly response to novel strain antigens.[6 8–11] We previously showed in a pilot study, where MTX was discontinued for 4 weeks in different periods with respect to trivalent seasonal influenza vaccination, that a temporary discontinuation of MTX after vaccination could significantly increase immunogenicity in patients with RA who had been on a stable dose of MTX.[12] However, a 4-week discontinuation was associated with an increased risk of RA flare by up to 1.4-fold during the 16-week follow-up period, suggesting that a shorter MTX discontinuation strategy may be desirable. Based on this pilot study, we hypothesised that a 2-week discontinuation of MTX after vaccination would be as effective as the 4 weeks of discontinuation while minimising a flare risk.

Therefore, we investigated the effect of MTX discontinuation for 2 weeks after vaccination on the response to seasonal influenza vaccination in patients with RA in this randomised controlled clinical trial.

METHODS

Study rationale and design

This was a prospective multicentre randomised investigator-blind parallel-group intervention study that aimed to investigate the effects of a 2-week MTX discontinuation on vaccine response to 2016–2017 seasonal influenza vaccination in patients with RA. A pilot study was conducted to estimate the efficacy and time of temporary MTX discontinuation to improve vaccine response. In the pilot study, MTX discontinuation for 4 weeks before vaccination did not improve vaccination response, whereas MTX discontinuation for 2 weeks before and after vaccination or for 4 weeks after vaccination improved vaccine response. Therefore, the

period of 2 weeks after vaccination were considered a critical period, where MTX should be held in the current study (online Supplementary figure S1).

Patients were recruited by their primary rheumatologists in outpatient clinic setting in three tertiary medical centres in South Korea. The first patient was recruited on 7 October 2016 and the last on 7 January 2017. The study was registered with www.clinicaltrials.gov, protocol number: NCT02897011. The study was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines. Written informed consent was obtained from all patients before enrolment in the trial.

Participants

Patients with RA who were aged 19 years or older and had been on the same dose of MTX for 6 weeks or longer were eligible for inclusion. RA was defined according to the revised 1987 American College of Rheumatology criteria.[13] The exclusion criteria were pregnant or lactating women, patients with a previous anaphylactic response to vaccine components or to an egg component, evidence of an acute infection with temperature >38°C at the time of vaccination, history of Guillain-Barré syndrome or demyelinating syndromes and previous vaccination with any live vaccine 4 weeks before or any inactivated vaccine 2 weeks before start of the study. Patients who necessitated a change in their RA treatment regimen within 4 weeks before enrolment and patients with any other additional rheumatic disease except for secondary Sjogren's disease were also excluded.

Randomisation and masking

Medical Research Collaborating Center (MRCC) at Seoul National University Hospital generated a randomisation table that was stratified by centres. MRCC was not involved in the other processes of the trial. The eligible patients were randomly assigned to continue MTX or to discontinue MTX for 2 weeks after vaccination by a Central Interactive Web Response System (IWRS) at a 1:1 ratio according to the randomisation table. Information on the intervention was concealed from the investigators who enrolled or assessed the study patients. Investigators who performed the haemagglutination inhibition (HI) antibody titre assay were masked to the allocated treatment. Because of the nature of the study, patients were not masked to intervention. To measure the adherence to the study protocol, study participants were required to record their MTX administration in a diary.

Intervention

The 2016–2017 seasonal quadrivalent influenza vaccine (GC Flu, Green Cross, South Korea) contained four antigens: 15 µg of A/California/7/2009 Reassortant virus NYMC X-181 (H1N1), 15 µg of A/Hong Kong/4801/2014 NYMC X-263B (H3N2), 15 µg of B/Phuket/3073/2013 (B-Yamagata) and 15 µg of B/Brisbane/60/2008 in a 0.5 mL prefilled syringe. The vaccine was delivered as a single intramuscular injection in the deltoid muscle by healthcare providers.

After vaccination, patients in the MTX-continue group continued their MTX in their current dose, whereas patents in the MTX-hold group suspended it for 2 weeks and then resumed it at previous dose.

Before (week 0) and at 4 weeks after vaccination, the serum of the patients was collected. The HI antibody titres against each of the four influenza strains in the vaccine were measured in duplicate by an independent laboratory (the Vaccine Bio Research Institute of the Catholic University, Seoul, Korea) according to standard procedures. The average of the duplicate measurements for each antigen was used for analyses.

Adding or changing DMARDs were not allowed until post-vaccination serum was obtained. During MTX discontinuation, acetaminophen (650 mg up to three times per day), non-steroidal anti-inflammatory drugs (in standard dosing) and/or prednisolone (or its equivalent) up to 10 mg per day were allowed to treat RA flares. Medications for other comorbid conditions were allowed.

Outcomes

The primary outcome was the frequency of satisfactory vaccine response to influenza antigens 4 weeks after vaccination. A satisfactory vaccine response was a priori defined as greater than or equal to fourfold increase in HI antibody titre at 4 weeks after vaccination relative to the baseline in two or more of four influenza vaccine antigens. Secondary endpoints included satisfactory response in ≥1 antigen, ≥3 antigens, ≥4 antigens of influenza vaccine, vaccine response to each antigen, frequency of seroprotection (defined as HI titres of ≥1:40) and fold change in postvaccination HI antibody titres against each vaccine antigen relative to baseline as well as incidence of influenza infection during influenza season 2016–2017.[14] Influenza-like illness was defined clinically as the presence of fever >38°C and cough with 48 hours of symptom onset.[15] Patients were interviewed using a structured questionnaire in March 2017 and between July and September 2017 during their follow-up visit in the clinic or per telephone. Adverse events that were associated with vaccination were captured from the patients at each visit. A RA flare was defined as an increase in Disease Activity Score in 28 joints (DAS28) of >1.2 (or >0.6 if the baseline DAS28 was ≥3.2).[16]

Statistical analysis

All analyses were conducted according to a predefined protocol. The analysis population was the modified intention-to-treat (mITT) population that included all study subjects who received the influenza vaccine and in whom both prevaccination and post-vaccination HI titres were available. The safety was summarised for all participants who received the vaccination.

In a prior pilot study, the satisfactory vaccine response to a trivalent seasonal influenza vaccination (defined by greater than or equal to fourfold increase in HI antibody in ≥2 of three influenza vaccine antigens) in patients with RA continuing MTX and those patients holding MTX treatment for 4 weeks were 54% and 71%, respectively.[12] Assuming that 2 weeks of MTX discontinuation would improve the vaccination response to that seen in patients who discontinued it for 4 weeks and assuming an alpha level of 0.05 (two-tailed), a power of 0.80 and dropout rate of 20%, 160 patients per group would be required for the study with a total target number of 320 patients.

Continuous variables were analysed by using a t-test. For vaccine titres, the reciprocal of HI titres were log-transformed for group comparisons. The binary secondary efficacy variables (frequency of satisfactory vaccine response and frequency of disease flare) were analysed by using X^2 tests or Fisher's exact test, as appropriate. P value <0.05 was considered to indicate statistical significance. All analyses were performed by using SPSS V.20.

RESULTS

Baseline characteristics

We enrolled 320 patients with RA (159 in MTX-continue group and 161 in MTX-hold group) between 7 October 2016 and

9 January 2017. All patients received the vaccination. Three patients in the MTX-continue group and one patient in MTX-hold group withdrew their consent and were excluded from analysis. Accordingly, 316 patients (156 in the MTX-continue group and 160 in the MTX-hold group) were included in the mITT population (figure 1). Patients were predominantly female (82.7% in the MTX-continue group and 87.5% in the MTX-hold group). The mean age was 52.2 years for the MTX-continue group and 53.7 years for the MTX-hold group. The two groups did not differ at baseline in terms of demographic or disease characteristics, including seropositivity for rheumatoid factor (RF) or anticyclic citrullinated peptide antibody (ACPA) or DAS28-C-reactive protein (CRP). The groups were also comparable in terms of their treatment regimen at baseline, including their use of systemic corticosteroids and MTX dose (table 1).

Impact of MTX discontinuation on vaccine response

Higher proportion of patients in the MTX-hold group achieved satisfactory vaccine response, defined as greater than or equal to fourfold increase in HI antibody titre in ≥2/4 influenza antigens, compared with the MTX-continue group (75.5% vs 54.5%, p<0.001; difference 21.0%, 95% CI 10.6% to 31.7%). Similarly, the proportion achieving vaccine response (ie, greater than or equal to fourfold increase in HI antibody titre) in ≥1/4 (89.4% vs 75.6%, p=0.001; difference 13.8%, 95% CI 5.4% to 22.1%), ≥3/4 (61.9% vs 36.5%, p<0.001; difference 25.4%, 95% CI 14.3% to 36.4%) and 4/4 influenza antigens (45.6% vs 21.8%, p<0.001; difference 23.8%, 95% CI 13.4% to 34.3%) was higher in the MTX-hold group than the MTX-continue group (figure 2).

In terms of the responses to individual vaccine antigens, the MTX-hold group showed a higher frequency of satisfactory response to all four influenza antigens than the MTX-continue group (H1N1: difference 11.9%, 95% CI 0.9% to 22.8%, p=0.033; H3N2: difference 16.8%, 95% CI 6.1% to 27.4%, p=0.002; B-Yamagata: difference 22.7%, 95% CI 11.7% to 33.7%, p<0.001; B-Victoria: difference 32.8%, 95% CI 21.8% to 43.6%, p<0.001). Compared with the MTX-continue group, the MTX-hold group had significantly higher fold increases in their antibody titres against all four influenza antigens (table 2).

The overall baseline seroprotection against all four influenza antigens appeared to be similar between the both groups (table 2). Postvaccination seroprotection rate was higher in the MTX-hold group than the MTX-continue group for all four antigens (H1N1: difference 10.7%, 95% CI 2.0% to 19.3%, p=0.016; H3N2: difference 15.9%, 95% CI 5.9% to 26.0%, p=0.002; B-Yamagata: difference 13.7%, 95% CI 5.2% to 22.4%, p=0.002; B-Victoria: difference 14.7%, 95% CI 4.5% to 25.0%, p=0.005).

When the patients were divided into those with and without seroprotection, the response to individual antigen was higher in the MTX-hold group than the MTX-continue group in the subgroup without baseline protection (defined as HI antibody titre was <1:40 against the respective antigen), whereas the difference between both groups is less dominant in patients with seroprotection at baseline (online Supplementary table S1). The difference in vaccine response did not differ between patients in the MTX-continue group and MTX-hold group who took MTX 7.5 mg per week or less, whereas the difference was significant between the patients in the MTX-continue group and MTX-hold group who took MTX 15 mg per week or more (figure 3).

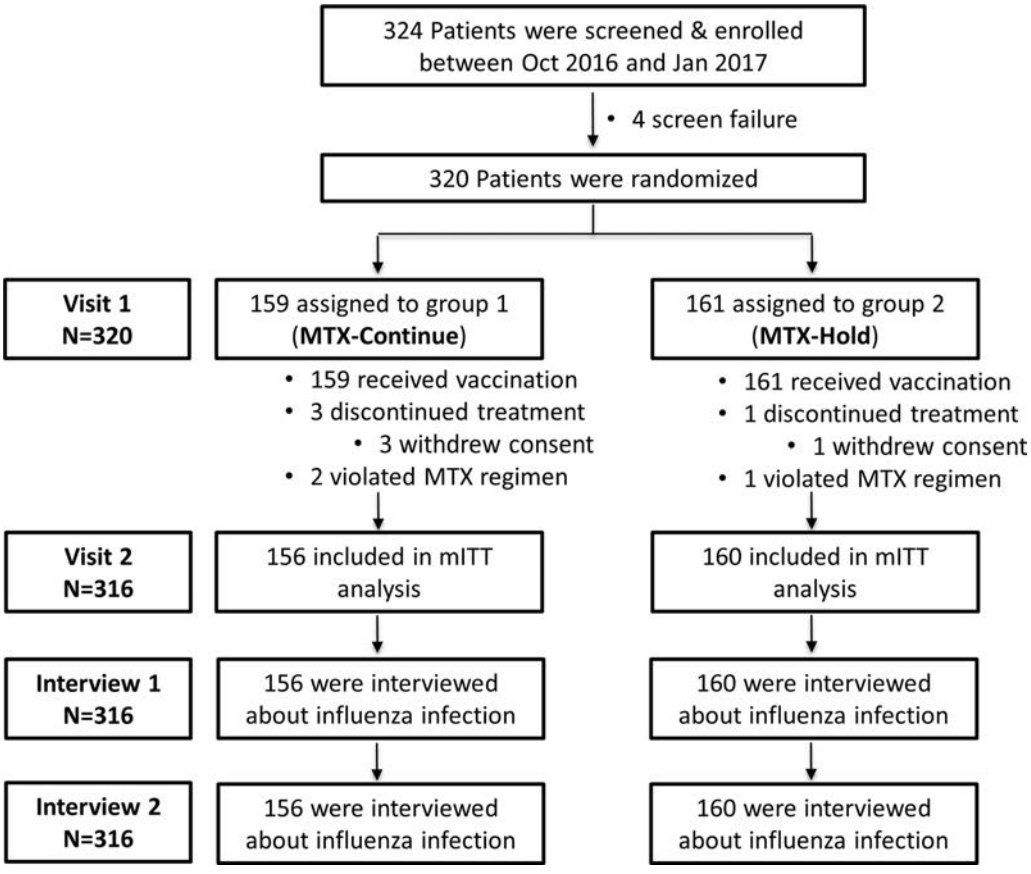

Figure 1 Patient flow. MTX, methotrexate; mITT, modified intention-to-treat.

Table 1 Baseline characteristics in the modified intention-to-treat population

	MTX continue (n=156)	MTX hold (n=160)
Female (%)	129 (82.7)	140 (87.5)
Age, years	52.2 (9.5)	53.7 (10.3)
Duration of RA, years	6.8 (6.5)	6.9 (6.2)
Body mass index, kg/m^2	23.3 (3.3)	23.2 (3.3)
Diabetes mellitus (%)	8 (5.1)	8 (5.0)
Smoking		
Never (%)	128 (82.1)	130 (81.3)
Current (%)	11 (7.1)	10 (6.3)
Former (%)	17 (10.9)	20 (12.5)
RF positivity (%)	120/154 (77.9)	132/157 (84.1)
Anti-CCP positivity (%)	105/121 (86.8)	111/135 (82.2)
DAS28-CRP	2.2 (0.9)	2.3 (1.1)
Treatment		
GC (%)	82 (52.6)	74 (46.3)
Mean GC dose, mg/day	1.8 (2.1)	1.7 (2.1)
MTX (%)	156 (100)	160 (100)
MTX dose, mg/week	13.3 (3.4)	13.1 (3.2)
Sulfasalazine (%)	8 (5.1)	10 (6.3)
Hydroxychloroquine (%)	35 (22.4)	31 (19.4)
Leflunomide (%)	33 (21.2)	37 (23.1)
Tacrolimus (%)	2 (1.3)	2 (1.3)
Biological DMARDs		
Tumour necrosis factor inhibitor (%)	11 (7.1)	13 (8.1)
Abatacept (%)	1 (0.6)	6 (3.8)
Tocilizumab (%)	4 (2.6)	7 (4.4)
Rituximab (%)	1 (0.6)	1 (0.6)
Tofacitinib (%)	0 (0)	1 (0.6)

The data are mean (SD) or number (%).
Anti-CCP, anticyclic citrullinated peptide; CRP, C-reactive protein; DAS28, Disease Activity Score in 28 joints; DMARDs, disease-modifying antirheumatic drugs; ESR, erythrocyte sedimentation rate; GC, glucocorticoids; MTX, methotrexate; RA, rheumatoid arthritis; RF, rheumatoid factor.

During the follow-up period up to 1 year, one (0.6%) of 160 patients in the MTX-hold group and three (1.9%) in the MTX-continue group developed an influenza-like illness.

Safety

The vaccine was well tolerated. No serious adverse events related to the vaccination were reported during the follow-up period. In regard to RA disease activity, the mean DAS28 was only by 0.1 higher from baseline in both MTX-continue group and MTX-hold group (0.0±0.7 vs 0.1±0.8, p=0.365). However, eight (5.1%) of 156 in the MTX-continue group and 17 (10.6%) of 160 patients in the MTX-hold group experienced a flare during 4 weeks after vaccination (p=0.07). During the follow-up period, seven (4.5%) in the MTX-continue group and 10 (6.3%) patients in the MTX-hold group required rescue medications for increased joint pain (table 3). However, none (0%) of the eight patients with a flare in the MTX-continue group and three (17.6%) of 17 patients with a flare in the MTX-hold group used a rescue medication.

Sensitivity analysis to assess the primary and secondary endpoints in all patients who had no MTX-protocol deviation (the per-protocol population) provide almost identical observations to the main analysis (online Supplementary figure S2 and table S2).

DISCUSSION

Here, we demonstrated that MTX discontinuation for 2 weeks after vaccination significantly increases the immunogenicity of a seasonal influenza vaccine in patients with RA, who had been on a stable dose of MTX, without significantly increasing risk of disease activity.

Patients with RA experience more infection disease burden due to immune dysfunction associated with the underlying autoimmunity and immunosuppressive treatment than the general population; the rate of the infection causing hospital admission and death is 1.5–2.0 times higher in patients with RA than in general population.[2 17] Therefore, the increased susceptibility to

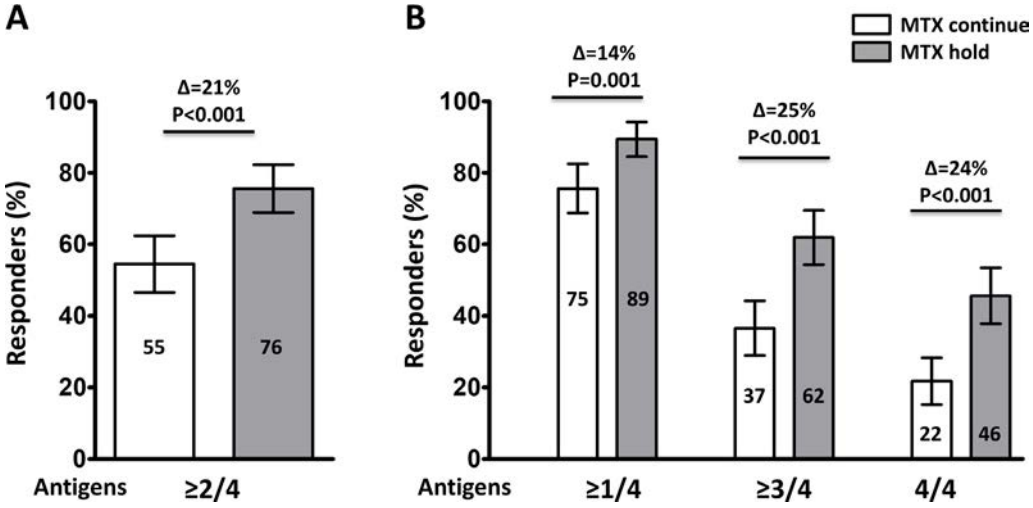

Figure 2 Frequency of vaccination responses to the influenza antigens. Satisfactory vaccine response, defined as greater than or equal to four fold increase of haemagglutination inhibition antibody titre at 4 weeks after vaccination against ≥2 of 4 vaccine strains (A) and against ≥1 of 4, ≥3 of 4 and 4 of 4 vaccine strains (B). Numbers in the bars indicate the percentage of satisfactory responders. Error bar represents 95% CI. P values were generated by X^2 test. MTX, methotrexate.

Table 2 Immunogenicity of influenza vaccine

	MTX continue (n=156)	MTX hold (n=160)	P values
H1N1			
Pre-vacc titre, GMT (95% CI)	14.8 (12.7 to 17.3)	16.2 (13.9 to 18.9)	0.422
Post-vacc titre, GMT (95% CI)	68.4 (56.8 to 82.4)	108.4 (90.7 to 129.5)	0.001
Fold increase, GM (95% CI)	4.6 (3.7 to 5.7)	6.7 (5.4 to 8.3)	0.018
Response, n (%)	79 (50.6)	100 (62.5)	0.033
Pre-vacc SP, n (%)	38 (24.4)	46 (28.8)	0.377
Post-vacc SP, n (%)	118 (75.6)	138 (86.3)	0.016
H3N2			
Pre-vacc titre, GMT (95% CI)	10.2 (8.8 to 11.8)	10.6 (9.1 to 12.3)	0.695
Post-vacc titre, GMT (95% CI)	43.9 (36.1 to 53.4)	84.3 (69.3 to 102.4)	<0.001
Fold increase, GM (95% CI)	4.3 (3.5 to 5.3)	8.0 (6.4 to 9.9)	<0.001
Response, n (%)	85 (54.5)	114 (71.3)	<0.001
Pre-vacc SP, n (%)	21 (13.5)	21 (13.1)	0.930
Post-vacc SP, n (%)	97 (62.2)	125 (78.1)	0.002
B-Yamagata			
Pre-vacc titre, GMT (95% CI)	22.4 (18.7 to 26.7)	20.8 (18.1– to 4.0)	0.534
Post-vacc titre, GMT (95% CI)	70.4 (57.8 to 85.7)	115.6 (97.4 to 137.3)	<0.001
Fold increase, GM (95% CI)	3.1 (2.6 to 3.8)	5.6 (4.7 to 6.6)	<0.001
Response, n (%)	66 (42.3)	104 (65.0)	<0.001
Pre-vacc SP, n (%)	60 (38.5)	51 (31.9)	0.220
Post-vacc SP, n (%)	116 (74.4)	141 (88.1)	0.002
B-Victoria			
Pre-vacc titre, GMT (95% CI)	13.8 (12.1 to 15.8)	11.7 (10.3 to 13.2)	0.065
Post-vacc titre, GMT (95% CI)	39.5 (33.3 to 46.9)	66.3 (56.8 to 77.4)	<0.001
Fold increase, GM (95% CI)	2.9 (2.4 to 3.4)	5.7 (4.9 to 6.7)	<0.001
Response, n (%)	64 (41.0)	118 (73.8)	<0.001
Pre-vacc SP, n (%)	33 (21.2)	21 (13.1)	0.058
Post-vacc SP, n (%)	95 (60.9)	121 (75.6)	0.005

Data are expressed in n (%) or value (95% CI). Antibody titres and fold increase are in GMT. Satisfactory vaccine response (ie, response=seroconversion) was defined as greater than or equal to fourfold improvement in titres relative to baseline. Seroprotection was defined as titres of ≥1:40. P values were generated by independent t-test for continuous variables and X^2 test for categorical variables.
Antibody titres, fold changes and vaccine response before and after vaccination to individual vaccine strains.
GM, geometric mean; GMT, geometric mean titre; n, number; pre-vacc SP, prevaccination seroprotection rate; post-vacc SP, postvaccination seroprotection rate.

infection urges that patients with RA be vaccinated for preventable infectious agents.[4 5 7]

MTX with its established efficacy and safety is commonly used as an anchor DMARD in the treatment of RA alone or in combination with the conventional or biologic DMARDs. While MTX is therapeutically used to prevent formation of antibodies against biologic DMARDs, it also significantly reduces the immunogenicity of various vaccines, including seasonal influenza vaccine.[13 14] It is recommended that vaccination should be done before a DMARD is started.[7] However, most patients with RA are already on MTX at the time when vaccination is considered. In a study, a second (booster) dose of adjuvant vaccine improves vaccine response but this approach is associated with a delay by 3–4 weeks to reach a protective immune status.[18] Therefore, a novel vaccination strategy is required to restore rapid and robust immunogenicity in patients with RA on MTX, especially when confronted with a devastating pandemic threat.[19]

In our previous pilot work, vaccine immunogenicity of trivalent influenza vaccine was significantly improved in patients with RA on stable dose of MTX, when MTX was suspended for 2 weeks before and 2 weeks after vaccination or 4 weeks after vaccination but not when it was suspended for 4 weeks before the vaccination.[12] A significant beneficial effect of MTX discontinuation could be definitively shown only for H3N2 strain and B/Yamagata but not for H1N1 due to the low number of the enrolled patients. This current trial done with higher subject number clearly demonstrated that even a 2-week discontinuation of MTX after vaccination significantly improves immunogenicity in all four strains of the quadrivalent influenza vaccine (H1N1, H3N2, B-Yamagata and B-Victoria) (table 2). In addition, holding MTX improved the vaccine response especially in those patients taking higher MTX dose (figure 2), indicating inhibition of vaccine response by MTX is dose dependent. Strikingly, improvement in satisfactory vaccine response was more prominent for the less exposed type B viral strains; response increased by 11.9% for H1N1% and 16.8% for H3N2 strains while it increased by up to 22.7% for B-Yamagata and 32.8% for B-Victoria strain (table 2). These results suggest that this MTX discontinuation strategy might be more crucial for response to influenza viruses relatively new to humans. Influenza pandemics occur when influenza strain undergoes major antigenic changes. Currently, influenza infections with avian H5N1 and H7N9 viruses with a reported mortality of 40%–60% have been rising in Asian countries.[20 21] The MTX discontinuation strategy therefore could be even more important when patients with RA should be vaccinated against these novel influenza strains. Further studies are needed to evaluate whether this short-term MTX discontinuation can be applied to other vaccines which has low immunogenicity such as herpes zoster.

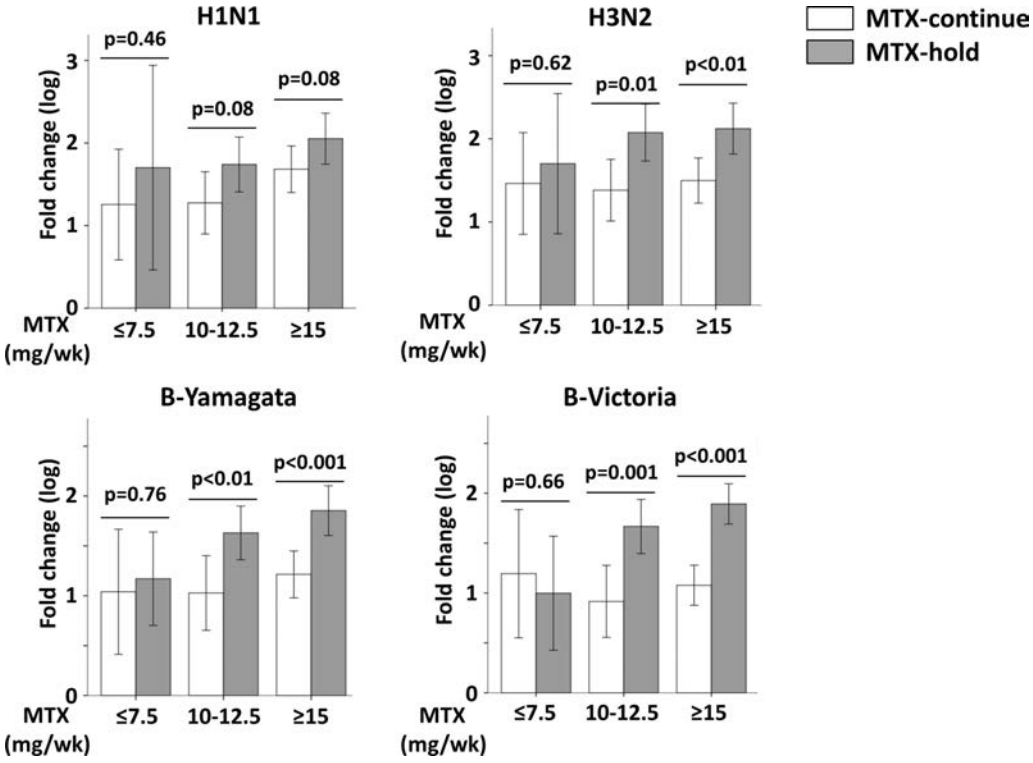

Figure 3 Impact of baseline methotrexate (MTX) dose on vaccination responses to the influenza antigens. Log-transformed fold change in antibody titre against each vaccine strain relative to baseline was depicted according to the baseline MTX dose per week (mg/week). Bar and whiskers represent mean and SD, respectively. P values were generated by t-test.

Patients tolerated the influenza vaccination well without a major complication. The profile of adverse events was similar between the MTX-continue group and the MTX-hold group. In regard to disease activity, the mean disease activity remained stable. However, at the individual level, 10.6% of the patients in the MTX-hold group and 5.1% in the MTX-continue group experienced a flare (table 3). All patients with a flare returned to their baseline disease activity when MTX was resumed. The higher flare rate relative to stable DAS28 change might be, in part, due to the flare definition which defines as an increase in DAS28 of >1.2 or >0.6 if the baseline DAS28 was ≥3.2. Therefore, smaller change in DAS28 in patients with baseline DAS28 >3.2 was considered a flare, although patients might not feel a clinical difference. Accordingly, none (0%) of the eight patients with a flare in the MTX-continue group and three (17.6%) of 17 patients with a flare in the MTX-hold group used a rescue medication, reflecting the relatively stable DAS28 during the study duration. However, the patients in this study had very low activity at baseline and the risk of flare could be higher in patients with higher activity.

Table 3 Adverse events and RA disease activity

	MTX continue (n=156)	MTX hold (n=160)	P values
Any AE (%)	34 (21.8)	45 (28.1)	0.194
SAE (%)	0 (0)	0 (0)	1.000
AE occurring in >1% of patients (%)			
Upper respiratory infection	12 (7.7)	9 (5.6)	0.461
Myalgia	5 (3.2)	10 (6.3)	0.203
Injection site reaction	4 (2.6)	6 (3.8)	0.750
Abdominal pain	3 (1.9)	1 (0.6)	0.366
Rash	2 (1.3)	2 (1.3)	1.000
Fatigue	0 (0)	2 (1.3)	0.498
Sore throat	0 (0)	2 (1.3)	0.498
Dizziness	2 (1.3)	0 (0)	0.243
DAS28 at visit 1 (0–100)	2.2 (0.9)	2.3 (1.1)	0.517
DAS28 at visit 2 (1–100)	2.3 (0.9)	2.4 (1.1)	0.220
Rescue medication (%)	7 (4.5)	10 (6.3)	0.487
RA flare at visit 2 (%)	8 (5.1)	17 (10.6)	0.070

The data are expressed as mean (SD) or number (%). RA flare was defined as an increase in DAS28 of >1.2 (or >0.6 if the DAS28 was ≥3.2).
AE, adverse event; DAS28, Disease Activity Score in 28 joints; MTX, methotrexate; RA, rheumatoid arthritis; SAE, serious adverse event.

The study has several limitations. First, all patients are Korean ethnicity. However, the clinical characteristics are similar to those in RA populations, and vaccine efficacy in our population is similar to that in other influenza vaccine studies.[8 12 22] Therefore, the result might be generalised to other ethnic groups. Second, the current population was composed of stable patients with RA with a mean baseline DAS28-CRP of 2.2 in the MTX-continue group and 2.3 in the MTX-hold group. This low disease activity might be a result of target-to-treat approach in routine clinical practice.[23] Therefore, the study population might be more similar to the general RA population than patients in clinical trials which include patients with higher disease activity. However, further studies testing the generalisability of our results to patients with moderate to high disease activity or with other ethnicities are warranted. Third, our study was not powered to detect a difference of influenza incidence between the two groups. A large-scale prospective study is needed to confirm whether the improved immunogenicity of MTX discontinuation can be translated into a decreased influenza incidence.

CONCLUSIONS

In conclusion, a temporary discontinuation of MTX for 2 weeks after vaccination improves the immunogenicity of a seasonal

influenza vaccine in patients with RA on stable dose of MTX without appreciably increasing disease activity.

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Contributors All authors contributed to the acquisition, analysis or interpretation of data and critical revision of the manuscript for important intellectual content. EBL had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. EBL, KLW and JKP were responsible for the study concept and design and drafting of the manuscript. EBL, JKP, YC and KLW were responsible for the statistical analysis.

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Competing interests EBL has acted as a consultant to Pfizer and received research grants from Green Cross Corporation and Hanmi Pharm.

Patient consent Obtained.

Ethics approval The study was approved by the institutional review board of the Seoul National University Hospital (IRB 1608-158-787).

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EXTENDED REPORT

Serious infection across biologic-treated patients with rheumatoid arthritis: results from the British Society for Rheumatology Biologics Register for Rheumatoid Arthritis

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ABSTRACT

Objectives To compare the incidence of serious infection (SI) across biologic drugs used to treat rheumatoid arthritis (RA) using data from the British Society for Rheumatology Biologics Register for Rheumatoid Arthritis (BSRBR-RA).

Methods The BSRBR-RA is a prospective observational cohort study. This analysis included patients with RA starting a new biologic. The primary outcome was SI defined as an infectious event requiring admission to hospital, intravenous antibiotics or resulting in death. Event rates were calculated and compared across biologics using Cox proportional hazards with adjustment for potential confounders. Secondary outcomes were the rate of infection by organ class and 30-day mortality following infection.

Results This analysis included 19 282 patients with 46 771 years of follow-up. The incidence of SI was 5.51 cases per 100 patient years for the entire cohort (95% CI 5.29 to 5.71). Compared with etanercept, tocilizumab had a higher risk of SI (HR 1.22, 95% CI 1.02 to 1.47) and certolizumab pegol a lower risk of SI (HR 0.75, 95% CI 0.58 to 0.97) in the fully adjusted model. The 30-day mortality following SI was 10.4% (95% CI 9.2% to 11.6%).

Conclusions The rate of SI was lower with certolizumab pegol than etanercept in the primary analysis but the result was no longer significant in several sensitivity analyses performed suggesting residual confounding may account for the observed difference. From these results, it would be wrong to conclude that certolizumab pegol has a lower rate of SI than other biologics; however, the risk does not appear to be significantly higher as has previously been suggested.

BACKGROUND

Infection represents a substantial source of both morbidity and mortality for patients with rheumatoid arthritis (RA). A recent real world study of UK patients with RA reported that 8% require hospital admission each year due to serious infection (SI).[1] The predictors of infection in RA include patient factors (older age, concomitant illness), disease-specific factors (level of disease activity and disability) and immunosuppression. Conventional synthetic disease-modifying anti-rheumatic drugs (cs-DMARDs) in RA have relatively little impact on infection risk; however, corticosteroid exposure appears to be an important predictor of infection.[2 3]

Anti-TNF therapy is associated with an increased rate of SI when compared with cs-DMARD therapy with the risk greatest during the first 6–12 months of therapy.[4–6] The infection risk with other biologics acting through different mechanisms in RA is less well established. One would hypothesise that biologic drugs acting on different cellular and cytokine targets would have a different pattern and rate of SI associated with them.

There are few head to head clinical trials between biologic therapies in RA. Those studies that do exist understandably focus on efficacy as their primary endpoint. While safety data are often included, the studies are not powered to detect significant differences in the rate and pattern of infection seen.

A large network meta-analysis of biologics found that the incidence of SI was comparable across biologic drugs used to treat RA with the exception of two drugs.[7] Anakinra and certolizumab pegol were both found to have a significantly higher rate of SI than other biologics. However, a network meta-analysis relies on an indirect comparison between drugs and if there are differences in study design it can be prone to error. In contrast, national registers use the same methodology for detecting and reporting of adverse events for each drug.

Early work from the German RABBIT register showed that the incidence of SI with anti-TNF was approximately 6% per year but the population of people receiving biologics has changed over the past 15 years and these analyses need updating.[8] There is a paucity of real world data on the infection risks associated with newer biologics such as tocilizumab. Results from a Japanese register showed no significant difference in the rate of SI with tocilizumab compared with anti-TNF therapy (HR 2.23, 95% CI 0.93, 5.37), but the CIs were wide and it is possible that the study was just underpowered to detect a difference.[9]

We set out to describe and compare the incidence and pattern of SI within the British Society for Rheumatology Biologics Register (BSRBR-RA) by biologic drug.

METHODS

Data source

We used data from the BSRBR-RA, a prospective observational study established to evaluate the safety of biologics. The methodology has been described in detail previously.[10] The inception of the register

in 2001 was to meet national recommendations that all individuals with RA starting anti-TNF therapy should be enrolled in a postmarketing surveillance study. Initial biologic cohorts were for etanercept and infliximab users. Adalimumab, rituximab, tocilizumab and certolizumab-pegol cohorts have since been recruited. Abatacept received a European product license in 2007 but was not approved as a first-line biologic in the UK until 2012 and as such was not adopted into the BSRBR-RA.

Patient and physician questionnaires are returned every 6 months for the first 3 years and an annual physician questionnaire is completed thereafter. All adverse events are coded according to Medical Dictionary for Regulatory Activities (MedDRA) definitions. The BSRBR-RA is linked to the national death register meaning that the date and cause of death were available for individuals who died during the study period.

Drug exposure

This analysis included patients with RA starting a new biologic. Individuals were considered 'at risk' from treatment start for 3 years or until the date SI, three half-lives after drug cessation, death or last follow-up before June 2016, whichever came first. For the rituximab cohort, patients were considered 'at risk' until 270 days after the last infusion. If two infusions were separated by more than 270 days, the subject was considered to have been continuously 'exposed', reflecting the varying dosing frequency of rituximab. Biosimilars have been included in the register since 2015 but at the time of analysis there was insufficient follow-up data to include them in this study. Patients who switched therapy were allowed to contribute follow-up time to more than one cohort. All patients provided written informed consent.

Outcome

The primary outcome was any SI—defined as an infection resulting in death, hospitalisation or requiring intravenous antimicrobial therapy. Events could be identified in one of three ways: from patient questionnaires, physician questionnaires or death certificates. When identified by questionnaire, event of interest forms were sent to the patient and treating clinician to gather more information. Events were divided into seven categories based on organ class of infection (sepsis, lower respiratory, skin, gastrointestinal, bone/joint, genitourinary and other). The MedDRA codes for each category are shown in the online Supplementary figure 1. Secondary outcome measures were the organ class of infection and the 30-day mortality following serious infection.

Statistical analysis

Event rates were calculated by dividing the number of SI by the time under observation for each drug with CIs calculated using a Poisson distribution. Rates were compared across biologics using Cox proportional hazards regression for time to first SI. Etanercept was chosen as the reference for comparison as it was the most widely used drug in the register. Potential confounders were identified a priori and adjustment was performed for age, gender, Disease Activity Score 28-erythrocyte sedimentation rate (DAS28-ESR), Health Assessment Score (HAQ), disease duration, smoking, seropositivity, polypharmacy and baseline steroid usage. Previous work with the BSRBR-RA has shown that polypharmacy is a good predictor of infections at a population level and can be used as a surrogate for comorbidity.[11] Individuals were divided into three categories (0–5, 6–9 and >10) based on the number of medications they were taking excluding therapy for RA, and this category was used in the adjusted model. Multiple imputation of missing baseline variables was performed with 20 cycles using the ICE package in Stata V.14. Assumptions of the Cox model were tested graphically using Nelson-Aalen plots and with Schoenfeld residuals.

Sensitivity analyses

As the patient characteristics of those receiving biologics have changed since the inception of the register, a sensitivity analysis looking only at individuals starting a biologic from 2010 onwards was performed.

Confounding by indication is a major problem in observational studies that can be partially addressed using propensity scores (PS). A separate PS model was created for each treatment comparison using an inverse probability of treatment weights model. A single PS for each patient was calculated based on the following baseline covariates: age, gender, DAS28, HAQ, disease duration, seropositivity and non-biologic DMARD therapy. Further information on the PS model is available in the online Supplementary table 4.

Patients starting their first biologic will have different disease characteristics compared with those who have failed on a previous biologic. Certain biologics tend only to be used as second or third line options and therefore comparing users of these drugs to new biologic users will be affected by channelling bias. A second sensitivity analysis was performed excluding those who were biologic naive, therefore, limiting only to individuals who have 'failed' at least one biologic agent.

A further sensitivity analysis was performed adjusting for individual comorbidities instead of polypharmacy. Comorbidities that predicted infection in a single variable model (diabetes, asthma and chronic obstructive pulmonary disease) were included in the multivariable model.

Previous studies have shown that the infection risk is greatest shortly after starting a new biologic. A sensitivity analysis was performed limiting to patients in their first year of follow-up in the register.

RESULTS

A total of 19 282 individual patients were included in the analysis contributing 46 771 patient-years follow-up. The baseline characteristics of patients separated by drug therapy are shown in table 1. There were no missing data for age, gender or number of drugs. For the remaining covariates used in the adjusted analyses, there were less than 5% missing data.

Incidence of serious infection

In total, 2606 events were classified as SI at a rate of 5.51 events per 100 patient years of follow-up (95% CI 5.29 to 5.71).

Etanercept was the largest cohort and was set as the reference group for other comparisons. In this cohort, the crude incidence of SI was 5.56 per 100 patient years (table 2). Three drugs had significantly different rates of SI compared with etanercept. These were rituximab, tocilizumab and certolizumab.

Both rituximab (6.29 cases per 100 patient years) and tocilizumab (6.98 cases per 100 patient years) had a higher rate of infection than etanercept in the unadjusted model. In the adjusted model, rituximab no longer had an increased rate of SI (HR 0.91, 95% CI 0.80 to 1.03).

Certolizumab pegol had the lowest crude incidence of infection at 3.80 cases per 100 patient years. The rate was significantly lower than etanercept in both the unadjusted and fully adjusted model (table 2). The adjusted relative risks by drug is shown in figure 1.

Table 1 Baseline demographics of the cohort

	Etanercept	Infliximab	Adalimumab	Rituximab	Tocilizumab	Certolizumab
Number exposed (n)	8630	4908	7818	5101	2174	1446
Years of follow-up	15314	8829	13071	5910	1963	1685
Mean age in years (SD)	56 (12)	56 (12)	56 (12)	60 (12)	57 (12)	56 (12)
Female (%)	77.5	76.8	77.4	78.6	79.6	76.1
Mean disease duration: years (SD)	13 (9)	13 (9)	13 (9)	16 (10)	15[10]	11 (9)
Mean DAS28 (SD)	6.5 (1.1)	6.6 (1.0)	6.4 (1.1)	6.4 (1.1)	6.1 (1.3)	5.9 (1.1)
Mean HAQ (SD)	2.0 (0.6)	2.1 (0.6)	1.9 (0.6)	2.0 (0.6)	1.9 (0.6)	1.6 (0.8)
Steroid user (%)	43.6	46.7	39.4	42.5	38.6	28.5
Current smoker (%)	21.3	22.1	22.7	22.4	22.6	21.4
Seropositive (%)	64.0	66.3	63.0	67.3	61.4	58.4
DMARD therapy						
Methotrexate (%)	48.5	79.0	55.7	59.0	58.2	63.4
Sulfasalazine (%)	15.4	13.9	18.6	14.1	14.0	27.4
Leflunomide (%)	8.8	6.7	10.0	8.7	7.3	9.0
Hydroxychloroquine (%)	11.3	8.8	13.3	11.5	15.6	29.6
Number of drugs						
0–5 drugs (%)	51.4	46.9	49.8	45.9	44.5	48.1
6–10 drugs %	42.0	49.3	43.6	45.6	44.5	45.1
More than 10 drugs (%)	6.6	4.6	6.6	8.5	11.0	6.8
Comorbidities						
Asthma (%)	11.2	9.9	10.5	11.8	13.7	12.1
COPD (%)	5.5	4.6	4.4	6.0	5.0	4.1
Diabetes (%)	6.1	5.0	6.0	6.6	7.6	6.1

COPD, chronic obstructive pulmonary disease; DAS28, Disease Activity Score 28; DMARD, disease-modifying antirheumatic drugs; HAQ, Health Assessment Questionnaire.

The results of the primary analysis were supported by the sensitivity analysis using propensity scores to adjust for potential confounding. Certolizumab was again found to have a significantly lower rate of SI, while tocilizumab had a higher rate of SI (see online Supplementary table 2). A sensitivity analysis limiting to new starters from 2010 found certolizumab still had a lower point estimate of serious infection rate but findings were no longer statistically significant in the fully adjusted model (HR 0.85, 95% CI 0.60 to 1.21).

In the sensitivity analysis excluding biologic-naive patients, the rate of infection was comparable across the drugs with the exception of tocilizumab which had a higher incidence of SI than etanercept (HR 1.85, 95% CI 1.48 to 2.31). Full results for all the sensitivity analyses are shown in the online Supplementary table 2.

Infection by organ class

Respiratory infections accounted for 42% of all SI and were the most frequently recorded class of infection for all drugs in the register followed by soft tissue and skin infections (see online Supplementary figure 1). The incidence of serious infection by organ class for the entire cohort of biologic users is shown in figure 2.

A full breakdown of the relative risk by drug class for each of the organ classes is shown in table 3. Etanercept had a comparatively low rate of respiratory tract infections at 1.81 cases per 100 patient years. This was significantly lower than adalimumab and tocilizumab in the fully adjusted model but not significantly different from the other drugs (table 3).

The rate of sepsis was significantly higher with rituximab than etanercept (HR 2.08, 95% CI 1.14 to 3.80). The reverse was seen when looking at skin infections with rituximab showing a significantly lower rate of events than etanercept (HR 0.54, 95% CI 0.39 to 0.75).

Adalimumab (HR 0.65, 95% CI 0.52 to 0.82) and certolizumab (HR 0.27, 95% CI 0.11 to 0.67) were associated with a significantly lower rate of skin infections than etanercept.

30-Day mortality by drug

The 30-day mortality following a serious infection for the whole cohort was 10.4% (95% CI 9.2% to 11.6%). The organ class of infection was a strong predictor of subsequent mortality. Sepsis/bacteraemia had the highest mortality at 45% (95% CI 33% to 61%) compared with just 2% (95% CI 1% to 3%) following skin

Table 2 Incidence of serious infection by drug

	Etanercept	Infliximab	Adalimumab	Rituximab	Tocilizumab	Certolizumab
Number of patients	8630	4908	7818	5101	2174	1446
Follow-up time in years	15314	8829	13071	5910	1963	1685
Infections: single failure	852	472	709	372	137	64
Incidence per 100 patient years (95% CI)*	5.56 (5.20 to 5.95)	5.35 (4.89 to 5.85)	5.42 (5.04 to 5.84)	6.29 (5.69 to 6.97)	6.98 (5.90 to 8.25)	3.80 (2.97 to 4.85)
Unadjusted HR (95% CI)	Ref	0.94 (0.84 to 1.06)	0.97 (0.88 to 1.07)	1.15 (1.01 to 1.30)	1.22 (1.02 to 1.47)	0.65 (0.51 to 0.84)
Adjusted HR (95% CI)	Ref	0.89 (0.79 to 1.00)	1.00 (0.90 to 1.10)	0.91 (0.80 to 1.03)	1.21 (1.01 to 1.46)	0.75 (0.58 to 0.97)

Covariates included in the adjusted model were age, gender, Disease Activity Score 28-erythrocyte sedimentation rate, Health Assessment Questionnaire, disease duration, smoking, seropositivity, polypharmacy and baseline steroid usage.
*Unadjusted incidence.

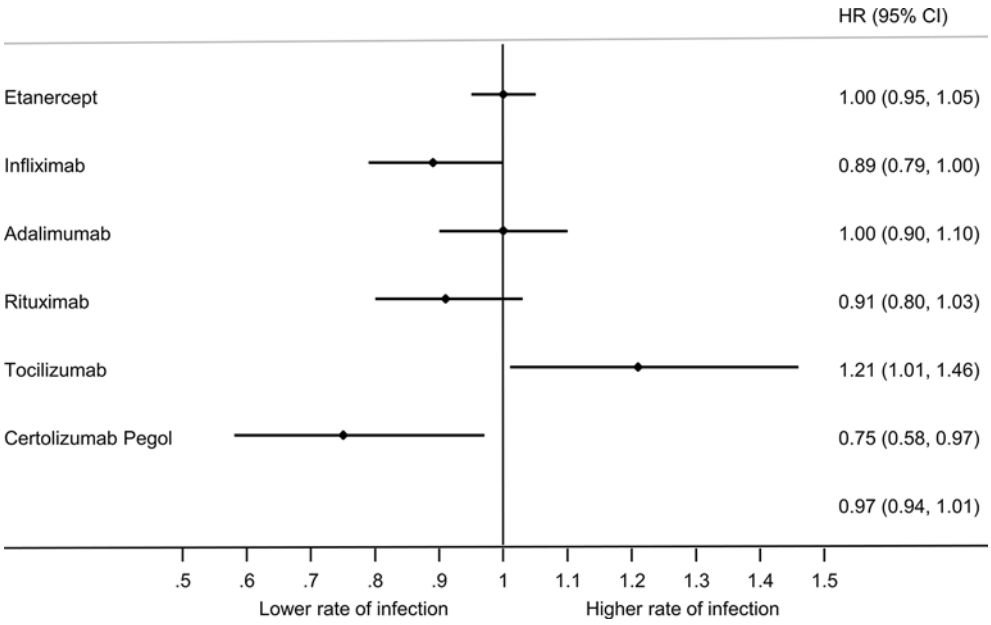

Figure 1 Adjusted relative risk of infection by drug.

infections. Sepsis remained a significant predictor of mortality in the fully adjusted model. The choice of biologic therapy was not a significant predictor of death following serious infection.

CONCLUSIONS

The rate of serious infection in this analysis was similar to that published in comparable cohorts of patients with RA treated with biologics.[6 8] The pattern of infection was also similar to previously published work with respiratory infections the most frequently documented class of infection followed by skin and soft tissue infection.[12] Small differences were observed between the drugs. It is important to appreciate the difference between relative and absolute risk when considering biologic choice. For patients whose baseline risk of infection is low, the choice of biologic will have very little impact on their subsequent infection risk. However, for patients with multiple risk factors who have a high baseline risk of infection, then choosing a drug with a slightly higher relative risk of infection can have a much larger impact on their infection risk.

In the unadjusted analysis, rituximab had a higher incidence of infection than etanercept but in the adjusted analysis the difference was no longer statistically significant. This suggests that patient factors as opposed to the drug itself were responsible for the observed difference. It is important to remember that drugs that are normally given second and third line are given to a different population than those starting a first biologic. Table 1 shows that the patients receiving rituximab tended to be older and with longer disease duration than those receiving other biologics.

A higher rate of infection was observed with tocilizumab compared with etanercept in both the adjusted and unadjusted models. Given the large sample size, this is unlikely to have occurred just by chance. It is possible that there was confounding but the result remained significant in a sensitivity analysis limiting to contemporary practice from 2010 onwards (HR 1.34, 95% CI 1.01 to 1.79) and a second sensitivity analysis excluding biologic-naive patients (table 2). These would suggest a true association. Our results were comparable with data from the German biologics register which found a higher relative risk of infection among tocilizumab users compared with those receiving anti-TNF therapy (relative risk 1.15).[13] There is little directly comparative literature of infection risk between tocilizumab and other biologics. The ADACTA trial was an RCT comparing tocilizumab monotherapy with adalimumab

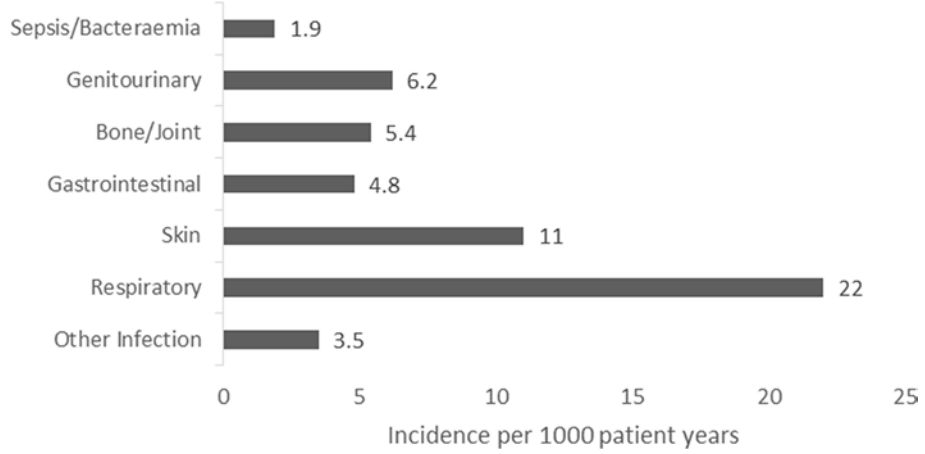

Figure 2 Incidence of infection by organ class.

Table 3 Adjusted relative risk of infection by organ class for each drug

Organ class	Etanercept	Infliximab	Adalimumab	Rituximab	Tocilizumab	Certolizumab
Number of patients	8630	4908	7818	5101	2174	1446
Sepsis/bacteraemia (95% CI)	Ref	0.83 (0.41 to 1.66)	1.04 0.57 to 1.91)	2.08 (1.14 to 3.80)	1.83 (0.63 to 5.35)	1.03 (0.24 to 4.41)
Respiratory (95% CI)	Ref	1.16 (0.96 to 1.39)	1.23 (1.04 to 1.45)	1.03 (0.83 to 1.28)	**1.61 (1.15 to 2.25)**	0.96 (0.63 to 1.46)
Skin (95% CI)	Ref	0.84 (0.66 to 1.06)	0.65 (0.52 to 0.82)	0.54 (0.39 to 0.75)	0.71 (0.40 to 1.24)	**0.27 (0.11 to 0.67)**
Gastrointestinal (95% CI)	Ref	0.95 (0.66 to 1.38)	0.77 (0.54 to 1.11)	0.93 (0.61 to 1.42)	1.45 (0.72 to 2.90)	0.51 (0.16 to 1.63)
Bone/joint (95% CI)	Ref	0.56(0.38 to 0.83)	0.80 (0.58 to 1.09)	0.67 (0.43 to 1.02)	0.46 (0.17 to 1.27)	0.73 (0.32 to 1.68)
Genitourinary (95% CI)	Ref	0.74 (0.50 to 1.07)	1.18 (0.87 to 1.59)	1.15 (0.79 to 1.68)	0.67 (0.27 to 1.66)	0.55 (0.20 to 1.52)
Other (95% CI)	Ref	0.54 (0.31 to 0.91)	1.08 (0.74 to 1.58)	0.72 (0.41 to 1.29)	1.15 (0.49 to 2.67)	0.50 (0.16 to 1.60)

Adjusted HR for the relative risk of serious infection for each organ class. Model adjusted for age, gender, Disease Activity Score 28-erythrocyte sedimentation rate, Health Assessment Questionnaire, disease duration, smoking, seropositivity, polypharmacy and baseline steroid usage CI. The unadjusted incidence of infection by organ class is shown in the online Supplementary table 3.

monotherapy in RA.[14] It found that tocilizumab had superior efficacy compared with adalimumab suggesting that it may be a more potent drug which could provide a biologically plausible link to a higher infection rate. In the ADACTA trial, no difference was observed in the rate of serious infection between the drugs but event numbers were small with just 13 SIs recorded throughout the entire study.

In the primary analysis, certolizumab pegol had a significantly lower incidence of SI than etanercept. This is in direct contradiction to the 2011 Cochrane review which found that certolizumab had a higher rate of infection.[7] In that review, the incidence of infection with certolizumab pegol was approximately 3–4 times higher than other anti-TNF drugs. It would seem unusual for drugs acting on the same pathway with similar efficacy to have such drastically different infection risks. In the EXXELERATE study, a direct head to head clinical trial of certolizumab pegol versus adalimumab the rate of serious infection was the same with both drugs at 3% though event numbers were small.[15] The Cochrane review was a network meta-analysis using indirect comparisons between biologics to estimate the relative risk of infection. Differences in how the control groups are treated, for example, different levels of glucocorticoid therapy as well as the crossover design of studies included can give misleading relative risks when comparing drugs between different studies.

A large number of patients in the certolizumab pegol cohort had never previously been on a biologic. In the sensitivity analysis limiting only to individuals who had failed at least one biologic, certolizumab pegol no longer had a favourable infection rate compared with etanercept. This suggests that unmeasured confounders may be responsible for the difference that was observed in the primary analysis. It is notable that etanercept entered the register in 2001 but certolizumab pegol recruitment did not commence until 10 years later in 2011.

A different pattern of infection was seen between the drugs. It was interesting to observe that a lower rate of sepsis was seen with the anti-TNF drugs compared with rituximab. Previous work from the German RABBIT register found that the risk of developing sepsis among individuals with SI and mortality from sepsis was lower with anti-TNF therapy compared with non-biologic DMARDs.[16] This is perhaps not surprising as TNF is thought to be an important cytokine in the development of sepsis and clinical trials have been carried out to evaluate the use of TNF blockade in treating patients with severe sepsis.[17 18] Many of the initial trials of anti-TNF in sepsis found no significant change in the mortality rates but they may have been underpowered. A recent meta-analysis has shown a small but significant reduction in mortality with anti-TNF among patients with sepsis.[19] These clinical trials were looking at the benefit of giving a single dose of anti-TNF to patients who already had developed sepsis. It is possible that greater benefit would be seen in individuals who were already taking anti-TNF therapy when they developed an infection.

The all-cause mortality in the overall cohort was low at 0.84% per year but was significantly increased immediately following a serious infection. Over 10% of patients who suffered a serious infection died within 30 days of the event highlighting the significance of these events. Sepsis was a significant predictor of mortality.

Strengths

The large sample size, robust method of data capture and accurate coding of adverse events including SI is a strength of the BSRBR-RA and allowed comprehensive analysis of SI in biologic-treated patients. What sets this study apart from many others looking at the safety of biologics is our choice of etanercept as the comparator arm. Previous studies using registries have mostly compared the risk of starting a biologic with the risk of continuing current DMARDs in patients with severe disease. This is an increasingly irrelevant comparison as most clinicians faced with a patient who has not responded to DMARDs will opt to start a biologic. Cost and efficacy will have a big influence in drug decision but in those who are at high risk of infection, safety will also play an important role. It is therefore vital to have directly comparative studies.

Limitations

As the previously mentioned Cochrane review showed a varying infection risk with different biologics, clinicians may have channelled higher risk patients away from drugs that they perceive to have the greatest infection risk towards supposedly 'safer' drugs. We have adjusted for baseline variables that predict infection but there will always be a degree of unmeasured confounding that we cannot adjust for. This could help to explain why such a drastically different infection risk was seen with certolizumab pegol in our analysis and the 2011 Cochrane review.

Caution needs to be exercised when interpreting the relative risk of infection by organ class. This secondary analysis was performed on smaller numbers of events and is more prone to error from misclassifications than the primary analysis. Multiple comparisons were performed and as such the chance of finding statistically significant results by chance increases. In efficacy studies, one would normally adjust the p value when performing multiple comparisons to reduce the risk of false positives. However, in pharmacovigilance studies, one is more concerned about missing a potential safety signal and false negatives are the

greater concern. We have therefore not adjusted the significance level despite performing multiple comparisons.

This study has identified differences in the incidence and patterns of SI between biologic drugs in a large RA cohort. For the majority of patients, biologic therapies remain a safe and efficacious treatment strategy. However, in a population of 'high-risk' individuals, differences in the relative risk of infection can have a significant impact on the absolute risk of SI. Recognising the subtleties in the differential infection risk profiles between drugs is a step towards personalised medicine and safer prescribing habits.

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Contributors All authors were involved in the design and statistical analysis of the study as well as manuscript drafting and gave approval to the final version.

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Competing interests The BSR commissioned the BSRBR-RA as a UK-wide national project to investigate the safety of biological agents in routine medical practice. BSR receives restricted income from UK pharmaceutical companies, presently Abbvie, Celltrion, Hospira, Pfizer, UCB and Roche, and in the past Swedish Orphan Biovitrum and Merck. This income finances a wholly separate contract between the BSR and the University of Manchester. The pharmaceutical company funders had no role in the design and conduct of the study; collection, management, analysis and interpretation of the data; preparation, review or approval of the manuscript and decision to submit the manuscript for publication. KH has received honoraria from Pfizer and Abbvie (<US$10 000). JG has received honoraria for speaking or attending conferences from Pfizer, BMS, UCB and Celgene (<US$10 000).

Patient consent Not required.

Ethics approval The study was approved by the North-West Multicentre Research Ethics Committee (MREC 00/8/053, IRAS: 64202).

Provenance and peer review Not commissioned; externally peer reviewed.

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CONCISE REPORT

Assay variation in the detection of antinuclear antibodies in the sera of patients with established SLE

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ABSTRACT

Objective The expression of antinuclear antibodies (ANA) is considered almost constant in systemic lupus erythematosus (SLE), although recent experience has suggested that many subjects with SLE considered for clinical trials are ANA negative at screening. The objective of this study is to determine whether assay variation can influence ANA detection in patients with established SLE.

Methods Sera from 103 patients with established SLE were tested using three different immunofluorescence assays (IFA) for ANA determination. ANA determinations were also performed by an ELISA and bead-based multiplex assay.

Results With IFA kits, the frequency of ANA negativity varied from 5 to 23 of 103 samples (4.9%–22.3%). The ELISA and multiplex assays showed that 12 (11.7%) and 14 (13.6%) samples were negative, respectively. Samples positive in all assays differed from those with discordant assay results in the frequency of historical anti–double-stranded DNA positivity and low complement levels at the time of blood sampling.

Discussion These findings indicate that ANA negativity occurs in patients with established SLE although the frequency varies depending on the assay kit. Given the range of negativity with well-validated assays, these findings raise questions about whether ANA positivity should be employed to determine eligibility for clinical trials.

INTRODUCTION

Antinuclear antibodies (ANA) are important biomarkers for systemic lupus erythematosus (SLE) and represent a criterion for patient classification.[1] While ANAs are not specific for SLE, patients with SLE are thought to be almost invariably positive. ANA testing is usually performed only at the time of diagnosis, however, because of the apparent lack of changes in ANA titres over time. In contrast, repeat testing of anti–double-stranded DNA (dsDNA) antibodies is common since levels of these antibodies are associated with disease activity. Among technologies for ANA determination, the immunofluorescence assay (IFA) is often viewed as the 'gold standard'.[2–4]

In addition to its role in patient evaluation, ANA testing has recently been used to assess the eligibility of patients for entry into clinical trials of new therapeutic agents, deriving from the experience with the development of belimumab. Belimumab is a monoclonal antibody directed against B-cell activating factor/B lymphocyte stimulator and received regulatory approval for the treatment of patients with active, autoantibody-positive SLE receiving standard therapy. After failure of a phase II study, re-analysis of the data showed that patients who were serologically positive (ANA and/or anti-DNA) responded to the agent; in the phase II study, approximately 30% of patients were serologically negative, defined as an ANA with a titre of ≤1:80. Subsequent phase III trials enrolled only serologically positive individuals at screening and met their endpoints.[5–8] Other sponsors conducting clinical trials are now enrolling only patients who are ANA and/or anti-DNA positive.[9] As such, serological testing is being used as a companion diagnostic or theranostic biomarker although existing tests have not been validated for this purpose.

The high frequency of ANA negativity in patients screened for trials is surprising and differs from the usual conceptualisation of the serology of SLE (ie, a frequency of ANA positivity of 95%–99%). One possibility for a discrepancy between historical and screening results may relate to a transition to a serologically negative status, reflecting the natural history of disease or the effects of therapy.[10 11] Alternatively, ANA variability may reflect the performance characteristics of the test kits.[12–14]

In view of the increasing use of ANA for determining trial eligibility, an explanation of these observations is important since it can impact both trial enrolment and eventual utilisation of a product approved for autoantibody-positive patients. To define further the serology of SLE as revealed by different ANA assay platforms and kits, we evaluated the detection of ANAs in patients with established disease rather than at the time of diagnosis.

METHODS

The study involved 103 patients from a cohort of patients with SLE who had historical ANA positivity followed at The Ohio State University. SLE was determined on the basis of four or more American College of Rheumatology criteria for classification. Table 1 presents a description of the patient population. Of the patients, approximately one half had a history of renal disease. Sera from patients were screened by two experienced observers with three commercially available IFA assays at a dilution of 1:40 in a single laboratory. We used results from the 1:40 dilution which is the recommended screening dilution by kit manufacturers and has maximum

Table 1 Characteristics of patients who were ANA positive in all assays and patients who showed discordance between assays

	ANA positive all assays	Disagreement among assays	P value
Age (years)*	34.2 (20.2–67)	38.4 (21.6–62.5)	NS†
Duration of SLE (years)*	6.3 (0.1–33.4)	5.7 (1.6–19.8)	NS
Male (%)	6.3% male	11.4% male	NS‡
Caucasian (%)	56%	66%	NS‡
African American (%)	38%	31%	NS‡
Lupus nephritis (%)	67%	51%	NS‡
Ever anti-dsDNA positive	59%	23%	0.0006‡
C3§ (SD)	99 (32)	121 (34)	0.0058¶

*Median (range).
†Mann-Whitney test.
‡Fisher's exact test.
§Complement component C3, measured in a serum sample taken at the same time the sample for ANA was obtained.
¶Unpaired t test.
ANA, antinuclear antibody; ds-DNA, double-stranded DNA; SLE, systemic lupus erythematosus.

sensitivity. In addition, the sera were tested by an ELISA as well as a bead-based multiplex assay.

RESULTS

As the results in table 2 indicate, the frequency of ANA positivity varies markedly depending on the assay platform and kit used. Among IFA kits, the frequency of negativity varied from 5 to 23 of the 103 (4.9%–22.3%) samples tested although some samples were considered to have indeterminate results. For the ELISA, 12 (11.7%) had negative values. For the multiplex assays, 14 (13.6%) of the samples were reported as negative; in this assay, limited elevations of anti-dsDNA lead to a result called indeterminate.

To determine any features associated with serological status, the patients were divided into those who were consistently ANA positive in all assays and those who showed discordancy among assays. Using this categorisation, a preliminary analysis indicated that those patients who demonstrated consistent ANA positivity differed from those who had disagreements among assays in the likelihood of historical anti-dsDNA positivity and low levels of C3 complement (table 1). Disease duration and the occurrence of nephritis did not differ significantly in the two patient groups.

Table 2 Antinuclear antibody testing kits

Test result	IFA kit 1	IFA kit 2	IFA kit 3	ELISA	Multiplex
Negative	23 (22.3)	10 (9.7)	5 (4.9)	12 (11.7)	14 (13.6)
Indeterminate	9 (8.7)	10 (9.7)	2 (1.9)	0	8 (7.8)

Data shown in brackets are the percentages of the total number of samples analysed.
IFA, immunofluorescence assay.
The 103 samples were analysed using three commercially available ANA IFA kits, an ELISA and a multiplex assay called the BioPlex 2200. The following IFA kits were used: kit 1, ImmunoConcepts (distributed by GFMD, Novi, Michigan, USA); kit 2, Inova Diagnostics, San Diego, California, USA; and kit 3, Bio-Rad Kallestad (Bio-Rad, Hercules, California, USA). For the ImmunoConcepts kit, the Hep-2000 ANA-Ro kit was used. All assays were performed according to the manufacturer's instructions, using secondary antibodies provided. For the IFA analysis, the serum samples were diluted 1:40 with 1× PBS to allow for the determination of whether the samples were positive or negative for ANA antibodies, as instructed by the manufacturer. Further titration of ANA-positive serum samples was not performed at this point of the studies. IFA was performed in one laboratory by two experienced observers, one of whom read kits 1 and 2; the other read kit 3. ANA-positive samples were defined by positive staining of the nucleus; staining of cytoplasm was not considered in this study in view of studies indicating the uncertain reliability of IFA in detecting antibodies to ribosomal P proteins (anti-P), a specificity that can lead to cytoplasmic staining.[15] Only four sera had anti-P antibodies by the multiplex assays. Since these samples were all consistently ANA positive, our consideration of only nuclear staining appears to reasonably capture antibodies to relevant target antigens.
The IFA slides were examined using the EVOS FL Cell Imaging System (Thermo Fisher, Waltham, Massachusetts, USA). An objective lens of ×20 was used, and the light source was an adjustable intensity LED. The samples also underwent ANA assessment by an ANA EIA as well as the BioPlex 2200 ANA Screen (both products of Bio-Rad); these assays were performed at Bio-Rad. The number (%) of samples identified as negative for each kit is shown. For IFA assays, indeterminate samples showed weak or borderline staining and could not be consistently classified as either negative or positive. Assays with modest elevations of anti-dsDNA are reported as indeterminate by the BioPlex 2200.

DISCUSSION

Our findings provide new insights into ANA expression in SLE and indicate differences among ANA assay kits in the detection of ANA reactivity in sera of patients with established disease. These differences are likely related to technical features of the assays which may differ in variables such as conditions for cell fixation, reagents and ambient assay conditions[12–14]; the array of ANAs in patient sera can also impact on detection. In the routine clinical setting, these findings indicate that the serological evaluation of lupus could be misleading depending on the kit used, an issue not well appreciated by clinicians despite reports in the literature.[13]

In general, ANA testing is performed at initial evaluation; if positive, repeat testing is usually not considered necessary since the criterion for classification or diagnosis has been met. A re-evaluation of ANA status could occur if a patient seeks care from a new provider or undergoes screening for a clinical trial.[9 16] Although the relationship between the patients we studied and those in belimumab trials is speculative, the data clearly show that, depending on the kit, ANA negativity can occur in established lupus not infrequently.

The use of certain ANA assays could affect the frequency of screen failures in the trial setting as well as the eventual utilisation of an agent if approved for serologically active patients.[9] Since the ANA assay used for screening is often not specified in protocols, the selection of a kit could lead to as much as a 17% change in the number of screen failures. Correspondingly, for products approved for serologically active SLE, the use of certain assays could determine whether a patient meets criteria for its use.

Often, the identity of the kit used in published studies is not available since it is generally believed that each has similar performance characteristics. In this regard, we have performed another study on 181 patients enrolled in a clinical trial for a new agent of SLE and found a wide variation in levels of ANA negativity using five kits (0.6%–27.6%).[17] Another study has reported that, while samples from patients with SLE with a high titre ANA are consistently detected by different testing laboratories, those with lower titres are more likely to be identified as negative or equivocal. That study also reported significant variation in the detection of staining patterns.[18] In this regard, in the current study, we found that sera with variable detection by IFA had, in general, low values in the multiplex assay and infrequently expressed antibodies to RNA-binding proteins; these findings suggest that consistent ANA detection depends on ANA titre as well as specificity.

Because of the growing use of ANA as a theranostic and the absence of guidance on which kits can be used for this purpose, our findings suggest that clinical trials using ANA assays for

screening should specify the kit used and its performance characteristics, especially with patients with established disease. We further suggest exploration of different assays as theranostics since our studies suggest that patients who are consistently ANA positive may differ immunologically from those whose ANA responses are variably detected; these differences could relate to past or current disease activity as reflected in anti-DNA or complement levels.

Our findings, along with those obtained during the development of belimumab, suggest that ANA status may identify features that are associated with either disease activity or the likelihood of responding to a therapeutic. This situation can create an uncertainty in the selection of the ANA test for use in screening. An assay that produces a low frequency of negativity can reduce screen failures but could allow entry of patients who are immunologically distinct and display less disease activity. Indeed, the use of such a test could be similar to a reliance on historical ANA positivity.

While the serological changes preceding the onset of SLE have been an area of extensive investigation of 'pre-autoimmunity',[19 20] few studies have addressed events after diagnosis and treatment, a phase of disease that can be called 'post-autoimmunity'. The setting of clinical trials may thus reveal an immunological feature of SLE (ie, frequency of seronegativity in established disease) that has been previously underappreciated. Whether this seronegativity reflects a response to prolonged therapy or natural exhaustion of autoimmune clones is unknown but merits further study. Future studies are therefore needed to determine the ANA assays most informative as theranostic biomarkers. Closely related issues are whether ANA positivity should be a criterion for trial entry in subjects with long-standing SLE and, indeed, whether subjects with long-standing seronegative disease should be studied separately.

Contributors DSP, PEL and BHR designed the study. DMS performed the assays and prepared the data for publication. DSP, PEL and BHR reviewed and interpreted the data and wrote the manuscript.

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Competing interests None declared.

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Data sharing statement Data will be available to interested investigators who can submit requests.

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CONCISE REPORT

Similar efficacy, safety and immunogenicity of adalimumab biosimilar BI 695501 and Humira reference product in patients with moderately to severely active rheumatoid arthritis: results from the phase III randomised VOLTAIRE-RA equivalence study

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ABSTRACT

Objective To demonstrate clinical equivalence of adalimumab biosimilar candidate BI 695501 with Humira.

Methods Patients with active rheumatoid arthritis on stable methotrexate were randomised to BI 695501 or Humira in a double-blind, parallel-group, equivalence study. At week 24, patients were rerandomised to continue BI 695501 or Humira, or switch from Humira to BI 695501. The coprimary endpoints were the percentage of patients achieving the American College of Rheumatology 20% response criteria (ACR20) at weeks 12 and 24. Further efficacy and safety endpoints and immunogenicity were assessed up to week 58.

Results 645 patients were randomised. At week 12, 67.0% and 61.1% (90% CI −0.9 to 12.7) of patients receiving BI 695501 (n=324) and Humira (n=321), respectively, achieved ACR20; at week 24 the corresponding values were 69.0% and 64.5% (95% CI −3.4 to 12.5). These differences were within prespecified margins (week 12: 90% CI (−12% to 15%); week 24: 95% CI (−15% to 15%)), demonstrating therapeutic bioequivalence. 593 patients were rerandomised at week 24. Up to week 48, mean change from baseline in Disease Activity Score 28-erythrocyte sedimentation rate and ACR20/ACR50/ACR70 response rates were similar across the switched (n=147), continuous BI 695501 (n=298) and continuous Humira (n=148) groups. Similar immunogenicity (antidrug antibodies (ADAs), ADA titres and neutralising antibodies) was seen between BI 695501 and Humira (to week 24) and across rerandomised groups (to week 48). Safety and tolerability profiles were similar between groups.

Conclusions BI 695501 demonstrated similar efficacy, safety and immunogenicity to Humira; switch from Humira to BI 695501 had no impact on efficacy, safety and immunogenicity.

Trial registration number NCT02137226, Results.

INTRODUCTION

Biosimilars are reproductions of existing biologic molecules that have a high degree of similarity to their reference products, including their molecular structure, biological function and effect in patients, that is, efficacy, safety and immunogenicity. Development programmes for biosimilars are specifically designed to demonstrate similarity to the reference product[1]; they do not assess efficacy and safety profiles versus a current standard of care. These requirements, defined by the Food and Drug Administration (FDA)[1] and European Medicines Agency (EMA),[2] include a phase III clinical trial comparing clinical efficacy and safety of the biosimilar with its reference product in a clinical model that is sensitive to detect any potential clinically meaningful differences between the two versions of the molecule.[1 3]

The wide use of biologics across a number of diseases has led to significant improvements in patients' health. This has come with an increase in healthcare expenditure.[4] However, the advent of biosimilars to infliximab, etanercept and rituximab has introduced more treatment choice[5] and led to cost reductions.

The tumour necrosis factor inhibitor Humira (adalimumab, AbbVie) is an established biologic treatment for a number of immune-mediated inflammatory diseases, including rheumatoid arthritis (RA), psoriatic arthritis and inflammatory bowel disease. A number of biosimilar candidates to Humira are currently in development, including the recently approved BI 695501 (Cyltezo, adalimumab-adbm, Boehringer Ingelheim).[6] Extensive comparison of the physicochemical structure and biologic function of BI 695501 and Humira showed structural similarity and comparable functionality (Sonderegger I, Wittner M, 2018. Manuscripts in preparation). Furthermore, the VOLTAIRE-PK study (NCT02045979) established three-way pharmacokinetic similarity between BI 695501, and European Union (EU)-approved and USA-approved Humira.[6]

The VOLTAIRE-RA trial constituted the final step of the biosimilarity assessment for BI 695501.

METHODS

Study design

VOLTAIRE-RA was a randomised, double-blind, parallel-arm, 58-week equivalence trial of BI 695501 and USA-sourced Humira (NCT02137226; figure 1A) in 14 countries (115 sites). Patients with moderate-to-severe RA on

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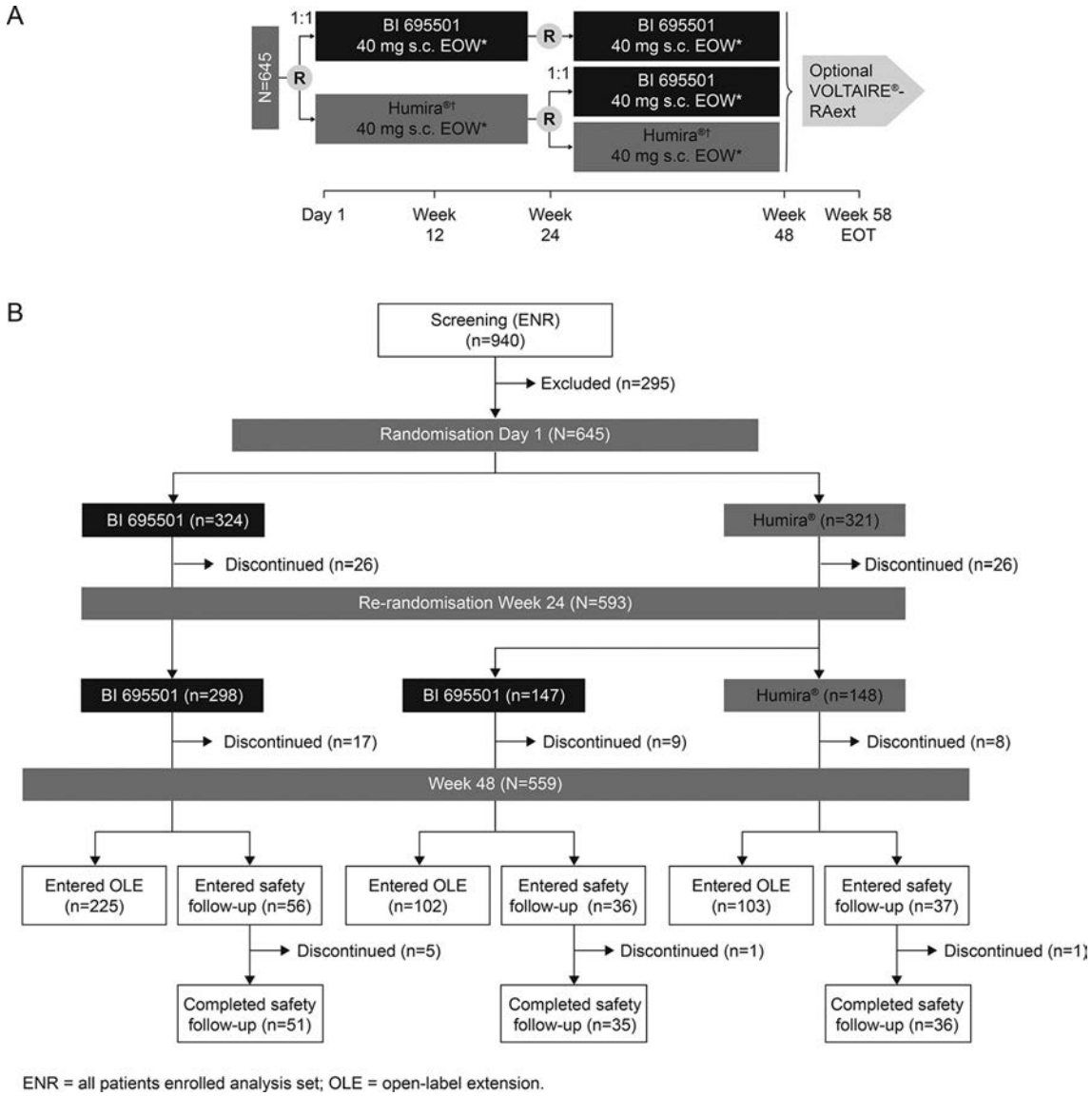

Figure 1 VOLTAIRE-RA study design (A) and patient disposition (B). *Patients continued with methotrexate 15–25 mg/week. Methotrexate 10–14 mg/week was permitted for patients with documented intolerance to higher doses of methotrexate. †Humira 40 mg/0.8 mL solution for subcutaneous injection. EOT, end of treatment; EOW, every other week; n, number of patients per group.

stable methotrexate (MTX) were randomised 1:1 to receive BI 695501 or Humira 40 mg subcutaneously by prefilled syringe once every 2 weeks for 24 weeks by suitably qualified, designated blinded trial personnel either on-site or at the patient's home. First doses of trial medication were administered at the site. Randomisation (via an interactive response technology system; Almac Clinical Technologies, Souderton, Pennsylvania, USA) included stratification according to region (Asia, EU, Latin America, USA) and prior exposure to a biologic agent (yes/no) (see online supplementary appendix A for further details). Patients originally randomised to Humira were rerandomised at week 24 to either continue Humira (continuous Humira) or transition to BI 695501 (Humira to BI 695501). Patients originally randomised to BI 695501 were dummy-rerandomised to continue BI 695501 (continuous BI 695501). Rerandomisation was stratified by prior exposure to a biologic agent only.

At the end of the trial, qualifying patients could enter an open-label extension (OLE; VOLTAIRE-RAext; NCT02640612), where all patients received BI 695501 for ≤48 weeks; otherwise, safety follow-up occurred at week 58. The study was conducted in accordance with the Declaration of Helsinki. All patients provided written informed consent.

Objectives and endpoints

The primary objective of the study was to demonstrate equivalent clinical efficacy of BI 695501 and Humira. Coprimary endpoints were the percentage of patients achieving the American College of Rheumatology 20% response criteria (ACR20) at week 12 (requested by FDA) and week 24 (requested by EMA). Prespecified secondary endpoints were change from baseline in Disease Activity Score in 28 joints (DAS28) using erythrocyte sedimentation rate (ESR) at weeks 12 and 24, and the percentage of patients with drug-related treatment-emergent adverse events (AEs). Further endpoints included ACR-based and DAS-based parameters at various time points, quality of life (36-Item Short-Form Health Survey (SF-36) V.2), AEs (including infections/serious infections, hypersensitivity reactions, drug-induced liver injury, injection site reactions) and immunogenicity (antidrug antibodies (ADAs), neutralising antibodies (nAbs), drug levels).

Patients

Adults (18–80 years) with moderately to severely active RA for ≥6 months, defined by ≥6 swollen joints (66 joint count) and ≥6 tender joints (68 joint count), at screening and baseline, and either ESR >28 mm/hour or C reactive protein (CRP) >1.0 mg/dL at screening, were enrolled. Patients must have received 15–25 mg/week MTX background treatment for ≥12 weeks prior to enrolment. MTX 10–14 mg/week was permitted for patients intolerant to higher doses. Patients could have been on oral corticosteroids ≤10 mg/day prednisolone or equivalent (stable for 4 weeks prior to day 1) and stable non-steroidal anti-inflammatory drugs for 2 weeks prior to day 1.

Exclusion criteria included previous RA treatment with adalimumab or >1 other biologic, active infection, hypersensitivity reactions or AEs to agents similar to the study drugs or their excipients (full criteria available in online supplementary appendix B).

Statistical analyses

For determination of the primary endpoint, non-responder imputation was used for patients who discontinued prior to that time point. For patients who had not discontinued but had missing data, multiple imputation was used. At each time point (weeks 12 and 24), and on each of the complete data sets following the imputation, logistic regression was applied, including fixed, categorical effects of treatment and prior exposure to a biologic agent (yes/no), and continuous effects of baseline DAS28-ESR. The multiple risk differences and CIs on the individual complete data sets were calculated using the Reeve method,[7] and combined using Rubin's rules.[8]

Region was not included in the model due to sparse data in some regions. This was known shortly after final recruitment and included in a protocol amendment prior to database lock.

The primary endpoint, analysed as described above and based on the full analysis set (FAS), was met if the upper and lower CIs of both coprimary endpoints were contained within the prespecified margins. Equivalence was achieved when the difference in ACR20 response rates (BI 695501 minus Humira) was within −12% and 15% (90% CI; week 12 per FDA consultation) and within −15% and 15% (95% CI; week 24 per EMA consultation). An FDA-agreed asymmetrical margin at week 12 was defined, with a slightly higher upper bound of +15% to allow for variations in techniques and response rates used in the calculation of the margins. For this test to be performed with adequate power (86%–91%), a sample size of ~650 patients was needed (FAS). This sample size was based on an assumed treatment difference in ACR20 response rates of 0%, a standard proportion of 59% and an asymmetrical equivalence margin of (−12% to 15%) at week 12, with corresponding values of 0%, 63% (−15% to 15%) at week 24.

The FAS contained all patients who received at least one dose of trial drug and who had all measures required for the efficacy endpoints (ACR20 at weeks 12 and 24) at baseline and at least once postbaseline. The per-protocol analysis set (PPS) contained all patients in the FAS who did not experience any important protocol deviations relevant for efficacy (eg, severe deviation to the restricted disease-modifying anti-rheumatic drug (DMARD) therapy prior to primary endpoint assessment). The safety analysis set (SAF) contained all patients who received at least one dose of trial drug. Descriptive safety data were coded according to MedDRA V.19.0. Data were analysed using SAS software Version 5.0.

Table 1 Baseline demographics and clinical characteristics (SAF)

Characteristics (unit)	BI 695501 (n=324)	Humira (n=321)
Mean age, years (SD)	53.7 (12.0)	53.6 (11.3)
Age, n (%)		
<65 years	264 (81.5)	275 (85.7)
≥65 years	60 (18.5)	46 (14.3)
Women, n (%)	267 (82.4)	269 (83.8)
Mean weight, kg (SD)	73.1 (16.9)	75.1 (17.1)
Mean BMI, kg/m^2 (SD)	27.0 (5.4)	27.9 (6.3)
Race, n (%)		
Asian	8 (2.5)	6 (1.9)
Black or African–American	6 (1.9)	7 (2.2)
White	309 (95.4)	304 (94.7)
Other*	1 (0.3)	4 (1.2)
Ethnicity, n (%)		
Hispanic or Latino	45 (13.9)	44 (13.7)
Not Hispanic or Latino	274 (84.6)	276 (86.0)
Not reported	5 (1.5)	1 (0.3)
Geographical region, n (%)		
Asia	6 (1.9)	6 (1.9)
Europe	231 (71.3)	228 (71.0)
Latin America	25 (7.7)	26 (8.1)
USA	62 (19.1)	61 (19.0)
Mean duration of RA, years (SD)	7.3 (7.2)	7.0 (6.8)
Duration of RA category, n (%)		
<2 years	87 (26.9)	76 (23.7)
≥2 years	234 (72.2)	238 (74.1)
Missing	3 (0.9)	7 (2.2)
Patients with autoantibodies, n (%)		
RF-positive	281 (86.7)	281 (87.5)
Anti-CCP positive	218 (67.3)	237 (73.8)
DAS28 and components		
DAS28-ESR, mean (SD)	6.6 (0.8)	6.6 (0.8)
ESR, mm/hour, mean (SD)	45.5 (19.2)	43.2 (18.0)
HAQ-DI, median (IQR)	1.5 (0.8)	1.5 (0.9)
Swollen joint count, mean (SD)	17.1 (10.4)	15.9 (9.1)
Tender joint count, mean (SD)	25.3 (13.7)	24.9 (13.3)
Prior exposure to a biological† agent, n (%)		
Yes	85 (26.2)	86 (26.8)
No	239 (73.8)	235 (73.2)
Prior cDMARD‡ therapies, mean (SD)	2.2 (1.4)	2.4 (1.5)
Mean MTX dose, mg/week (SD)	16.3 (3.6)	16.8 (3.9)
Patients with ADAs, n (%)		
ADA-positive	11 (3.4)	21 (6.5)
Neutralising ADA-positive	9 (2.8)	16 (5.0)

*Not American Indian or Alaska Native, or Native Hawaiian or Other Pacific Islander.
†Prior biologics included etanercept, tocilizumab, infliximab, certolizumab, rituximab, abatacept and golimumab.
‡Prior cDMARDs included methotrexate, sulfasalazine, leflunomide, chloroquine, hydroxychloroquine and gold.
ADA, antidrug antibody; BMI, body mass index; CCP, cyclic citrullinated peptide; cDMARD, conventional disease-modifying anti-rheumatic drug; DAS28, Disease Activity Score 28-joint count; ESR, erythrocyte sedimentation rate; HAQ-DI, Health Assessment Questionnaire-Disability Index; MTX, methotrexate; n, number of patients per group; RA, rheumatoid arthritis; RF, rheumatoid factor; SAF, safety analysis set.

The secondary efficacy endpoint of change from baseline in DAS28-ESR was assessed via an analysis of covariance model, using multiple imputation method for missing data. ACR20 at

week 48, ACR50 and ACR70 and further efficacy endpoints were computed using the same missing data methodology. Exploratory endpoints were analysed by descriptive statistical methods.

Immunogenicity

Immunogenicity evaluations were performed (SAF) as previously described (overview available in online supplementary appendix C).[6]

RESULTS

Patient disposition and baseline characteristics

The first patient was screened on 4 February 2015. Across 137 centres, 645 patients were randomised (3 March 2015–18 October 2015) 1:1 to BI 695501 (n=324) and Humira (n=321) (SAF). Six patients were excluded from the FAS (lack of postbaseline efficacy assessment); 38 patients were excluded from the PPS (protocol deviations). At week 24, 593 patients were rerandomised to continuous BI 695501 (n=298), continuous Humira (n=148) and Humira to BI 695501 (n=147); 85 (13.2%) patients discontinued the trial prematurely. Last patient, last visit occurred on 18 October 2016. There were no differences in the rate of treatment or trial discontinuation between treatment groups. Patient disposition and geographical distribution are presented in figure 1B and online supplementary table S1, respectively.

Table 2 Primary efficacy endpoint: estimate and CIs for differences in ACR20 response rate at week 12 and week 24 (FAS)

		n	Proportion (%)	Difference in proportions (BI 695501 – Humira, %) Estimate	CI
Week 12	BI 695501	321	67.0	5.9	90% CI (–0.9 to 12.7)
	Humira	318	61.1		
Week 24	BI 695501	321	69.0	4.5	95% CI (–3.4 to 12.5)
	Humira	318	64.5		

ACR20, American College of Rheumatology 20%; FAS, full analysis set.

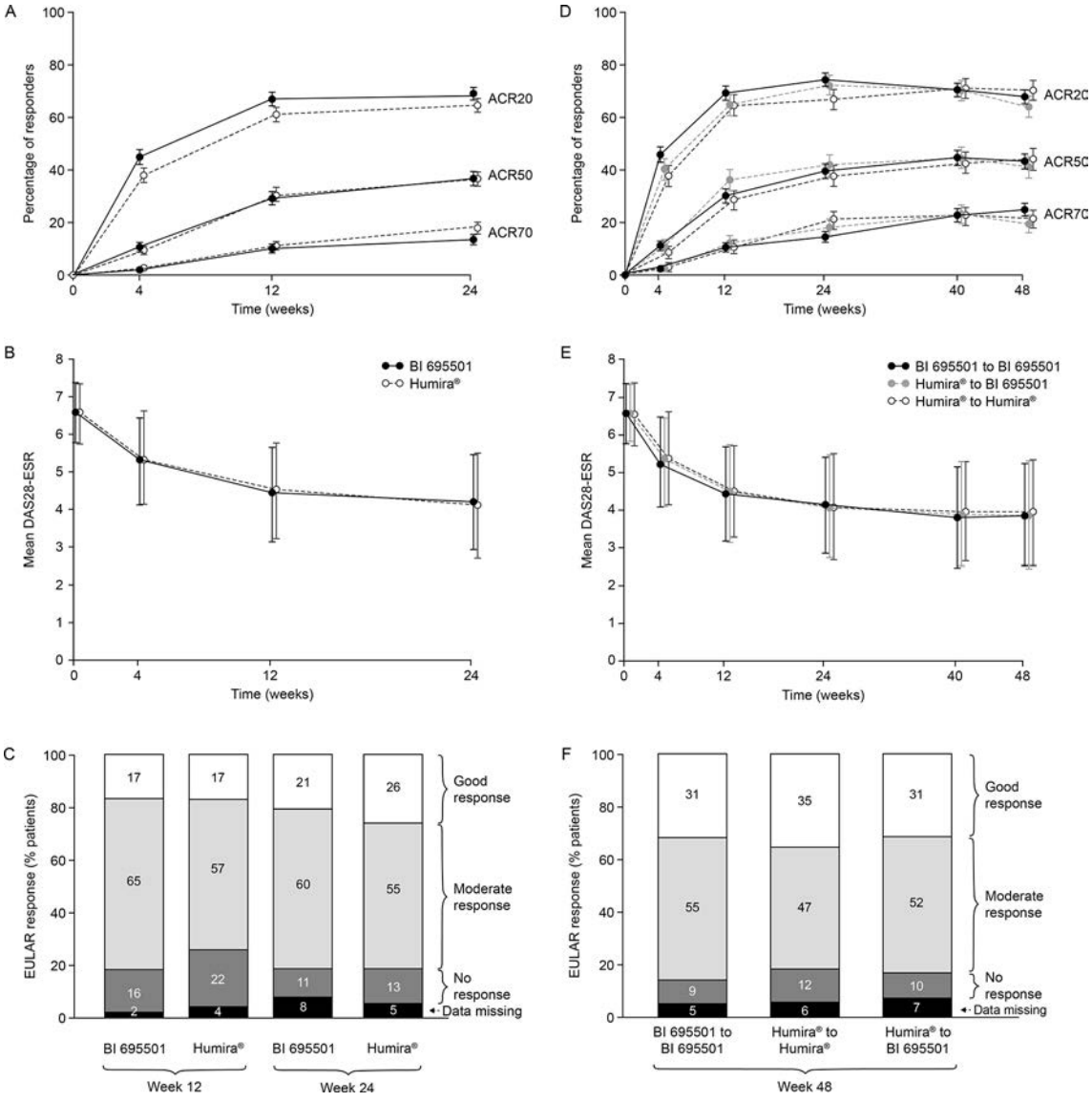

Figure 2 Week 24 results (A–C). Percentage of patients with ACR20/ACR50/ACR70 responses; bars show SEs (A). Mean DAS28-ESR; bars show SDs (B). EULAR responses (C). Week 48 results (D–F). Percentage of patients with ACR20/ACR50/ACR70 responses. Bars show SEs (D). Mean DAS28-ESR; bars show SDs (E). EULAR responses (F). ACR, American College of Rheumatology; DAS28-ESR, Disease Activity Score in 28 joints-erythrocyte sedimentation rate; EULAR, European League Against Rheumatism.

Table 3 Overview of AEs (SAF)

Patients with, n (%)	AEs occurring day 1 to week 58			AEs occurring week 24 to week 58		
	BI 695501 to BI 695501 (n=324)	Humira to BI 695501 (n=146)	Humira to Humira (n=175)	BI 695501 to BI 695501 (n=298)	Humira to BI 695501 (n=146)	Humira to Humira (n=148)
At least one AE	193 (59.6)	93 (63.7)	105 (60.0)	126 (42.3)	62 (42.5)	51 (34.5)
At least one drug-related AE	62 (19.1)	28 (19.2)	40 (22.9)	39 (13.1)	17 (11.6)	17 (11.5)
At least one serious AE	18 (5.6)	10 (6.8)	17 (9.7)	6 (2.0)	6 (4.1)	5 (3.4)
At least one serious drug-related AE	2 (0.6)	1 (0.7)	6 (3.4)	1 (0.3)	0 (0.0)	2 (1.4)
AE leading to study drug discontinuation	13 (4.0)	6 (4.1)	12 (6.9)	5 (1.7)	6 (4.1)	1 (0.7)

AE, treatment-emergent adverse event; n, number of patients per group; SAF, safety analysis set.

Baseline demographics and clinical characteristics were balanced between treatment groups (table 1).

Efficacy

Results at week 24

Both coprimary endpoints met the predefined criteria, demonstrating therapeutic equivalence of BI 695501 and Humira at weeks 12 and 24 (table 2). The difference in the proportion of patients achieving an ACR20 response was within the prespecified interval at week 12 (90% CI −0.9 to 12.7) and week 24 (95% CI −3.4 to 12.5). Primary and sensitivity analyses of the coprimary endpoints are presented in online supplementary figure S1. A post-hoc analysis to determine relative risk is presented in online supplementary table S2.

As a sensitivity analysis, the primary efficacy analysis was repeated on the PPS (same imputation methodology; online supplementary figure S1). The similarity of ACR20 responses in the two groups at weeks 12 and 24 was independent of baseline demographic and clinical characteristics (online supplementary figure S2). The analysis of the secondary efficacy endpoints supported the findings of the primary efficacy analysis. The mean percentage of patients meeting the ACR20/50/70 response criteria was similar in each treatment group at weeks 12 and 24 (figure 2A). The mean change from baseline in DAS28-ESR was similar between the two treatment groups at weeks 12 and 24 (online supplementary table S3; figure 2B).

The percentage of patients with European League Against Rheumatism (EULAR)-defined 'good' and 'moderate' response rates was similar at weeks 12, 24 and 48 (figure 2C; ACR/EULAR Boolean definition[9] of remission available in online supplementary table S4). Both treatment groups showed a similar increase in the SF-36 physical and mental component scores (weeks 12 and 24), indicating a similar improvement in quality of life (online supplementary figure S3).

Results at week 48

Results from baseline to week 48 are presented as per the three treatment groups generated at rerandomisation (week 24). ACR20/50/70 response rates and mean change from baseline in DAS28-ESR were similar across the switched and the continuous groups (figure 2D,E). Similar percentages of patients had 'good' and 'moderate' EULAR response rates at week 48 in each group (figure 2F).

Safety

Safety follow-up was to week 58 for all patients who did not enter the OLE. The proportion of patients with drug-related AEs was similar between the treatment groups. Overall, safety findings were similar between the continuous BI 695501 and Humira arms from day 1 to week 58, and between the rerandomised groups from week 24 to 58 (table 3). The frequency of AEs with an incidence of ≥3% to week 58 is presented in online supplementary table S5. Among serious AEs, infections and infestations was the most common system organ class (0.6% for BI 695501 vs 4.0% for Humira). No deaths were reported during the study.

The most frequently reported AEs leading to drug discontinuation were acute pyelonephritis (n=2) and urticaria (n=2) (both in the Humira group only). Up to week 24, serious infections were pneumonia (n=4), acute pyelonephritis (n=2), and appendicitis, infective arthritis and bronchitis (each, n=1) (Humira group only). Cellulitis was reported for one patient (BI 695501 group). From week 24 to week 58, serious infections were pneumonia in one patient (continuous Humira group), and influenza, viral pneumonia and salmonella sepsis in one patient (Humira to BI 695501 group).

Immunogenicity

Immunogenicity data were available at week 24 (SAF population; 92.2%; n=595/645) and week 48 (87.8% of total randomised; n=566). At baseline, 32 (5.0%) patients had ADAs against adalimumab (BI 695501 group, n=11; Humira group, n=21). In 25/32 patients these ADAs were neutralising (9 BI 695501; 16 Humira).

Overall 50.2% of the patients were ADA-positive at any time point up to week 24. The ADA frequencies up to week 24 were similar in the BI 695501 (47.4%) and in the Humira groups (53.0%) (figure 3A). ADA titres at week 24 (figure 3B) and nAb frequencies up to week 24 (figure 3A) were also similar between the groups. Whether or not patients transitioned from Humira to BI 695501 or continued on Humira did not influence subsequent ADA frequency and titres. Similar immunogenicity was observed after week 24 in all rerandomised groups (ADA-positive patients at any time point up to week 48, figure 3D; nAb-positive patients at any time point up to week 48, figure 3D; ADA titres at week 48, figure 3E).

The impact of ADA on drug plasma levels at weeks 24 and 48 is shown in figure 3C,F. A lower drug concentration was measured in ADA-positive patients compared with ADA-negative patients. This effect was independent of treatment group. Overall, in a post-hoc exploratory analysis, ADA-positive patients had a numerically slightly lower median change from baseline in DAS28-CRP at week 48 than ADA-negative patients (week 48: ADA-positive −2.25, ADA-negative −2.52).

DISCUSSION

The efficacy, safety and immunogenicity results of this study demonstrate clinical equivalence of BI 695501 and Humira. In

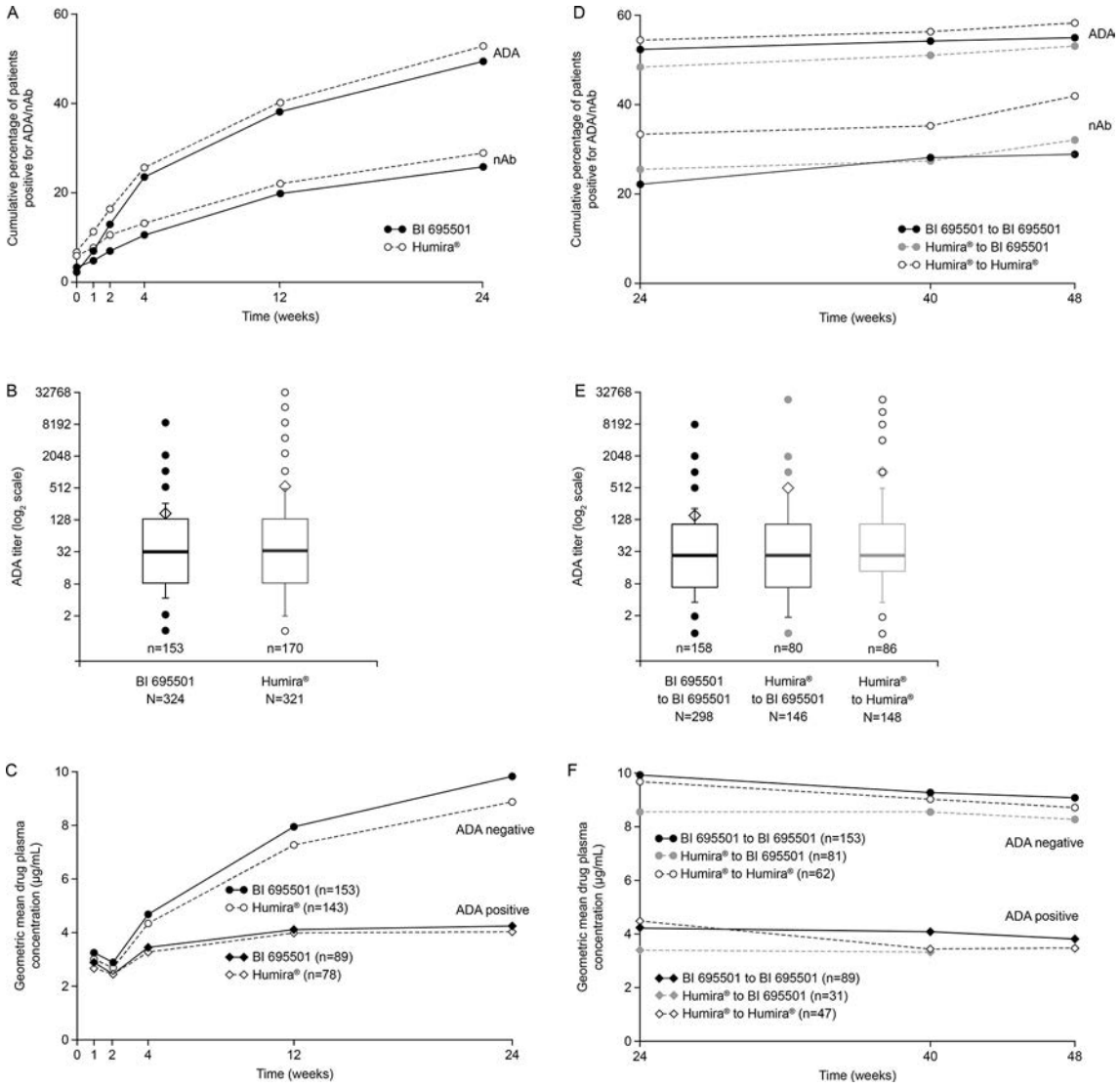

Figure 3 Week 24 results (A–C). Percentage of patients with positive ADA/nAb test (A). ADA titre (B). Drug plasma concentration by presence of ADAs (C). Week 48 results (D–F). Percentage of patients with positive ADA/nAb test (D). ADA titre (E). Drug plasma concentration by presence of ADAs (F). ADA, antidrug antibodies; n, number of patients per group; nAb, neutralising antibodies.

combination with the phase I VOLTAIRE-PK study data[6] plus prior physicochemical and functional analyses (manuscripts in preparation), VOLTAIRE-RA completes the similarity assessment of the adalimumab biosimilar BI 695501.

Selection of study design and endpoints

A biosimilar phase III study needs to be designed to optimise the chance of detecting potential clinical differences between the biosimilar candidate and reference product.[1 2] A suitable clinical model is characterised by a combination of certain factors, including a disease and a population that will respond to the treatment with a large effect size and endpoints that are sensitive to measure those responses. A homogeneous population increases the sensitivity to detect differences because of the reduction of interindividual variability. The clinical model is usually selected in consultation with regulatory agencies.

Adalimumab is a standard of care in a wide range of autoimmune diseases and is commonly used in adults and children. Phase III studies for other adalimumab biosimilar candidate molecules have been completed or are ongoing in adult patients with RA (eg, with SB5,[10] FKB327[11] and PF-06410293[12]) or plaque psoriasis (including with MYL-1401A,[13] CHS-1420,[14] M923[15] and MSB11022[16]) or both (ABP 501[17 18] and GP2017[19 20]). RA was selected for the main phase III study with BI 695501 due to its sensitivity to adalimumab, the availability of ACR20 as a well-established and sensitive measure of disease activity reduction, and operational feasibility. Supportive phase III studies are ongoing with BI 695501 in plaque psoriasis (NCT02850965) and Crohn's disease (NCT02871635).

Switching from reference product Humira to BI 695501

When switching from the reference product to a biosimilar for which clinical similarity has been established (as presented here for Humira to BI 695501), one would not expect a change in efficacy or safety, although natural fluctuation of disease activity in individual patients may occur. It is important that such treatment switches are studied and understood as this may become common practice within routine care (eg, many patients transitioned from Remicade to its biosimilars as they became available).

Here, patients who had been treated with Humira (baseline–24 weeks) were randomised to either continue Humira or switch to BI 695501 (weeks 25–48). No differences were

detected between these two groups with regard to adherence to treatment, efficacy, safety and immunogenicity. Future, observational studies could help confirm these findings in the real-world setting. Since biosimilars to Remicade have become available, a number of observational and interventional studies (such as the NOR-SWITCH study)[21] have confirmed the findings of the development programme of CT-P13 (Remsima, Inflectra), suggesting that developing biosimilars in a programme that relies on analytical, preclinical and limited clinical studies is a robust concept.

Overall assessment of efficacy and safety

The efficacy data from VOLTAIRE-RA indicate that BI 695501 and Humira have therapeutic equivalence at week 12 and week 24. Up to week 48, the mean change from baseline in DAS28-ESR and ACR20/50/70 response rates was similar across the switched and continuous groups. BI 695501 and Humira demonstrated similar safety and tolerability; there were no new safety findings for adalimumab. The frequency of hypersensitivity or injection site reactions was similarly frequent in all treatment groups.

Overall assessment of immunogenicity

Immunogenicity is a key aspect of the clinical similarity evaluation of a biosimilar agent. Therefore, a highly sensitive and drug-tolerant ADA assay was developed and applied during clinical development of BI 695501. Different and often less sensitive ADA and nAb assays were used in historical trials (eg, pivotal trials with Humira[22]). This can explain previous reports of different frequencies of ADA-positive and nAb-positive patients detected in earlier studies. Overall, similar immunogenicity (ADA frequency and titres, and nAb frequency) was observed between BI 695501 and Humira throughout this study. Patients switching from Humira to BI 695501 did not demonstrate increased immunogenicity or more hypersensitivity reactions compared with patients continuing to receive Humira.

As expected, an inverse correlation between ADAs and drug plasma concentration was detected. This effect was similar between the BI 695501 and Humira at week 24 and between the three study groups at week 48. This confirms previous data from the VOLTAIRE-PK study[6] showing a comparable impact of ADA on key pharmacokinetic parameters for BI 695501, and USA-approved and EU-approved Humira. An explanation of the pre-existence of antibodies in otherwise drug-naïve subjects is given in online supplementary appendix D.

Adalimumab biosimilar landscape

The introduction of adalimumab was a major step forward for patients suffering from certain chronic immune-mediated diseases. Its benefits for patients, along with the prevalence of its indications, led to healthcare system costs exceeding US$15 billion (2016). It is therefore unsurprising that several companies are developing biosimilars to Humira. Currently, Amgen's ABP 501 (Amgevita/Amjevita) is FDA-approved and EMA-approved.[22 23] Clinical study results are typically similar for different biosimilar candidates due to comparable study designs (regulator agency requirement) and inclusion of AbbVie's reference product Humira as the common comparator.

CONCLUSION

VOLTAIRE-RA showed that BI 695501 and Humira are highly similar in terms of efficacy, safety and immunogenicity. The switch from Humira to BI 695501 had no impact on efficacy, safety and immunogenicity. These data, together with the analytical and the phase I data, suggest that BI 695501 and Humira are biosimilar and thus therapeutically equivalent.

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Contributors SBC and DA were involved in the acquisition, analysis or interpretation of the data. All authors were involved in the critical revision of the manuscript, approval to submit and in agreement to be accountable.

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Competing interests SBC and ECL received funding from Boehringer Ingelheim, study sponsor, as principal investigators of this study. AA-R and PAK have no competing interests to declare. NP, IS and DA are (or were) employees of Boehringer Ingelheim, study sponsor.

Patient consent Obtained.

Ethics approval The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the institutional review boards of all participating centres. All patients provided written informed consent.

Provenance and peer review Not commissioned; externally peer reviewed.

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CONCISE REPORT

'Deep Koebner' phenomenon of the flexor tendon-associated accessory pulleys as a novel factor in tenosynovitis and dactylitis in psoriatic arthritis

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ABSTRACT

Objectives Skin and joint involvement in psoriasis (PsO) and psoriatic arthritis (PsA) are thought to relate to the so-called Koebner response. Given that dactylitis is non-randomly distributed in the digits, this study tested the hypothesis that the accessory pulleys linked to the flexor tendons were thickened in PsA and thus exhibited koebnerisation.

Methods Ninety-six subjects (27 PsA, 27 rheumatoid arthritis (RA), 23 PsO and 19 healthy controls (HCs)) were enrolled. The A1, A2 and A4 pulley thickness was measured using a high-resolution probe (22 MHz). All patients were in remission or low disease activity with current dactylitis being excluded.

Results Within 864 pulleys investigated, patients with PsA had thicker pulleys in every digit compared with both RA (P<0.001 and P=0.003) and HCs (P<0.001). RA and PsO groups had some pulleys in some digits thicker than HCs whereas some others were comparable. The second digit A1 pulley thickness was higher in patients with PsA with previous dactylitis (P=0.020). More pulleys were thickened in the PsA group (165/243, 68%) than RA (41/243, 17%; P<0.001) and HCs (13/171, 7.6%; P<0.001).

Conclusions In established PsA, the accessory pulleys are thickened compared with RA, PsO or HCs and especially in subjects with a history of dactylitis. These findings implicate the involvement of pulleys in PsA-related tenosynovitis and dactylitis supporting the idea of deep koebnerisation in dactylitis and sites of high physical stress.

INTRODUCTION

Dactylitis is a hallmark of psoriatic arthritis (PsA) occurring in around 40% of cases at some point in the disease course.[1] The original MRI studies of dactylitis suggested that the main cause of this pathology was flexor tenosynovitis in addition to soft-tissue oedema, joint synovitis and osteitis.[2 3] It has been suggested that enthesitis is the primary lesion in spondyloarthritis (SpA) including PsA.[4] In support for a role for the enthesis in dactylitis pathogenesis, high-resolution MRI studies showed that enthesitis was common in distal interphalangeal joints PsA involvement pointing towards a link between small joint swelling and dactylitis.[5] Also, recent MRI studies showed entheseal changes in the digital pulleys at flexor tendons in dactylitis.[6]

The digital pulleys have constituent fibrocartilage and have the role of tracking the tendon accurately maintaining the apposition of tendon and bone across the joint to restrict 'bowstringing' of tendon during flexion.[6] Ultrasonography (US) has increasingly being used for its ability and sensitivity to visualise soft tissues in the early stages of inflammatory arthritis.[7 8] The A1 pulley can be visualised using high-frequency US,[9] a structure that was demonstrated to be abnormal in PsA dactylitis.[6] Also, the pulleys are frequently thickened as determined by US imaging of trigger fingers.[10 11]

It has long been considered that disease expression in PsA might relate to abnormal responses to physical stress with Koebner response in the skin and 'deep Koebner' response in the joints.[12] The small digital pulleys are sites of high physical stress. Therefore, we investigated the digital pulleys in PsA including cases with previous dactylitis and compared this with rheumatoid arthritis (RA) (where flexor tenosynovitis is also common), psoriasis (PsO) subjects[13] and healthy controls (HCs). Since our pilot work on the reliability of scanning the pulleys had shown a better agreement for A1, A2 and A4 pulleys, we only focused to those for this study.[14]

METHODS

Following local ethical committee approval, written informed consent was obtained from all participants. Consecutive patients with RA (n=27) fulfilling the American College of Rheumatology/European League Against Rheumatism criteria,[15] patients with PsA according to CASPAR[16] criteria (n=27), PsO without arthritis (n=23) and HCs without any inflammatory rheumatological disease (n=19) were enrolled from two rheumatology and dermatology centres (Verona and Reggio Emilia, Italy).

The inclusion criterion for PsA and RA groups was a history of hand involvement; however, only patients with PsA with minimal disease activity[17] for at least 6 months prior to US examination and patients with RA with low disease activity[18] were recruited. Patients on biologics, with present active dactylitis, chronic renal failure and diabetes mellitus, were excluded.

Clinical assessments were done by a rheumatologist in each centre (AM, DC). All US was performed blinded to the clinical assessment and diagnosis, by the same experienced investigators all the time

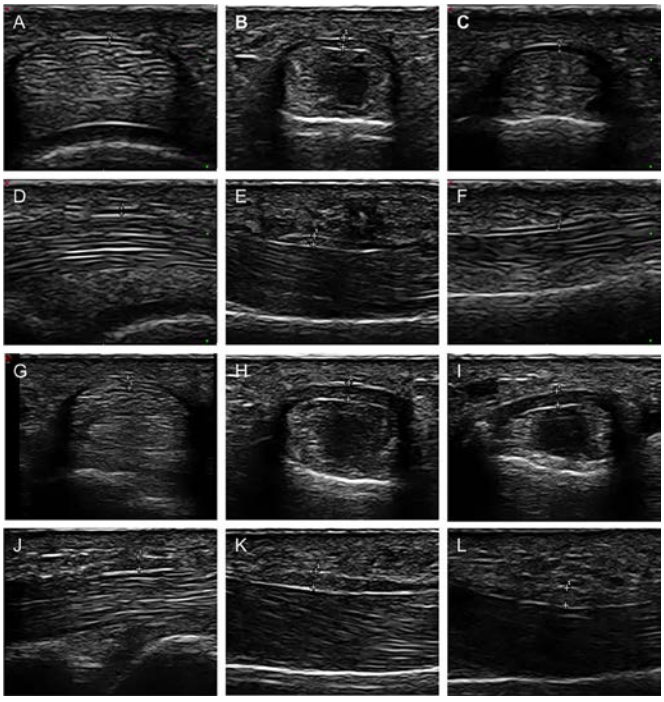

Figure 1 Transverse (A, B and C) and longitudinal (D, E and F) views of A1, A2 and A4 pulleys, respectively, in a healthy control. The thickened pulleys can be seen in transverse (G, H and I) and longitudinal (J, K, L) views, in the same order of pulleys, in a patient with psoriatic arthritis.

Table 1 Demographic and clinical characteristics of the different groups of patients

	PsA n=27	RA n=27	PsO n=23	HCs n=19
Sex distribution (M/F)	16/11	7/20	14/9	7/12
Age, mean (SD)	56.8 (10)	58.3 (11)	56.6 (9)	55.7 (10)
BMI (SD), kg/m^2	26.9 (3.3)	26.3 (3.3)	25.9 (3.2)	26.4 (3.6)
Employment (n, %)				
Manual	6 (22.2)	4 (14.8)	6 (26.1)	4 (21)
Intellectual	11 (40.7)	12 (44.4)	9 (39.1)	8 (42.1)
Unemployed	10 (37)	11 (40.7)	8 (34.8)	7 (36.8)
Duration of arthritis symptoms, years (mean (SD))	5.6 (4.1)	6.8 (5.1)	–	–
Duration of psoriasis, years (mean (SD))	10.3 (10.1)	–	13.4 (10.7)	–
Previous hand dactylitis, n (%)	14 (51.8)	0	–	–
Trigger finger, n (%)	5 (18.5)	3 (11.1)	0	0
Nail involvement, n (%)	11 (40.7)	–	2 (8.7)	–
Scalp psoriasis, n (%)	18 (66.7)	–	13 (56.5)	–
Palmoplantar psoriasis, n (%)	10 (37)	–	7 (30.4)	–
Swollen joint count ≤1, n (%)	3 (11.1)	1 (3.7)	–	–
Tender joint count ≤1, n (%)	4 (14.8)	4 (14.8)	–	–
Increased CRP, n (%)	1 (3.7)	1 (3.7)	–	–
DAS-28 CRP, mean (SD)	2.42 (0.48)	2.48 (0.5)	–	–
RA test positive, n (%)	0	20 (74.1)	–	–
APCA positive, n (%)	0	19 (70.4)	–	–

APCA, anticitrullinated peptide antibody; BMI, body mass index; CRP, C reactive protein; DAS-28, disease activity score on 28 joints; HC, healthy control; PsA, psoriatic arthritis; PsO, psoriasis; RA, rheumatoid arthritis.

(IT, PM) using a MyLabClassC (Esaote, Genova, Italy) equipped with a 10–22 MHz linear transducer using a pre-agreed scanning protocol with reliability testing (details in online supplementary text).

The dominant-hand second to fourth digit volar aspects were imaged in longitudinal and transverse planes. Two measurements (transverse and longitudinal) were done for the A1 pulley at the metacarpophalangeal joint level. The border between the inner rim of the A1 pulley and the flexor tendon was defined by dynamic examination. The A2 and A4 pulley thicknesses were assessed in full extension, in correspondence of the proximal and middle phalanx in both transverse and longitudinal planes measuring them from their thickest site (figure 1A–F).

Statistical analysis

The intraclass correlation coefficient values between longitudinal and transverse measurements were analysed. To compare the measurements between the two groups with a continuous scale, the independent two-sample Student t-test or the Mann-Whitney U test were used and dichotomous data were compared using the χ^2 test or Fisher exact test, as appropriate. The upper normal value for the thickness of the pulleys was defined as 2 SD above the measurements obtained from HCs. The number of pulleys with increased thickness was compared for patients with PsA and RA. The following possible explanatory variables were entered in a multiple regression analysis with the number of pulleys with increased thickness per patient as the dependent variable: age, sex, body mass index (BMI), duration of disease, clinical diagnosis and manual work. The study was explorative in nature and a power calculation was not performed. Statistical analysis was performed using SPSS V.22 (SPSS, Chicago, Illinois, USA).

RESULTS

The patient characteristics are given in table 1. Patients and HCs were matched for BMI and age. The patients with PsA and PsO were predominantly men and patients with RA and HCs were predominantly women (table 1).

The pulleys were identifiable in both planes in all patients and all HCs. A total of 1732 measurements were done in 864 pulleys. The intraclass correlation coefficient values between longitudinal and transverse measurements showed excellent agreement (0.82). As the longitudinal scans permitted detection of the thickest site displaying the whole length of the pulley, the longitudinal scan results are given below (table 2). The transverse measures are also shown in online supplementary table 1.

Patients with PsA had thicker pulleys in every digit compared with RA and HCs (figure 1G–L). Patients with RA had thicker pulleys than HCs, especially in the A1 pulley, but A2 pulley of the second digit and A4 pulley of the fourth digit were not different. Patients with PsO had thicker pulleys than HCs but not as thick as patients with PsA. In particular, patients with PsA had thicker A1 and A4 pulleys than PsO, whereas A2 pulleys were not statistically different in every digit. More pulleys were thickened in the PsA group (165/243, 68%) than RA (41/243, 17%; P<0.001) (online supplementary table 2). The multiple regression analysis entering age, sex, BMI, duration of disease, diagnosis and manual work demonstrated that only the diagnosis of PsA was associated with the number of thickened pulleys (OR 4.8 (95% CI 3.3 to 6.3); P=0.001).

In order to understand if previous episodes of dactylitis could modify pulley anatomy and increase their thickness, we performed a subanalysis by comparing patients PsA with or without a dactylitis history. Only the second-digit A1 pulley was found thicker in patients who had previous dactylitis. The mean thickness of PsA pulleys still remained significantly higher compared with RA when patients with PsA with previous

Table 2 Pulley thickness in different groups of patients and comparisons

Digit/pulley	PsA n=27	RA n=27	PsO n=23	HCs n=19	PsA vs RA P value	PsA vs PsO P value	RA vs HCs P value	PsA vs HCs P value	PsO vs HCs P value
II/A1 longitudinal	0.62±0.18	0.44±0.16	0.39±0.10	0.32±0.09	0.0001	0.012	0.004	0.0001	0.012
II/A2 longitudinal	0.57±0.16	0.40±0.10	0.38±0.07	0.34±0.12	0.003	0.022	0.458	0.0001	0.022
II/A4 longitudinal	0.48±0.14	0.34±0.08	0.29±0.06	0.27±0.10	0.0001	0.052	0.017	0.0001	0.052
III/A1 longitudinal	0.68±0.21	0.42±0.09	0.39±0.11	0.31±0.09	0.0001	0.075	0.0001	0.0001	0.075
III/A2 longitudinal	0.58±0.17	0.41±0.11	0.34±0.13	0.31±0.10	0.0001	0.312	0.004	0.0001	0.312
III/A4 longitudinal	0.47±0.13	0.36±0.08	0.32±0.06	0.27±0.11	0.001	0.016	0.010	0.0001	0.016
IV/A1 longitudinal	0.64±0.18	0.40±0.11	0.41±0.13	0.29±0.11	0.0001	0.372	0.002	0.0001	0.372
IV/A2 longitudinal	0.53±0.14	0.36±0.09	0.34±0.08	0.29±0.12	0.0001	0.109	0.047	0.0001	0.109
IV/A4 longitudinal	0.46±0.13	0.31±0.07	0.30±0.06	0.27±0.11	0.0001	0.028	0.870	0.0001	0.028

Values are expressed in millimetres (mean±SD).
HC, healthy control; PsA, psoriatic arthritis; PsO, psoriasis; RA, rheumatoid arthritis.

dactylitis were excluded, except for A1 pulley of the second finger (online supplementary table 3). Patients with trigger finger did not have more frequent dactylitis in the past and the thicknesses in any of the pulleys were not higher than those of patients without trigger finger (data not given).

DISCUSSION

Given the emerging link between the flexor tendon pulleys in dactylitis in PsA, we hypothesised that these structures may be abnormally thickened in PsA cases compared with RA, PsO and HCs and thus may exhibit deep Koebner response. The findings herein confirmed that the pulleys were indeed thickened in subjects with PsA compared with RA. The thicker pulleys in RA compared with HCs might point towards a non-specific effect of a chronic autoimmune tenosynovitis on the adjacent pulleys. However, the greater magnitude of pulley thickening in PsA, especially in the setting of dactylitis, suggests an intrinsic pathology in the pulley contributing to PsA-related tenosynovitis.

Skin injury in PsO is associated with epidermal hyperplasia and thickening. Likewise, the skeletal phenotype in PsA and SpA is often associated with excessive repair responses that manifest not as epidermal hyperplasia but as postinflammation skeletal hyperplasia. Also, subjects with PsO, but without clinical arthritis, often exhibit thickening of non-diseased large entheses. Given that the accessory pulleys are a type of mini-entheses network that anchor the tendons to the bone and are subjected to very high physical stress, we tested the hypothesis that these likewise exhibit 'deep Koebner' phenomena and are thickened. Indeed, these findings suggest that such a mechanism is possible given the frequency of thickening of these structures in PsA. It will be important to show that such changes are present in early PsA where their presence would support the idea of early biomechanical alterations in tendon function that could contribute to dactylitic tenosynovitis.

This study had a number of limitations. First, we scanned three digits of the dominant hand. Including patients with PsA with hand involvement may not be representative of all PsA population, although hands are frequently involved in peripheral disease. Studies in early PsA to ascertain whether these pulley changes trigger PsA tenosynovitis or whether they represent a reactive process are needed. We defined the cut-off for the thickness of a pulley as 2 SD above the measurements in HCs; this is not a validated measure as there are no data published on it and need replication. In addition, our sample size was not very large, yet we were still able to demonstrate differences among groups. Despite these limitations, our study suggests a high frequency of micro-entheseal changes in PsA pulleys.

In conclusion, pulleys are involved in PsA, even in patients who are in minimal disease activity suggesting a finding of tissue damage rather than inflammation. The difference between PsA and RA suggests that pulley involvement is not purely dependent on tenosynovitis. The difference between PsO and HCs may point to pulley involvement being an early finding, as previously shown with enthesitis.

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EXTENDED REPORT

Comorbid TNF-mediated heart valve disease and chronic polyarthritis share common mesenchymal cell-mediated aetiopathogenesis

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ABSTRACT

Objectives Patients with rheumatoid arthritis and spondyloarthritisshow higher mortality rates, mainly caused by cardiac comorbidities. The TghuTNF (Tg197) arthritis model develops tumour necrosis factor (TNF)-driven and mesenchymalsynovial fibroblast (SF)-dependent polyarthritis. Here, we investigate whether this model develops, similarly to human patients, comorbid heart pathology and explore cellular and molecular mechanisms linking arthritis to cardiac comorbidities.

Methods Histopathological analysis and echocardiographic evaluation of cardiac function were performed in the Tg197 model. Valve interstitial cells (VICs) were targeted by mice carrying the *ColVI-Cre*transgene. Tg197 *ColVI-Cre Tnfr1*$^{fl/fl}$ and Tg197 *ColVI-Cre Tnfr1*$^{cneo/cneo}$ mutant mice were used to explore the role of mesenchymal TNF signalling in the development of heart valve disease. Pathogenic VICs and SFs were further analysed by comparative RNA-sequencing analysis.

Results Tg197 mice develop left-sided heart valve disease, characterised by valvular fibrosis with minimal signs of inflammation. Thickened valve areas consist almost entirely of hyperproliferative *ColVI*-expressing mesenchymal VICs. Development of pathology results in valve stenosis and left ventricular dysfunction, accompanied by arrhythmic episodes and, occasionally, valvular regurgitation. TNF dependency of the pathology was indicated by disease modulation following pharmacological inhibition or mesenchymal-specific genetic ablation or activation of TNF/TNFR1 signalling. Tg197-derived VICs exhibited an activated phenotype *ex vivo*, reminiscent of the activated pathogenic phenotype of Tg197-derived SFs. Significant functional similarities between SFs and VICs were revealed by RNA-seq analysis, demonstrating common cellular mechanisms underlying TNF-mediated arthritides and cardiac comorbidities.

Conclusions Comorbidheart valve disease and chronic polyarthritis are efficiently modelled in the Tg197 arthritis model and share common TNF/TNFR1-mediated, mesenchymal cell-specific aetiopathogenic mechanisms.

INTRODUCTION

Chronic inflammatory joint diseases are associated with articular inflammation leading to joint damage and increased mortality rates, which are mainly attributed to cardiovascular comorbidities.[1 2] Cardiac disease manifestations are detected in 70%–80% of patients with rheumatoid arthritis (RA) and spondyloarthritis (SpA) and symptoms can vary greatly, including arrhythmias, ischaemic heart failure as well as valvular diseases, such as valve insufficiency and stenosis.[3 4] The mechanisms mediating the co-occurrence of cardiac comorbidities in patients with chronic inflammatory joint diseases remain unknown.

The critical role of tumour necrosis factor (TNF) in RA and SpA pathologies is now well established both in transgenic animal models[5–8] and by the highly positive clinical responses of human patients to anti-TNF therapies.[9 10] Interestingly, recent studies in mice with deregulated TNF expression indicated a pivotal role of TNF also in cardiovascular diseases.[11–15] It, therefore, appears that TNF may commonly underlie arthritis and arthritis-related cardiac manifestations in human patients, which could also explain the amelioration of both of these comorbidities in patients treated with anti-TNF biologics.[16]

Mesenchymal cells are active participants in the structure and function of almost all tissues and contribute to their homeostasis.[17] The mesenchymal cells of the joint are the synovial fibroblasts (SFs).[18 19] Several studies have implicated SFs as key pathogenic cells, capable of initiating and driving the development of joint pathologies both in mouse models and human patients.[8 20 21] Interestingly, using TNF-driven models of comorbid arthritis and inflammatory bowel disease (IBD), we have previously established that TNF signals, uniquely operating in SFs or intestinal mesenchymal cells (IMCs), are sufficient to orchestrate the full pathogenic process of these two complex pathologies.[8] Yet another mesenchymal cell type, which is known to form the heart valves and is responsible for the maintenance of valve extracellular matrix structures, is the valve interstitial cells (VICs).[22–24] Recent *in vitro* studies have suggested that TNF can activate quiescent VICs into myofibroblasts inducing their pathogenic contribution to heart valve diseases (HVDs).[23 25] We have therefore hypothesised that the huTNF-driven, Tg197 model of arthritis[5] may exhibit heart valve pathology and that a common mesenchymal cell-specific TNF-mediated mechanism, operating on VICs, could explain the comorbidity.

We show here that Tg197 mice develop spontaneous left-sided heart valve pathology, characterised by extensive fibrosis and thickening of the aortic valve (AV) and mitral valve (MV) and associated with activated and hyperproliferating VICs. Valvular stenosis was associated with deterioration of cardiac function due to valvular degeneration and left ventricular (LV) dysfunction, simulating comorbid valvular diseases detected in patients with RA/SpA. Moreover, we show that this cardiac phenotype is ameliorated on Ab-mediated inhibition of TNF or by genetic mesenchymal-specific ablation of TNFR1. We further demonstrate that Tg197 VICs cultured *ex vivo* exhibit an activated phenotype characterised by increased huTNF production as well as increased proliferative and migratory capacities, similar to the one exhibited by arthritogenic SFs. Comparison of RNA-sequencing profiles between Tg197-derived SFs and VICs revealed similar pathogenic genes and pathways being activated in the two cell types. Overall, our studies establish a common TNF-driven mesenchymal cell-specific mechanism that may underlie aetiopathogenesis of comorbid joint and HVDs also in human patients.

MATERIALS AND METHODS

Mice

Tg197,[5] *ColVI-Cre*,[8] *Tnfr1*$^{fl/fl}$[26] and *Tnfr1*$^{cneo/cneo}$[27] mice were previously described; *Rosa26*$^{mT/mG}$ mice[28] were purchased from the Jackson Laboratories. Mice were maintained on a C57BL/6J or C57BL/6×CBA genetic background in the animal facilities of Biomedical Sciences Research Center (BSRC) 'Alexander Fleming' under SPF conditions. All animals were sacrificed at 11–12 weeks of age. Further details can be seen online in supplementary methods.

Antibodies

Antibodies used for immunohistochemistry, fluorescence-activated cell sorting (FACS) and immunofluorescence can be found in online supplementary methods.

Immunohistochemistry and immunofluorescence

Paraffin-embedded tissue sections and heart transverse optimal cutting temperature (OCT) cryosections were stained and evaluated according to protocols found in online supplementary methods.

Echocardiography and ECG

Echocardiography assessment and ECG were performed in the Department of Pharmacology, Medical School NKUA, Greece. Further details can be seen in online supplementary methods.

Isolation and culturing of SFs and VICs

SFs and VICs were isolated and cultured up to the third or fourth passage when they were used for cellular assays and sequencing. Detailed protocol is described in online supplementary methods.

FACS

See details in online supplementary methods.

Proliferation assay

To determine cellular proliferation, the Cell Proliferation ELISA, BrdU kit (Sigma-Aldrich) was used.

ELISA

Detection of hTNF was performed using the hTNF Quantikine Elisa (R&D Systems).

Wound-healing assay

To determine the migratory capacity of the cells, we used the wound-healing assay as described in online supplementary methods.

3′ RNA-sequencing and analysis

RNA-seq was performed in three biological replicates of cultured VICs and SFs isolated from Tg197 mice and WT littermates at their eighth week of age. Further analysis is found in online supplementary methods.

Statistical analysis

Data are presented as mean±SEM, and Student's t test was used for the evaluation of statistical significance, with P values <0.05 being considered statistically significant. Analysis was performed using the GraphPad Prism V.6.

RESULTS

TNF-dependent left-sided heart valve pathology develops as a comorbid condition in the Tg197 arthritis model

Histopathological evaluation of heart tissue from Tg197 animals revealed pathological alterations localised in the left side of the heart, affecting specifically the AV (figure 1A) and MV area (figure 1B), while the pulmonary valve as well as the blood arteries and vessels appeared unaffected (online supplementary figure S1,4). Pathology was associated with AV and MV thickening (figure 1C) mainly due to fibrosis, which extended to the root of the valve, as shown by the intense Masson's staining (figure 1A,B). Inflammation appeared to have only a minimal contribution, as indicated by the limited number of infiltrating inflammatory cells in the valves at 12 weeks of age (online supplementary figure S3).

Signs of heart valve pathology were detected in the Tg197 mice already from 4 weeks of age and became progressively worse as animals aged (online supplementary figure S4) in parallel to their arthritis pathology. By 8 weeks of age, when Tg197 mice had established arthritis, pathology in both valves was manifested with 100% penetrance and without a gender bias. Treatment of Tg197 animals with the anti-TNF infliximab (Remicade), from 4 to 11 weeks of age, resulted in the amelioration of the HVD, demonstrated by the decrease in valvular thickening and fibrosis (figure 1C and supplementary figure S2)

Heart valve pathology leads to LV dysfunction in the Tg197 animals

To assess whether the valvular thickening and fibrosis observed in Tg197 animals also affect their cardiac function, we performed echocardiography and ECG analysis in 12-week-old mice. Tg197 mice displayed increased AV and MV velocities (figure 1D), indicative of valvular stenosis. Moreover, in approximately 15% of the transgenic mice examined, aortic and/or mitral regurgitation was detected (online supplementary figure S5), suggesting valvular insufficiency. An additional consequence of the MV dysfunction was the observed increased atrial pressure leading to dilation of the left atrium (LA) (table 1). Echocardiography data analysis consistently showed that Tg197 animals displayed LV dilation with some degree of hypertrophy, indicated by the increased LV dimensions (LV end-diastolic diameter (LVEDd), LV end-systolic diameter (LVEDs), LV length in diastole (LVLd),

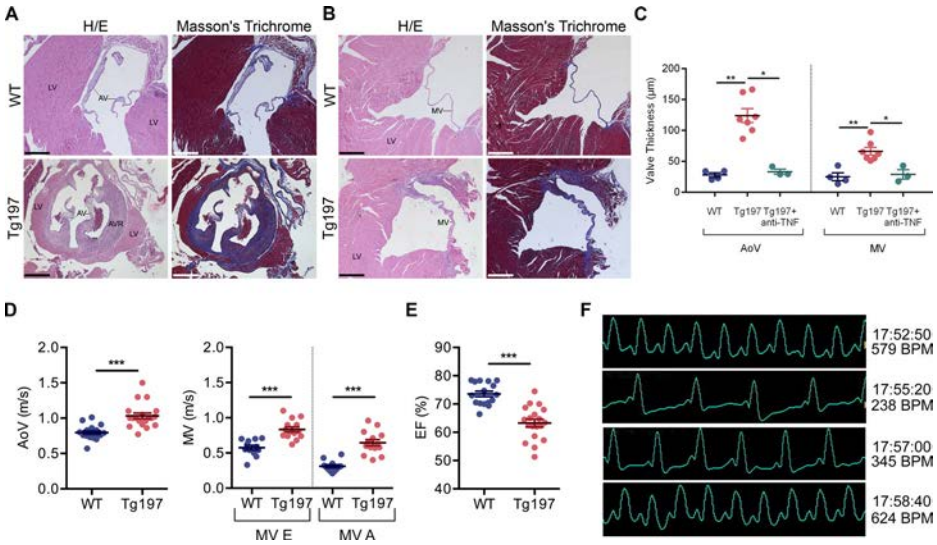

Figure 1 Tg197 arthritis model develops tumour necrosis factor (TNF)-dependent left-sided heart valve disease which leads to left ventricle (LV) dysfunction. (A, B) Representative images of H&E and Masson's trichrome-stained transverse heart sections showing the aortic valve (AV) (A) and the mitral valve (MV) (B) leaflets of Tg197 and WT littermate animals at 12 weeks old of age;(scale bar, 400 µm) (C) Comparison of the AoV and MV thickness between WT, Tg197 and Tg197 treated with anti-TNF infliximab (Remicade) animals at 11–12 weeks of age (data are presented as individual values, with mean±SEM; *P<0.02; **P<0.01). (D) Blood aortic (AoV) and mitral velocities (MV E and A) acquired by Doppler analysis of Tg197 mice and WT littermates at their 12 weeks of age (left and right panels, respectively; data are presented as individual values, with mean±SEM; ***P<0.0001). (E) Ejection fraction (EF%) of Tg197 mice and WT littermates at their 12 weeks of age, calculated by the modified Simpson equation, using 2D images in echocardiography analysis (data are presented as individual values, with mean±SEM; ***P<0.0001). (F) Representative ECGs of Tg197 animals with few minutes interval (four consecutive time points with ~1 min interval, starting from the upper panel), at their 12 weeks of age. AVR, aortic valve root; WT, wild type.

LV end-diastolic posterior wall thickness (LVPWd), end-diastolic interventricular septal thickness (IVSd)) and the significant increase of heart-to-body weight ratio compared to WT mice (table 1). Tg197 mice also exhibited significant reduction of the global cardiac function, as indicated by their reduced ejection fraction (EF%) (figure 1E) and, more importantly, by their reduced regional contractile function reflected in the lower systolic velocity of the posterior wall (SVPW) (table 1).

Interestingly, we have observed that Tg197 animals exhibit increased premature mortality of unknown aetiology starting at 10 weeks of age, reaching an ~50% incidence at 13 weeks of age (online supplementary figure S6). Notably, assessment of cardiac function of Tg197 animals showed that they were prone to exhibit fatal episodes of arrhythmias in advanced disease stages (12 weeks), mainly switching from bradycardia (~200 bpm) to tachycardia (~500–650 bpm) in a few minutes interval during ECG (figure 1F). Therefore, arrhythmic episodes could be associated with the premature deaths observed in Tg197 animals.

Collectively, our data show that the histopathological findings in Tg197 heart valves are associated with left-sided valvular degeneration and dysfunction and are accompanied by echocardiographic findings of LV cardiomyopathy.

Table 1 Echocardiographic parameters in Tg197 and WT mice at 12 weeks of age

	WT (n=16)	Tg197 (n=19)	P value
Body weight (g)	26.25±1.06	16.73±0.091	<0.0001
Heart weight (mg)	107.10±3.39	83.00±5.31	<0.0001
HW/BW (mg/g)	4.11±0.08	5.05±0.27	0.0054
LVEDd (mm/BW)	0.14±0.01	0.22±0.01	<0.0001
LVEDs (mm/BW)	0.08±0.01	0.14±0.01	<0.0001
LVLd (mm/BW)	0.28±0.01	0.38±0.01	<0.0001
LVPWd (mm/BW)	0.026±0.001	0.038±0.002	<0.0001
IVSd (mm/BW)	0.026±0.001	0.038±0.002	<0.0001
LA (mm/BW)	0.083±0.003	0.132±0.006	<0.0001
SVPW (cm/s)	3.02±0.08	2.14±0.07	<0.0001
E/A ratio	1.87±0.13	1.33±0.09	0.0009

Values were normalised with the body weight (except for SVPW), as indicated in the table. Data were expressed as mean±SEM.
E/A ratio, ratio between E (peak early diastolic flow) and A (peak late diastolic flow); HW/BW, heart weight-to-body weight ratio; IVSd, end-diastolic interventricular septal thickness; LA, left atrium; LVEDd, left ventricular end-diastolic diameter; LVEDs, left ventricular end-systolic diameter; LVLd, left ventricular length in diastole; LVPWd, left ventricular end-diastolic posterior wall thickness; SVPW: systolic velocity of the posterior wall.

Hypertrophic valves of Tg197 mice consist mainly of activated VICs

Since SFs have been previously established as drivers of arthritogenesis in the Tg197 model,[8] we investigated whether VICs play also a pathogenic role in the observed Tg197 heart valve pathology. To this end, we first crossed the reporter mouse *Rosa26*$^{mT/mG}$ which expresses green fluorescent protein (GFP) upon recombination, with the *ColVI-Cre* mouse, which has been previously used to target mesenchymal cells in the joints, small intestine,[8] colon[29] and other organs.[30] Examination of the heart valves of *ColVI-Cre-Rosa26*$^{mT/mG}$ mice revealed co-localisation of GFP expression with vimentin (figure 2A), a known marker of fibroblasts and VICs,[23 31] indicating efficient targeting of VICs by *ColVI-Cre*, and confirming their mesenchymal origin.[22] Efficient recombination was confirmed by further characterisation of GFP$^+$ cells derived from dissociated heart valve tissue from *ColVI-Cre-Rosa26*$^{mT/mG}$ mice using FACS analysis. GFP$^+$ cells strongly expressed VIC and mesenchymal cell markers (vimentin, CD29 and podoplanin), while displaying no expression of haematopoietic (CD45) and endothelial (CD31) markers

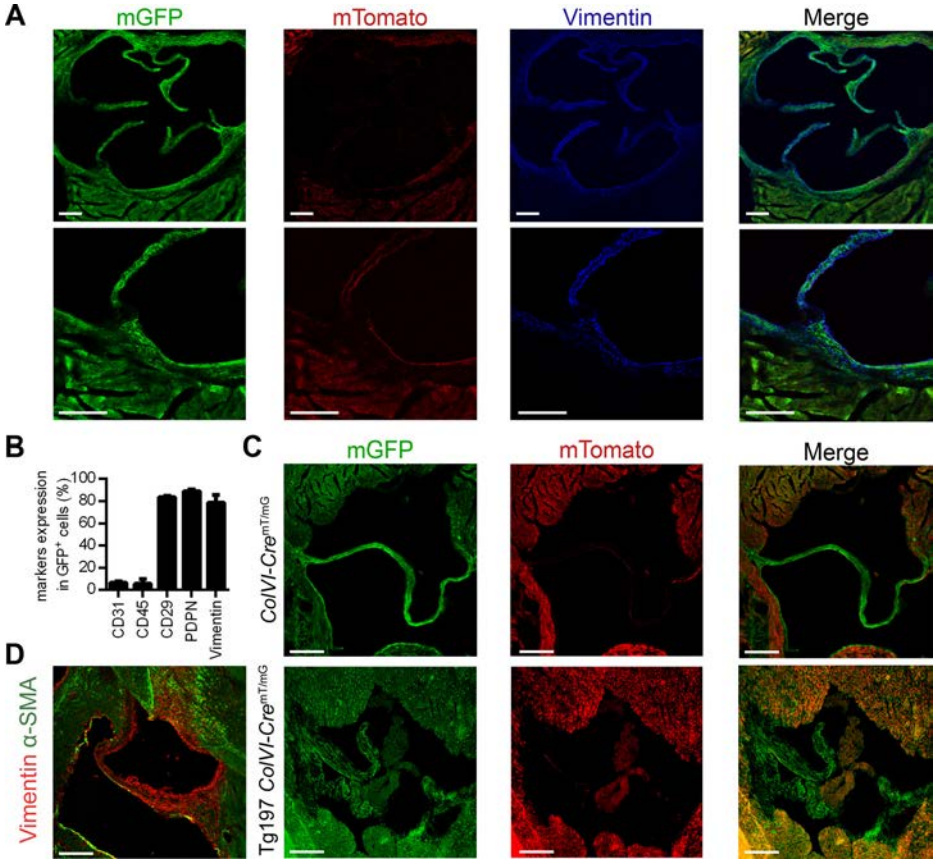

Figure 2 Heart valve disease of Tg197 mice is caused by accumulation of activated mesenchymal valve interstitial cells (VICs). (A) Representative images of transverse heart cryosections of *ColVI-Cre-Rosa26*$^{mT/mG}$ mice, at their 8 weeks of age, and colocalisation of GFP expression with vimentin expression in the heart valve (lower panel: higher magnification of the upper panel) (scale bar: 100 µm). (B) Fluorescence-activated cell sorting (FACS) analysis of ColVI-expressing cells (GFP$^+$) with markers for endothelial (CD31), haematopoietic (CD45) and fibroblast/mesenchymal cells (CD29, podoplanin [PDPN], vimentin) from dissociated heart valves of *ColVI-Cre-Rosa26*$^{mT/mG}$ mice, at their 8 weeks of age (data are presented as mean±SEM, n=3 from three individual experiments). (C) Representative images of transverse heart cryosections of *ColVI-Cre*$^{mT/mG}$ and Tg197 *ColVI-Cre*$^{mT/mG}$ mice at their 12 weeks of age (scale bar: 100 µm). (D) Representative image of transverse heart cryosections of Tg197 mice at their 12 weeks of age and colocalisation of smooth muscle actin (α-SMA) expression with vimentin expression in the heart valve and root (scale bar: 100 µm).

(figure 2B). These results suggest that the *ColVI-Cre* mouse effectively targets mainly vimentin$^+$, CD29$^+$ and podoplanin$^+$ mesenchymal-like VICs in the heart valve.

To explore the role of VICs in the Tg197 heart valve pathology, we crossed the *ColVI-Cre-Rosa26*$^{mT/mG}$ mice with Tg197 mice. The thickened fibrotic heart valves of these mice were mainly populated by GFP$^+$ VICs (figure 2C), supporting their central role in the heart valve phenotype. The pathogenic potential of VICs in Tg197 animals was further assessed by the expression of α-SMA, a well-established marker of activated myofibroblastic VICs.[32] Interestingly, α-SMA-expressing VICs were detected in the thickened valvular area and root of Tg197 mice (figure 2D), indicating that the pathology observed is mainly characterised by accumulation of activated VICs.

TNFR1 signalling in mesenchymal cells is necessary and sufficient for the development of Tg197 heart valve pathology

Having established the contribution of activated mesenchymal VICs and TNF dependency of the valvular hyperplasia in Tg197 mice, we further explored the role of mesenchyme-specific TNF signalling in the development of this pathology. To address whether TNF signalling in mesenchymal VICs is required for the development of heart valve pathology, Tg197 animals were crossed with *ColVI-Cre Tnfr1*$^{fl/fl}$ animals.[26] Tg197 *ColVI-Cre Tnfr1*$^{fl/fl}$ mice exhibited ameliorated heart valve pathology, as indicated by the lack of heart valve thickening and fibrosis (figure 3A, C). This finding suggests that TNF signalling, through TNFR1 in mesenchymal cells, is essential for the pathogenesis of HVD in the Tg197 model.

Next, we examined whether TNF signalling in mesenchymal cells was also sufficient to induce heart valve pathology in Tg197 mice. To this end, we crossed Tg197 with *ColVI-Cre Tnfr1*$^{cneo/cneo}$ mice to achieve specific reactivation of TNFR1 signalling only in mesenchymal cells.[27] Tg197 *ColVI-Cre Tnfr1*$^{cneo/cneo}$ mice developed valvular thickening and extensive fibrosis, while control Tg197 *Tnfr1*$^{cneo/cneo}$ did not show any signs of heart valve thickening and fibrosis (figure 3B,D), demonstrating that TNF signalling through TNFR1 in mesenchymal cells is sufficient to trigger heart valve pathology in Tg197 mice. Consequently, TNF signalling in the mesenchyme is both necessary and sufficient for the development of heart valve pathology in Tg197 animals.

Ex vivo-derived Tg197 VICs exhibit an activated phenotype

It is known that *ex vivo* human RA and mouse arthritogenic SFs exhibit increased proliferative and migratory capacities.[33–35] To investigate whether pathogenic VICs display a similar phenotype, we isolated VICs from Tg197 and WT animals at 8 weeks of age, when HVD is well established.

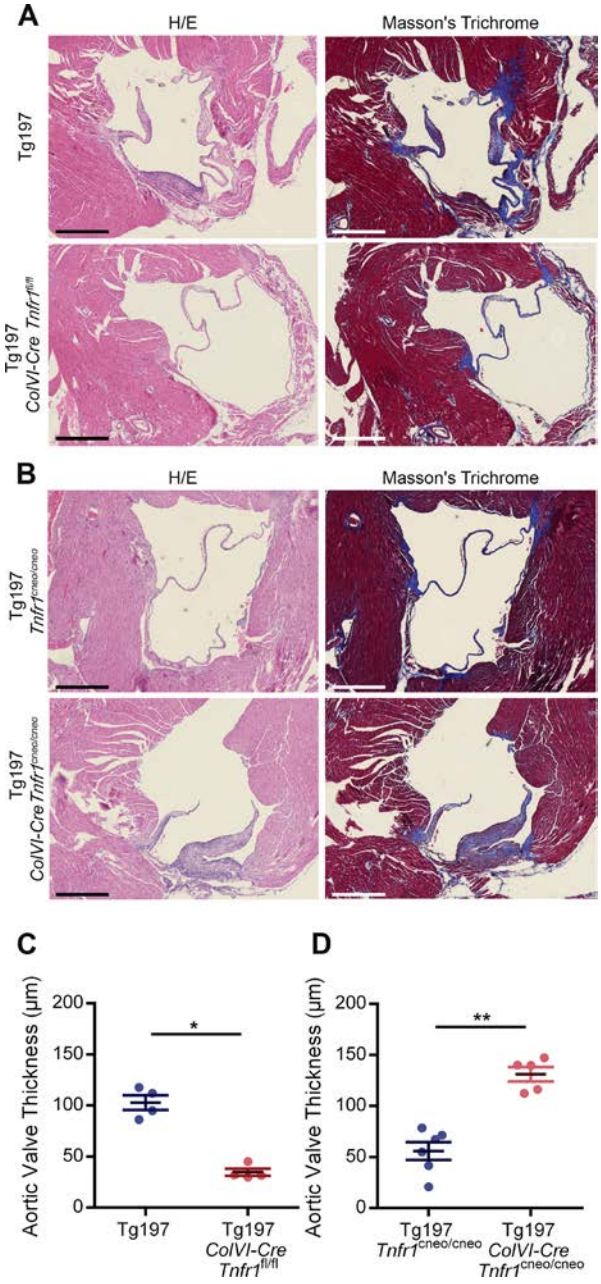

Figure 3 TNF signalling on valve interstitial cells (VICs) is required and sufficient for the development of heart valve disease of Tg197 animals. (A) Representative images of H&E and Masson's trichrome-stained transverse heart sections of Tg197 and Tg197 *ColVI-Cre Tnfr1*$^{fl/fl}$ and animals at 12 weeks of age (scale bar, 500 µm). (B) Representative images of H&E and Masson's trichrome-stained transverse heart sections of Tg197 *Tnfr1*$^{cneo/cneo}$ and Tg197 *ColVI-Cre Tnfr1*$^{cneo/cneo}$ and at 12 weeks of age (scale bar, 500 µm). (C) Comparison of the aortic valve thickness between Tg197 and Tg197 *ColVI-Cre Tnfr1*$^{fl/fl}$ animals at 12 weeks of age (data are presented as individual values, with mean± SEM; *P<0.03). (D) Comparison of the aortic valve thickness between Tg197 *Tnfr1*$^{cneo/cneo}$ and Tg197 *Tnfr1*$^{cneo/cneo}$ at 12 weeks of age (data are presented as individual values, with mean± SEM; **P<0.005).

We first confirmed the homogeneity of VICs cultures by characterising cultured VICs isolated from *ColVI-Cre-Rosa26*$^{mT/mG}$ animals. FACS analysis confirmed that approximately 80% of the isolated VICs were GFP^{+} and displayed high expression of known fibroblast and mesenchymal cell markers including CD29, vimentin, podoplanin, CD140a, CD90.2, CD105, vascular cell adhesion protein 1 (VCAM-1) and intercellular adhesion molecular 1 (ICAM-1) (figure 4A), while they lacked expression of haematopoietic (CD45) and endothelial (CD31) markers, thus preserving the observed *in vivo* expression marker profile (figure 2B).

We further assessed the activation status of Tg197-derived VICs. These cells were found to overexpress hTNF (figure 4B) and displayed increased proliferative and migratory capacities (figure 4C,D), similarly to Tg197-derived SFs.[34] Therefore, VICs are shown to exhibit an activated phenotype with similar characteristics to the one exhibited by the arthritogenic Tg197 SFs *ex vivo*.[33 34]

Tg197 VICs express common pathogenic signatures with Tg197 SFs

Arthritogenic SFs have been recently found to exhibit a distinct expression profile, characterised by pathogenic deregulation of genes affecting key pathways for the development of polyarthritis symptoms.[36] We, therefore, explored the commonalities of pathogenic Tg197 VICs and SFs at the gene expression, pathway and transcriptional regulation level. For this purpose, we isolated SFs and VICs from 8-week-old Tg197 animals, with established arthritis and HVD, and compared their expression profiles to those of SFs and VICs isolated from WT littermates by using RNA-sequencing.

Both Tg197 SFs and VICs displayed >500 significant differentially expressed genes (DEGs) compared to their WT controls (figure 5A). More specifically, a total of 408 and 381 genes were upregulated in Tg197 VICs and SFs, respectively, with almost 30% of them commonly upregulated in both cell types (figure 5B), while a total of 327 and 160 genes were downregulated in Tg197 VICs and SFs, respectively, with approximately 10% of them commonly downregulated in both cell types (figure 5B). Further functional enrichment analysis of the common upregulated genes placed immune and inflammatory responses, as well as nuclear factor (NF)-κB signalling at the top enriched pathways. Pathways enriched in the overlapping downregulated genes included extracellular matrix organisation and regulation of growth, indicating extracellular matrix (ECM) remodelling and deregulated cell growth (online supplementary figure S7).

To further explore the similarities of these two pathogenic cell types at the pathway level, functional enrichment analysis was performed for all DEGs in SFs and VICs. Interestingly, KEGG pathways enriched in SFs' and VICs' upregulated genes show a great overlap (60%) (figure 5C). These pathways were subsequently clustered into broader KEGG pathway categories. The most pronounced category was immune response, which included pathways such as chemokine and TLR signalling, while the most prominent correlation was to human 'rheumatoid arthritis' term, with known RA-related and cardiovascular disease-related genes (*Tnf*, *Il1b*[37] and *Acp5*[38]) being upregulated in both cell types. Other categories include cancer and infectious diseases, such as tuberculosis and pertussis which have also been associated with inflammation and TNF signalling. TNF and NF-κB signalling were also enriched in both cell types, with a distinct set of genes such as *Mmp9, Tnf, Il1b, Cxcl2;3;12, Ccl4 and Cd14* being upregulated in both Tg197 SFs and VICs (figure 5D). Interestingly, some of the functions enriched only in VICs' DEGs involve cardiovascular diseases, indicating the differences between VICs and SFs due to their different tissue of origin (online supplementary figure S7).

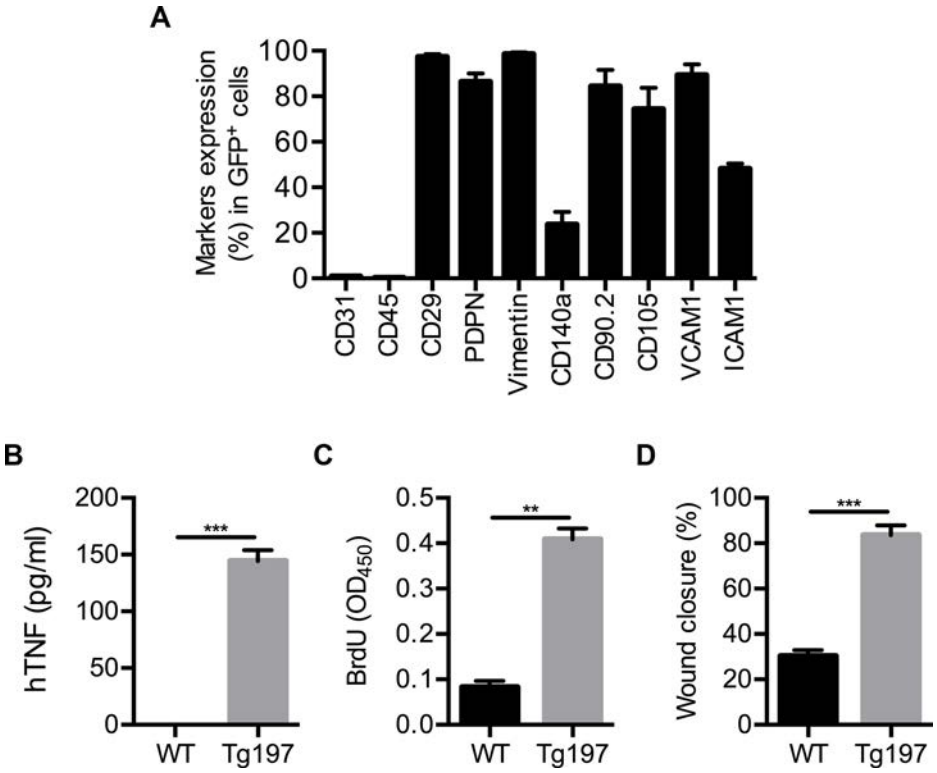

Figure 4 Activated phenotype of Tg197-derived valve interstitial cells (VICs) *ex vivo*. (A) Fluorescence-activated cell sorting (FACS) analysis of *ColVI*-expressing cells (GFP$^+$) with markers for endothelial (CD31), haematopoietic (CD45) and fibroblast/mesenchymal cells (CD29, podoplanin [PDPN], vimentin, CD140a, CD90.2, CD105, VCAM-1, ICAM-1) in isolated VICs from *ColVI-Cre-Rosa26*$^{mT/mG}$ mice at 8 weeks of age. (B–D) Levels of secreted hTNF in the supernatants (B), BrdU incorporation (C) and wound healing ability calculated by percentage of wound closure (D) of VICs isolated from WT and Tg197 mice at 8 weeks of age (data are presented as mean±SEM, n=3 from three individual experiments; **P<0.001; ***P<0.0005).

Furthermore, the RNEA tool,[39] which infers regulatory networks by predicting interactions between transcription factors and their target genes, was used to explore the similarities between VICs and SFs at the transcriptional regulation level. Regulatory networks were extracted from both cells' gene expression profile and their intersection is reported in figure 5E. Interestingly, *Sfpi1* and *Pparg*, which are known to regulate mesenchymal activation,[36] were revealed by this analysis as the two main common regulators of the two networks; *Tnf* was also found to be a central regulator. These findings strongly suggest that Tg197 VICs share a commonly altered expression profile with Tg197 SFs at the gene expression, functional pathways and transcriptional regulation circuit levels.

DISCUSSION

Patients with RA and SpA show a higher risk of developing associated cardiac diseases, which highly contribute to their increased mortality rates.[2] More specifically, they exhibit a 30% increased incidence of valvular pathologies, including non-specific valvular thickening and mild valve regurgitation.[3 40] Recent studies using sensitive imaging methods, such as transesophageal echocardiography, report an even greater prevalence of left-sided HVD in RA patients with valve thickening in half of the cases involving both mitral (47%) and aortic valves (32%) and valve regurgitation (21%).[41] The involvement of TNF in the pathogenesis of RA and SpA is well established; however, its role in the development of arthritis-related cardiac comorbidities remains unknown.

We demonstrate here that overexpression of TNF, in the TghuTNF (Tg197) arthritis model, in addition to the chronic polyarthritis[5] drives also the development of spontaneous left-sided HVD, which mainly leads to valvular thickening with some degree of stenosis and occasionally to valve insufficiency, comorbid pathologies often observed in patients with RA/SpA.[3 42] Interestingly, a similar left-sided heart valve pathology, exhibiting valvular thickening and fibrosis, was also observed in the TgA86, transmembrane TNF overexpressing, mouse model of SpA[6 43] (supplementary figure S8), further strengthening the pathogenic role of TNF in the development of arthritis-related cardiac comorbidities. The greater mechanical stress and haemodynamic pressures imposed on the left side of the heart is a likely explanation for the discrepancy between the diseased left-sided and unaffected right-sided valves, also observed in patients with RA/SpA.

AV stenosis and MV and/or AV regurgitation have been shown to result in LV hypertrophy with preserved EF and occasionally in LV dilation with some degree of contractile dysfunction.[44] Similarly, in the Tg197 model, valvular pathology contributes to the observed extensive LV dilation with some degree of LV hypertrophy as well as to the concomitant contractile dysfunction. However, additional mechanisms that have been proposed as contributing factors in the increased prevalence of global heart failure in patients with RA/SpA, such as myocardial fibrosis and oedema as well as arterial blood pressure, coronary heart disease and myocardial remodeling[45] remain to be studied for their contribution in the global heart impairment observed in Tg197 animals. We have also detected repeated arrhythmic episodes in Tg197 mice which could explain the premature sudden deaths observed in this model recapitulating the increased risk of sudden cardiac death experienced by patients with RA/SpA, due to atrial fibrillation and other types of tachyarrhythmias which suggest diffuse myocardial electrical instability.[46 47] Overall, our

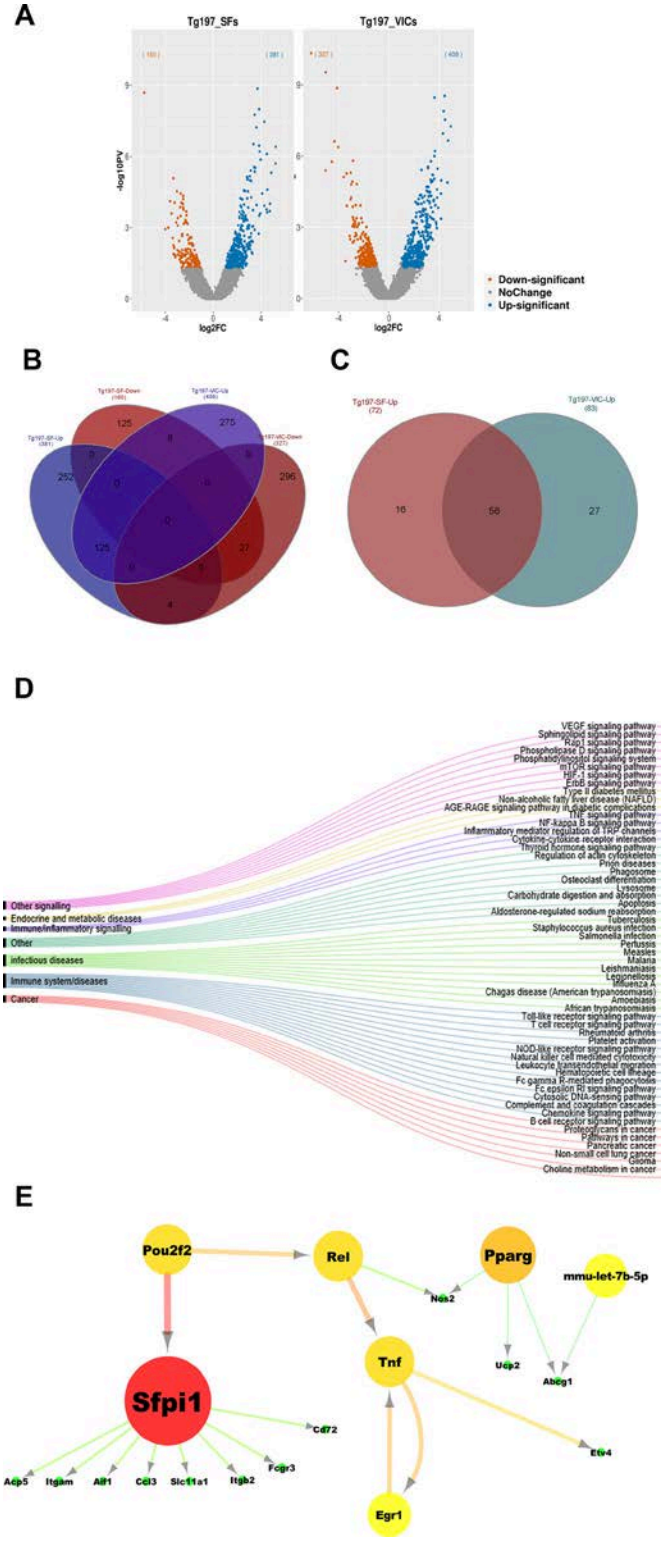

Figure 5 Tg197 valve interstitial cells (VICs) exhibit common pathogenic molecular signatures with Tg197 synovial fibroblasts (SFs) at 8 weeks of age. (A) Volcano plots with the number of differentially expressed genes (DEGs) of Tg197 VICs and SFs compared to WT. (B) Venn diagram showing the overlap of DEGs in Tg197 VICs and SFs. (C) Venn diagram showing overlap of enriched KEGG pathways derived from functional enrichment analysis of upregulated DEGs in Tg1917 VICs and SFs. (D) Alluvial diagram illustrating overlapping KEGG pathways in Tg197 VICs and SFs, grouped according to their KEGG broader categories. (E) Intersection of regulatory networks of Tg197 VICs and SFs.

data demonstrate that the Tg197 arthritis model develops HVD and cardiac arrhythmias that closely mimic the cardiac clinical findings and premature mortality observed in patients with arthritis, supporting the value of this model in providing mechanistic insights into the pathogenesis of these comorbidities. The reversal of the cardiac phenotype by pharmacological inhibition of TNF in this model supports the vital role of TNF in the development of RA/SpA-related cardiac valvular comorbidities and suggests that anti-TNF therapy could also prevent cardiac comorbidities and avoid adverse cardiovascular side effects caused by other drugs, such as DMARDs.[2] Our findings also highlight the importance of regular echocardiographic screening on patients with RA and SpA.

The association between elevated TNF levels and valvular pathology has been previously suggested in other mutant mice.[14 15] Notably, these mice exhibit an inflammatory valvulitis, in contrast to the hypertrophic valves of Tg197 mice, which consist mainly of activated mesenchymal VICs. This discrepancy could be attributed to various factors, such as differences in the genetic background or in the cytokine imbalances driving the pathology. More specifically, the inflammatory phenotype of IL1Ra-deficient mice[14] has been observed in the inflammation-susceptible[48] BALB/c genetic background whereas in the C57 background they show milder pathology.[14] Additionally, pathology in the IL1Ra-[14] as well as in the TTP-deficient[15] and K/BxN transgenic[49] mice could be driven by diverse upstream mechanisms providing additional pathogenic cytokine disbalances apart from TNF.

Extensive characterisation and comparison of the transcriptional profiles of pathogenic Tg197 VICs and SFs, compared with their healthy counterparts, revealed a shared altered and pathogenic profile of these two cell types. Inflammatory and immune responses were among the commonly enriched KEGG pathways in both Tg197 SFs and VICs, supporting their activated and pathogenic status. Our analysis further supports the central role of *Tnf* in both cell types and pathologies. Interestingly, *Sfpi1,* an NF-κB modulator,[50] emerged as a common transcriptional regulator of both activated VICs and SFs, highlighting the importance of NF-κB signalling in this process, as was also confirmed by the enrichment of the NF-κB signalling pathway in both cell types. Moreover, *Sfpi1*, encoding the myeloid-specific transcription factor PU.1,[51] has been found to be upregulated in RA-FLS,[52] while being also implicated in the pathogenesis of heart hypertrophy.[53] Collectively, the centrality of *Sfpi1*, in combination with the enriched immune and TLR signalling, as well as NF-κB signalling pathways, in both Tg197 VICs and SFs could support their conversion to activated mesenchymal cells possessing pathogenic innate immune properties. This hypothesis is further supported by recent findings suggesting that Tg197 SFs undergo a metabolic reprogramming,[54] similar to the metabolic alterations reported in both inflammatory heart diseases and RA.[55 56] Therefore, we hypothesise that VICs and SFs become pathogenic on common TNF-induced metabolic reprogramming acquiring a detrimental innate phenotype, which should be further explored.

We finally show here that mesenchymal-specific TNF signalling, through TNFR1, is both required and sufficient for the development of heart valve pathology. Notably, SF-specific and IMC-specific TNFR1 signalling has been previously demonstrated to be causal in orchestrating comorbid polyarthritis and Crohn's-like IBD in a TNF overexpressing mouse model.[8] It may, therefore, be strongly postulated that mesenchymal cell responses to TNF explain complex chronic inflammatory disease comorbidities involving joint, intestinal and, as shown in the

present study, also cardiac pathologies. Future detailed insights into the molecular and cellular mechanisms commonly underlying aetiopathogenesis of mesenchymal cell-driven comorbidities, such as those expressed under the RA/SpA paradigm, may also offer novel approaches to therapeutically target common pathogenic processes.

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Contributors GK, LN, MS, NK and MCD designed the study and interpreted the experimental results. LN, MS, PC and IM performed the experiments and data analysis. AP contributed to the data analysis. LN and MCD wrote the first draft of the manuscript and all authors were involved in critically revising its final preparation. All authors approved the final version to be published.

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EXTENDED REPORT

Tyrosine kinase Fyn promotes osteoarthritis by activating the β-catenin pathway

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ABSTRACT

Objectives To investigate the role of tyrosine kinase Fyn in the development of osteoarthritis (OA) and the underlying mechanisms, and to define whether targeting Fyn could prevent OA in mice.

Methods Cartilage samples from normal and aged mice were analysed with proteome-wide screening. Fyn expression was examined with immunofluorescence in human and age-dependent or experimental mouse OA cartilage samples. Experimental OA in Fyn-knockout mice was induced by destabilisation of the medial meniscus. Primary cultured mouse chondrocytes were treated with proinflammatory cytokine interleukin-1β. The inhibitor of Src kinase family, AZD0530 (saracatinib), and inhibitor of Fyn, PP1, were used to treat experimental OA in mice.

Results Fyn expression was markedly upregulated in human OA cartilage and in cartilage from aged mice and those with post-traumatic OA. Fyn accumulates in articular chondrocytes and interacts directly with and phosphorylates β-catenin at Tyr142, which stabilises β-catenin and promotes its nuclear translocation. The deletion of Fyn effectively delayed the development of post-traumatic and age-dependent OA in mice. Fyn inhibitors AZD0530 and PP1 significantly attenuated OA progression by blocking the β-catenin pathway and reducing the levels of extracellular matrix catabolic enzymes in the articular cartilage.

Conclusions Fyn accumulates and activates β-catenin signalling in chondrocytes, accelerating the degradation of the articular cartilage and OA development. Targeting Fyn is a novel and potentially therapeutic approach to the treatment of OA.

INTRODUCTION

Osteoarthritis (OA) is an age-related or post-traumatic degenerative joint disease that is characterised by the loss of articular cartilage, the hypertrophic differentiation of chondrocytes, subchondral bone remodelling, synovial inflammation and osteophyte formation.[1 2] Despite the identified risk factors, which include ageing, previous joint injury, obesity, genetics, sex and anatomical factors related to joint shape and alignment, the exact pathogenesis of OA remains undefined.[3 4] The well-accepted pathogenic mechanisms of OA include the increased production of matrix-degrading enzymes, such as the matrix metalloproteinases (MMPs) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS), the degradation of the cartilage-specific extracellular matrix (ECM) and chondrocyte apoptosis.[5–8] There is still no effective disease-modifying treatment for OA and a novel drug for its treatment is urgently required. Therefore, understanding the mechanisms that control chondrocyte hypertrophy and regulate the expression of ECM-degradation-related genes is important for developing effective therapies for OA.

Accumulating data suggest that canonical WNT/β-catenin signalling plays an important role in regulating the pathogenesis of OA.[9–13] Increased levels of β-catenin have been reported in chondrocytes within areas of degenerated cartilage. The activation of β-catenin signalling in articular chondrocytes in adult mice leads to premature chondrocyte differentiation and the development of an OA-like phenotype.[14] Furthermore, the activation of β-catenin in mature cartilage cells stimulates their hypertrophy, matrix mineralisation and the expression of MMP13 and vascular endothelial growth factor.[15–18] However, the mechanism by which WNT/β-catenin is activated during OA and cartilage degeneration has not been fully clarified.

In this study, we show that the non-receptor tyrosine kinase Fyn, a member of the Src family kinases (SFKs), accumulates in the cartilage of aged mice, mice with post-traumatic OA and human OA cartilage. Fyn directly phosphorylates and stabilises β-catenin, promoting its nuclear translocation and activation, leading to the enhanced expression of collagen X, MMP13 and ADAMTS5, and the accelerated degradation of the ECM. We thus identify, for the first time, the critical role of Fyn in OA through its activation of β-catenin signalling and provide a potential novel therapeutic target for OA.

RESULTS

Fyn accumulates in articular cartilage of aged mice, mice with post-traumatic OA and humans with OA

OA is strongly linked to ageing, but the mechanism of this link is poorly understood. We first used proteome-wide screening to identify proteins involved in cartilage degeneration by comparing the articular cartilage from young (2-month-old) and aged (12-month-old) mice. Among the 5015 proteins identified, 303 proteins were upregulated and 115 proteins were downregulated (>1.5-fold) in the cartilage of the aged mice compared with that of the young mice (online supplementary table 1). The tyrosine kinase Fyn, but no other member of the SFK family (including Fgr, Lyn, Csk and Hck), was the most strongly upregulated protein

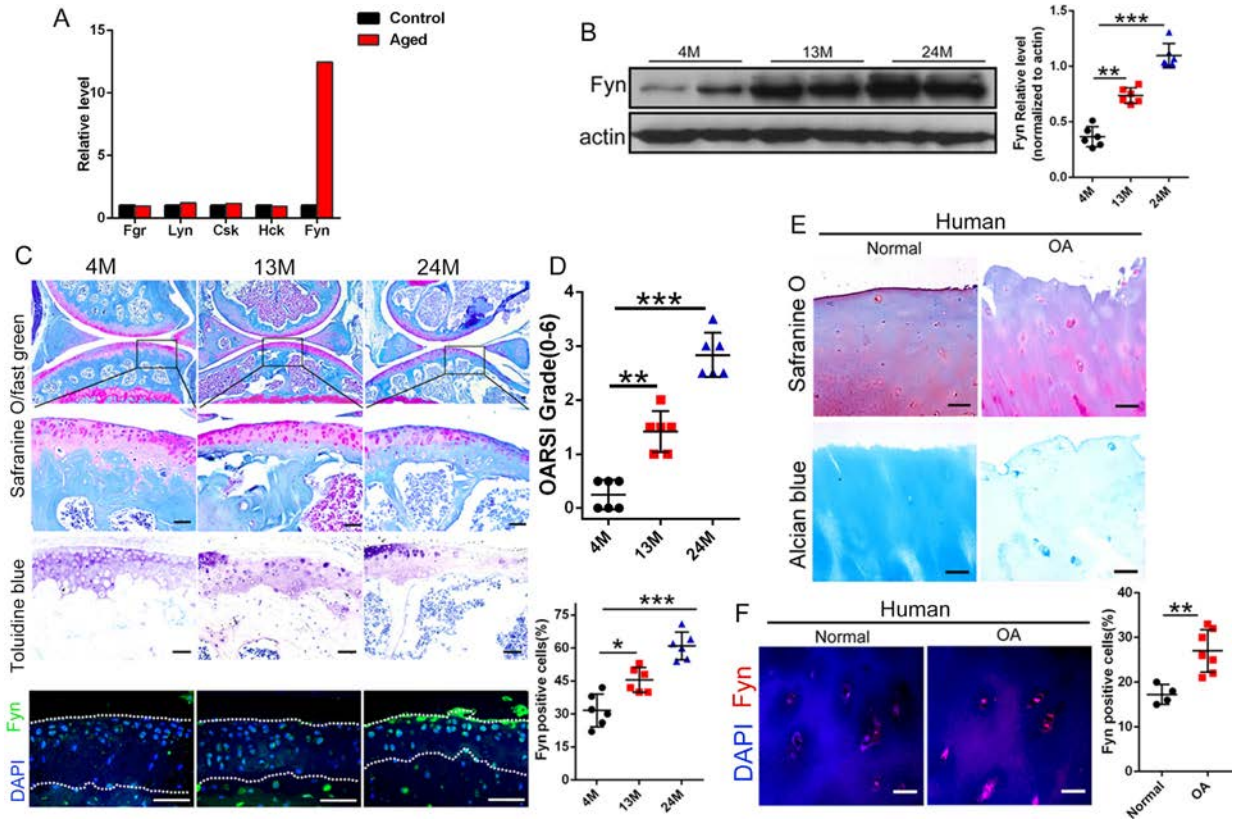

Figure 1 Fyn accumulates in articular cartilage of aged mice and patients with osteoarthritis (OA). (A) Quantitative analysis of Src family kinases expression with a proteome-wide screen for the proteins involved in cartilage degeneration by analysing the articular cartilage from young (2-month-old) and aged (12-month-old) mice. (B) Immunoblotting analysis (left) and quantification (right) of Fyn in dissected articular cartilage from mice 4, 13 and 24 months old. Data are representative of three independent experiments, **P<0.01, ***P<0.001. (C) Upper: Safranin O and Fast Green staining of sagittal sections of joints from mice collected at 4, 13 and 24 months of age; proteoglycan (red) and bone (blue). The tibia medial compartments of the mice are shown at higher resolution. Middle: toluidine blue staining of sagittal sections of joints from aged mice. Lower: immunofluorescence analyses (left) and quantification (right) of Fyn (green) in articular chondrocytes; n=6. *P<0.05, ***P<0.001. Scale bars, 50 µm. (D) Osteoarthritis Research Society International (OARSI) grades for mice 4, 13 and 24 months old, n=6. **P<0.01, ***P<0.001. (E) Safranin O and Fast Green staining (upper) and Alcian Blue staining (lower) of cartilage from normal humans and those with OA. Scale bars, 50 µm. (F) Immunofluorescence analysis (left) and quantification (right) of Fyn (red) in cartilage chondrocytes from normal humans (n=4) and patients with OA (n=7); **P<0.01. Scale bars, 50 µm. All data are shown as means±SD.

(12.47-fold) in the aged cartilage (figure 1A). The marked increased Fyn in aged cartilage was confirmed with a western blotting analysis of the articular cartilage from mice aged 4, 13 and 24 months (figure 1B). The accumulation of Fyn was accompanied by the degeneration of the cartilage and increased Osteoarthritis Research Society International (OARSI) grades in the aged mice (figure 1C,D). Immunofluorescent staining confirmed that Fyn accumulates in the articular chondrocytes of aged mice (figure 1C).

We then assessed the expression of Fyn in a surgically induced post-traumatic OA mouse model. Consistent with our previous finding, Fyn expression was low in the undamaged articular cartilage at baseline, but its level increased dramatically in OA mice along with the increased cartilage damage (4 and 8 weeks after surgery performed to destabilise the medial meniscus (DMM surgery)) (online supplementary figure 1A–D). To determine whether Fyn levels were elevated in human OA articular cartilage, we compared Fyn expression in aged (67.00±3.03 years) human OA cartilage from subjects undergoing total knee replacement and normal cartilage samples from young (30.25±8.18 years) traffic incident victims with no history of arthritic disease. Marked elevation of Fyn levels was detected in the aged and OA cartilage chondrocytes with an immunofluorescence analysis, together with the degeneration and loss of structure in the OA cartilage (figure 1E,F). Taken together, our findings demonstrate that the Src kinase family member Fyn accumulates in degenerated and damaged articular cartilage in aged mice and patients with OA.

Deletion of Fyn prevents the development of post-traumatic and aged-related OA in mice

To determine the significance of Fyn accumulation in the articular chondrocytes of degenerated or damaged cartilage, we used Fyn-knockout mice (Fyn-KO) from the Jackson Laboratory (*Fyn*$^{tm1Sor/J}$, stock no. 012468). In gross appearance, the Fyn-KO mice were slightly smaller than the control mice (online supplementary figure 2A), and their body weights and limb lengths were also slightly lower (online supplementary figure 2B,C). The deletion of Fyn in the cartilage was confirmed in 8-week-old mice with Fyn staining and western blotting (figure 2A,B). No discernible differences in the morphology or organisation of the articular cartilage or growth plates were apparent between the Fyn-KO mice and the littermate control mice at 8 weeks of age (online supplementary figure 2D–F). These results suggest that the deletion of *Fyn* did not induce skeletal developmental abnormalities in mice.

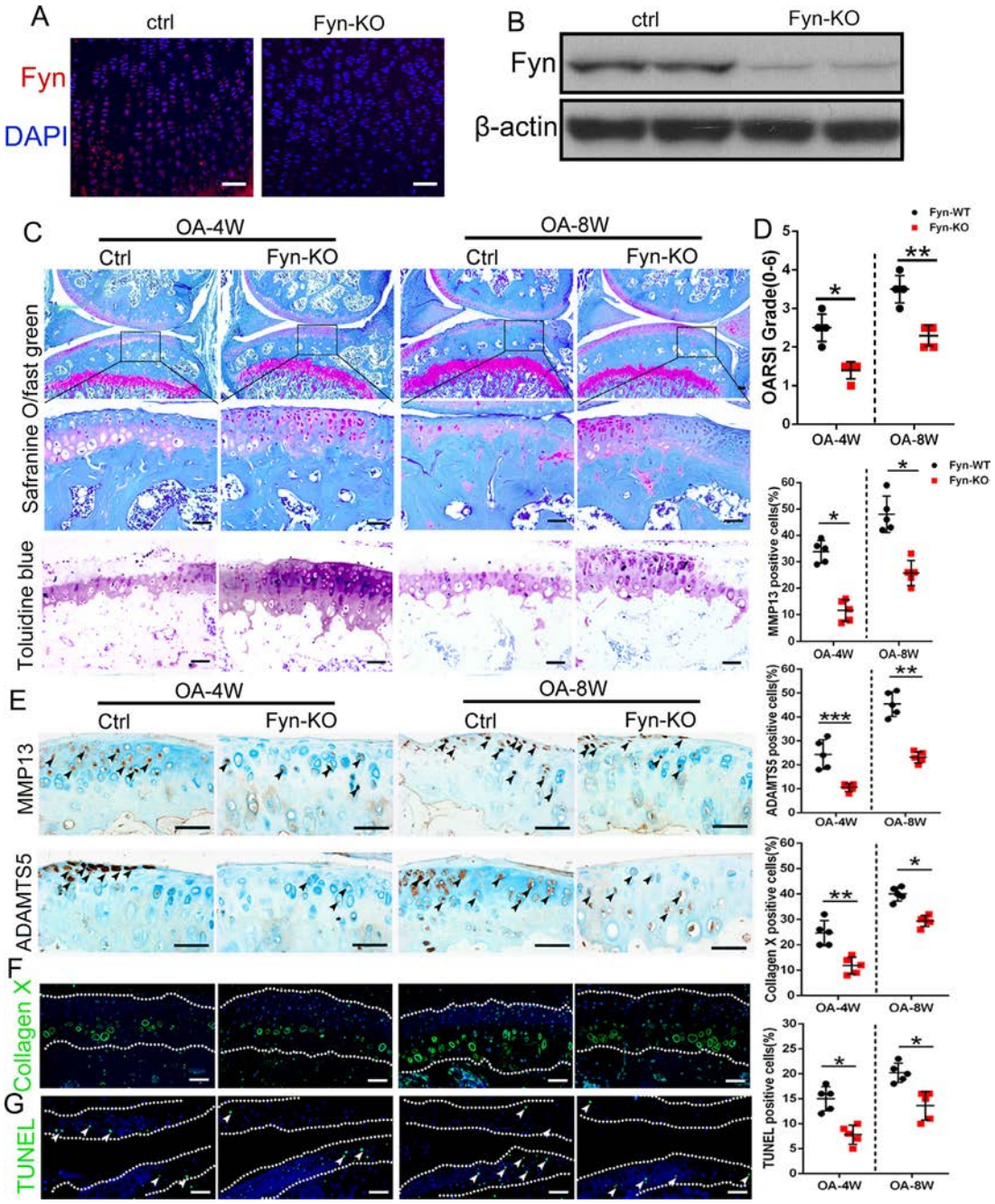

Figure 2 Deletion of Fyn delays development of post-traumatic osteoarthritis (OA) in mice. (A) Immunofluorescence analysis of Fyn (red) expression in joints of control and Fyn-knockout (Fyn-KO) mice. Data are representative of three independent experiments. (B) Immunoblotting analysis of Fyn levels in cartilage from control and Fyn-KO mice; the β-actin level was used as the internal control. Data are representative of three independent experiments. (C) Safranin O/Fast Green (upper) and toluidine blue staining (lower) of joints from control and Fyn-KO mice at 4 and 8 weeks after surgery performed to destabilise the medial meniscus (DMM surgery). n=5. Scale bars, 50 µm. (D) Osteoarthritis Research Society International (OARSI) grades for the joints of control and Fyn-KO mice at 4 and 8 weeks after DMM surgery. n=5. (E) Immunostaining (left) and quantification (right) of matrix metalloproteinase 13 (MMP13) (upper) and a disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS5) (lower) in the joint cartilage of control and Fyn-KO mice at 4 and 8 weeks after DMM surgery. Black arrowheads indicate positive cells. n=5. Scale bars, 50 µm. Immunofluorescence analysis (left) and quantification (right) of collagen X (F) and terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) staining (G) in joint cartilage from control and Fyn-KO mice at 4 and 8 weeks after DMM surgery. White arrowheads indicate TUNEL-positive cells. n=5. Scale bars, 50 µm. *P<0.05, **P<0.01, ***P<0.001. All data are shown as means±SD.

We next examined the effects of Fyn deficiency on the development of post-traumatic and age-dependent OA. DMM surgery was performed on 6-week-old Fyn-KO mice and their littermate control mice (Control). The control mice developed moderate and severe OA at 4 and 8 weeks after surgery, respectively. Interestingly, the Fyn-KO mice showed markedly reduced cartilage degradation (figure 2C), significantly lower OARSI grades (figure 2D) and decreased synovial inflammation (online supplementary figure 3A) at both 4 and 8 weeks after DMM surgery. Expression of MMP13 and ADAMTS5 (figure 2E), the chondrocyte hypertrophic marker collagen X (figure 2F) and osteocalcin in the subchondral bone (online supplementary figure 3B)

was dramatically reduced in the cartilage of the Fyn-KO mice compared with that of the control mice. Moreover, terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) staining showed that the number of apoptotic chondrocytes was significantly reduced in the articular cartilage of the Fyn-KO mice (figure 2G). We also noticed reduced cartilage degradation in 12-month-old Fyn-KO mice as compared with that in control mice (online supplementary figure 3C,D). Together, these data suggest that Fyn plays a role in OA development and that the loss of Fyn efficiently prevents the development of post-traumatic and age-dependent OA in mice.

Fyn interacts with, phosphorylates (Tyr142) and stabilises β-catenin in chondrocytes

To explore the mechanism by which Fyn promotes the development of OA, we combined immunoprecipitation (IP) with a proteomic analysis to identify the Fyn-interacting proteins in the chondroprogenitor cell line ATDC5. Interestingly, the peptide sequence of the β-catenin protein was found in the purified complex, indicating that β-catenin is a potential binding partner of Fyn (figure 3A). The interaction between β-catenin and Fyn was confirmed in ATDC5 cells (figure 3B), primary chondrocytes (online supplementary figure 4A) and cartilage tissues (online supplementary figure 4B) with IP analyses. Double staining for β-catenin and Fyn also showed that β-catenin colocalises with Fyn in ATDC5 cells (figure 3C). Previous studies have demonstrated that tyrosine kinases, such as Fyn, phosphorylate β-catenin at multiple sites (including Tyr142) and promote the disassociation of the E-cadherin–catenin complex, resulting in the loss of cadherin-mediated cell–cell adhesion and an increase in cytoplasmic β-catenin. This represents an alternative mechanism for activating β-catenin signalling.[19 20] To determine whether Fyn phosphorylates β-catenin in chondrocytes, we conducted an in vitro Fyn kinase assay in ATDC5 cells. As shown in figure 3D, Fyn immunoprecipitated β-catenin phosphorylated at Tyr142 in vitro, and this phosphorylation was inhibited by the Fyn kinase inhibitor, PP1, or AZD0530. Importantly, the expressions of β-catenin and its phosphorylation (Tyr142) were

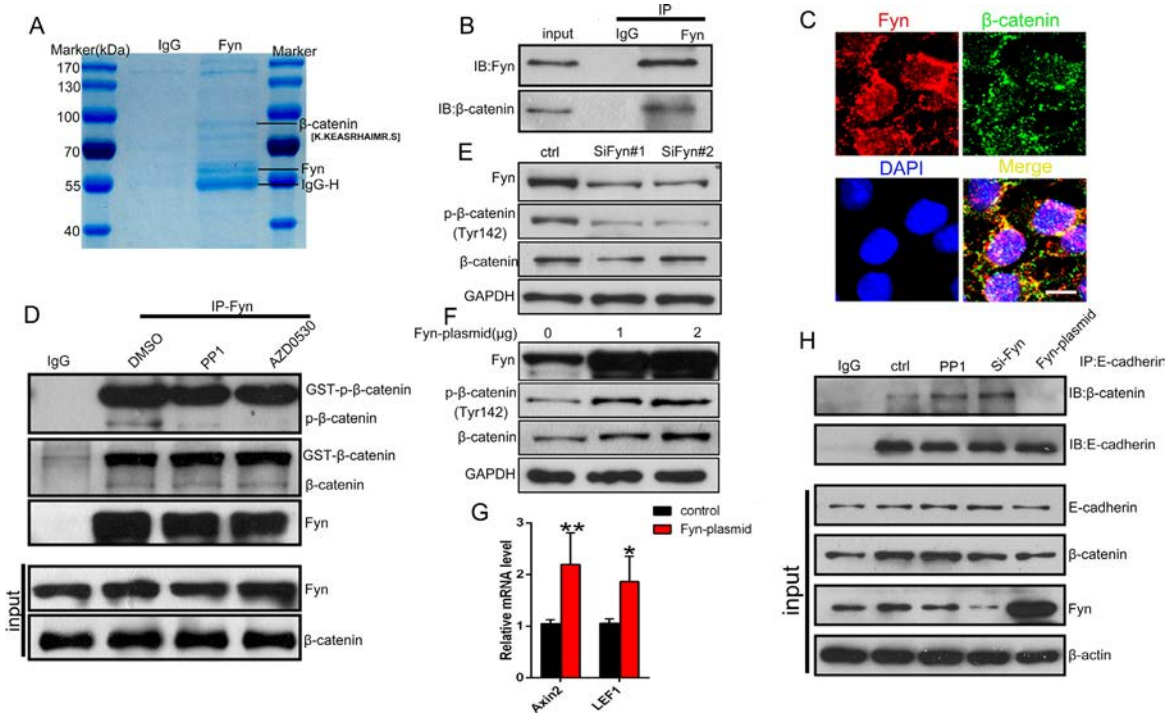

Figure 3 Fyn interacts with, phosphorylates (Tyr142) and stabilises β-catenin in chondrocytes. (A) ATDC5 cell extracts were subjected to immunoprecipitation with an anti-Fyn antibody. The immunoprecipitate–protein complex was separated with SDS–PAGE and the gel was then stained with Coomassie Brilliant Blue. The proteins were digested in-gel with proteases and identified with an LC–MS/MS analysis. The identified peptide sequence of β-catenin is shown (K.KEASRHAIMR.S). (B) Fyn was immunoprecipitated from ATDC5 cells with an anti-Fyn antibody. The presence of β-catenin and Fyn in these immunoprecipitates was evaluated with immunoblotting. Data are representative of three independent experiments. (C) ATDC5 cells were fixed for immunofluorescence analysis. Fyn was detected with a primary anti-Fyn antibody and Alexa-Fluor-594-conjugated goat anti-mouse IgG antibody, and β-catenin was detected with a primary anti-β-catenin antibody and Alexa-Fluor-488-conjugated goat anti-rabbit IgG antibody. Representative cells in the same field from each experimental group are shown. Scale bar, 10 μm. (D) ATDC5 cells were pretreated with PP1, AZD0530 or DMSO for 24 hours, and then immunoprecipitated with an anti-Fyn antibody. The precipitated Fyn was assayed for kinase activity against recombinant GST-tagged β-catenin. Data are representative of three independent experiments. (E) ATDC5 cells were transfected with negative control siRNA or two Fyn-specific small interfering RNAs (siRNAs) and different amounts (1 or 2 μg) of Fyn-encoding plasmid (F) for 60 hours. Expression levels of Fyn, β-catenin, p-β-catenin (Tyr142) and GAPDH were detected with immunoblotting. Data are representative of three independent experiments. (G) Relative mRNA expression levels of two Wnt canonical targets Axin2 and LEF1 in ATDC5 cells after transfected with a Fyn-encoding plasmid (1 μg). n=3, *P<0.05, **P<0.01. All data are shown as means±SD. (H) ATDC5 cells were pretreated with DMSO (ctrl) or PP1 for 24 hours, and transfected with siRNA or Fyn-encoding plasmid for 60 hours. The cell extracts were subjected to immunoprecipitation with an anti-E-cadherin antibody, and the levels of β-catenin and E-cadherin were analysed with immunoblotting. Data are representative of three independent experiments. DMSO, dimethyl sulfoxide; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GST, glutathione S-transferase; IB, immunoblotting; IP, immunoprecipitation; LC-MS/MS, liquid chromatography-tandem mass spectrometry; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

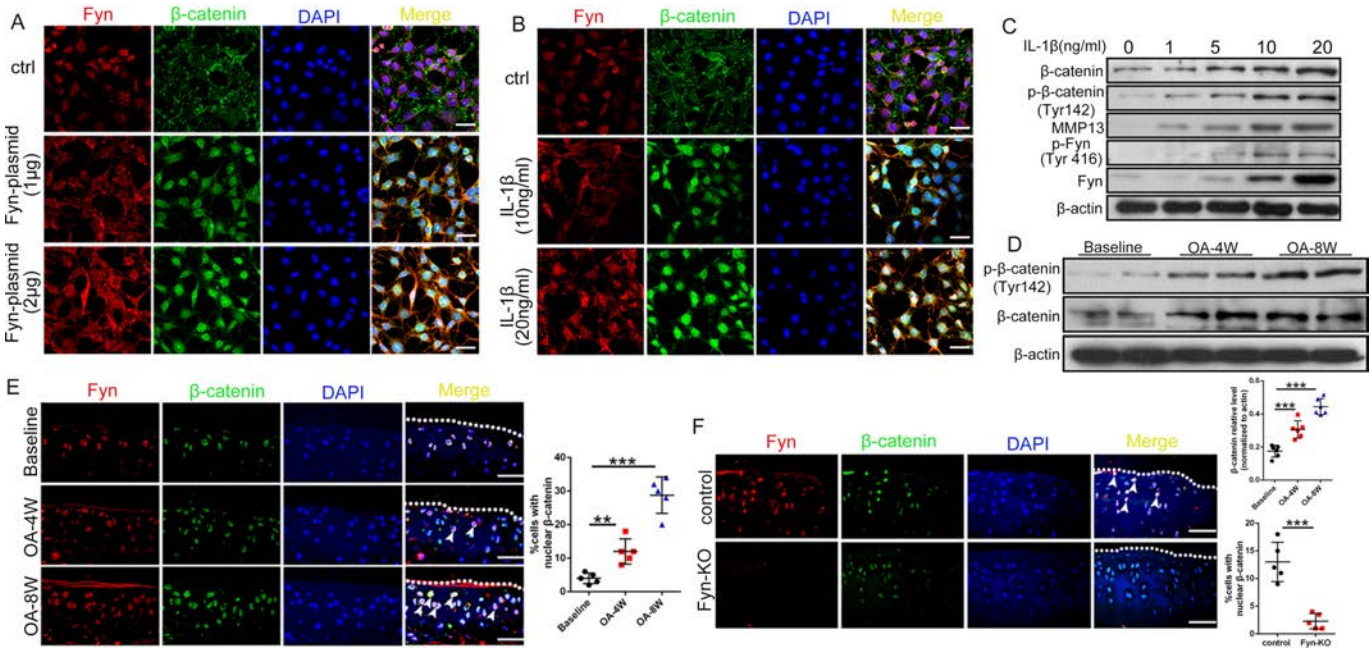

Figure 4 Fyn promotes the nuclear translocation of β-catenin and activates the β-catenin pathway in chondrocytes during osteoarthritis (OA) development. (A) Representative images of immunofluorescence for Fyn (red) and β-catenin (green) in ATDC5 cells after transfection with a Fyn-encoding plasmid (0, 1 or 2 μg) for 60 hours. Scale bar, 10 μm. (B) Representative images of immunofluorescence for Fyn (red) and β-catenin (green) in ATDC5 cells after treatment with different concentrations of IL-1β (0, 10 or 20 ng/mL) for 24 hours. Scale bar, 10 μm. (C) Immunoblotting analysis of β-catenin, p-β-catenin (Tyr142), matrix metalloproteinase 13 (MMP13), p-Fyn (Tyr416), Fyn, and β-actin in ATDC5 cells after incubation with different concentrations of IL-1β (0, 1, 5, 10 or 20 ng/mL) for 24 hours. Data are representative of three independent experiments. (D) Immunoblotting analysis of β-catenin, p-β-catenin (Tyr142) and β-actin in cartilage from sham-treated C57BL/6J mice or C57BL/6J mice at 4 or 8 weeks after induction of traumatic OA. Expression of β-catenin was quantified. Data are representative of three independent experiments. ***P<0.001. (E) Representative images of immunofluorescence (left) for Fyn (red) and β-catenin (green) and quantification (right) of cells with nuclear β-catenin in articular chondrocytes from sham-treated C57BL/6J mice or C57BL/6J mice 4 or 8 weeks after induction of traumatic OA. n=5. **P<0.01, ***P<0.001. Scale bar, 50 μm. (F) Representative images of immunofluorescence (left) for Fyn (red) and β-catenin (green) and quantification (right) of cells with nuclear β-catenin in articular chondrocytes from control and Fyn-KO mice. n=5. ***P<0.001. Scale bar, 50 μm. White arrows indicate β-catenin-positive nuclei (E and F). All data are shown as means±SD.

diminished by *Fyn* knockdown with a Fyn-directed small interfering RNAs (siRNAs) (figure 3E, online supplementary figure 4C), but were enhanced by Fyn overexpression from a Fyn-encoding plasmid in ATDC5 cells (figure 3F, online supplementary figure 4D). Additionally, the mRNA levels of Wnt canonical target Axin2 and LEF1 were also increased as Fyn was enhanced in ATDC5 cells (figure 3G), suggesting that Fyn activated β-catenin signalling. To verify the critical role of Fyn in the β-catenin signalling pathway, the formation of the cadherin–catenin complex was examined in ATDC5 cells. The inhibition of Fyn by PP1 or the downregulation of Fyn expression with siRNAs stabilised the cadherin–catenin complex, whereas Fyn overexpression disrupted the complex (figure 3H). Taken together, these data suggest that Fyn interacts with, phosphorylates (Tyr142) and stabilises β-catenin, causing the dissociation of the cadherin–catenin complex in chondrocytes.

Fyn activates the β-catenin pathway and promotes OA development

To evaluate the role of Fyn in β-catenin signalling in chondrocytes during OA, we doubly stained chondrocytes for Fyn and β-catenin in vitro and in vivo. When we transfected ATDC5 cells with increasing amounts of a Fyn-encoding plasmid (0, 1 or 2 μg), we observed that β-catenin translocated from the cell membrane to the nucleus as the level of Fyn increased (figure 4A). We then treated the ATDC5 cells with the proinflammatory factor interleukin 1β (IL-1β; 0, 10 and 20 ng/mL) to mimic the degradation of the ECMs and proteoglycans in OA.[21] IL-1β dose-dependently induced the accumulation of Fyn and β-catenin in both the cytoplasm and nuclei of the cells (figure 4B) and increased the levels of Fyn expression and phosphorylation (Tyr416), β-catenin expression and phosphorylation (Tyr142), and MMP13 expression (figure 4C). Similar results were observed in IL-1β-stimulated primary chondrocytes (online supplementary figure 5A,B). We then assessed the levels of β-catenin and its phosphorylation in cartilage from aged mice and mice with post-traumatic OA. As expected, the levels of β-catenin and its phosphorylation increased with ageing (online supplementary figure 5C) and the exacerbation of OA (figure 4D). Consistent with this, double staining for Fyn and β-catenin demonstrated the accumulation and nuclear translocation of β-catenin in the chondrocytes of aged mice (online supplementary figure 5D) and mice with surgically induced OA (figure 4E). Furthermore, the levels of β-catenin and its nuclear localisation were markedly lower in the articular chondrocytes of post-traumatic Fyn-KO mice than in the control mice (figure 4F). Wnt3a is an activator of canonical WNT/β-catenin pathway. Knockdown of Fyn did not reduce the Wnt3a-upregulated β-catenin (online supplementary figure 4E). Although WNT antagonist DKK-1 or SFRP1 prevented Wnt3a-induced accumulation of β-catenin, they did not affect upregulation of β-catenin caused by overexpression of Fyn in ATDC5 cells (online supplementary figure 4F). Thus, our results

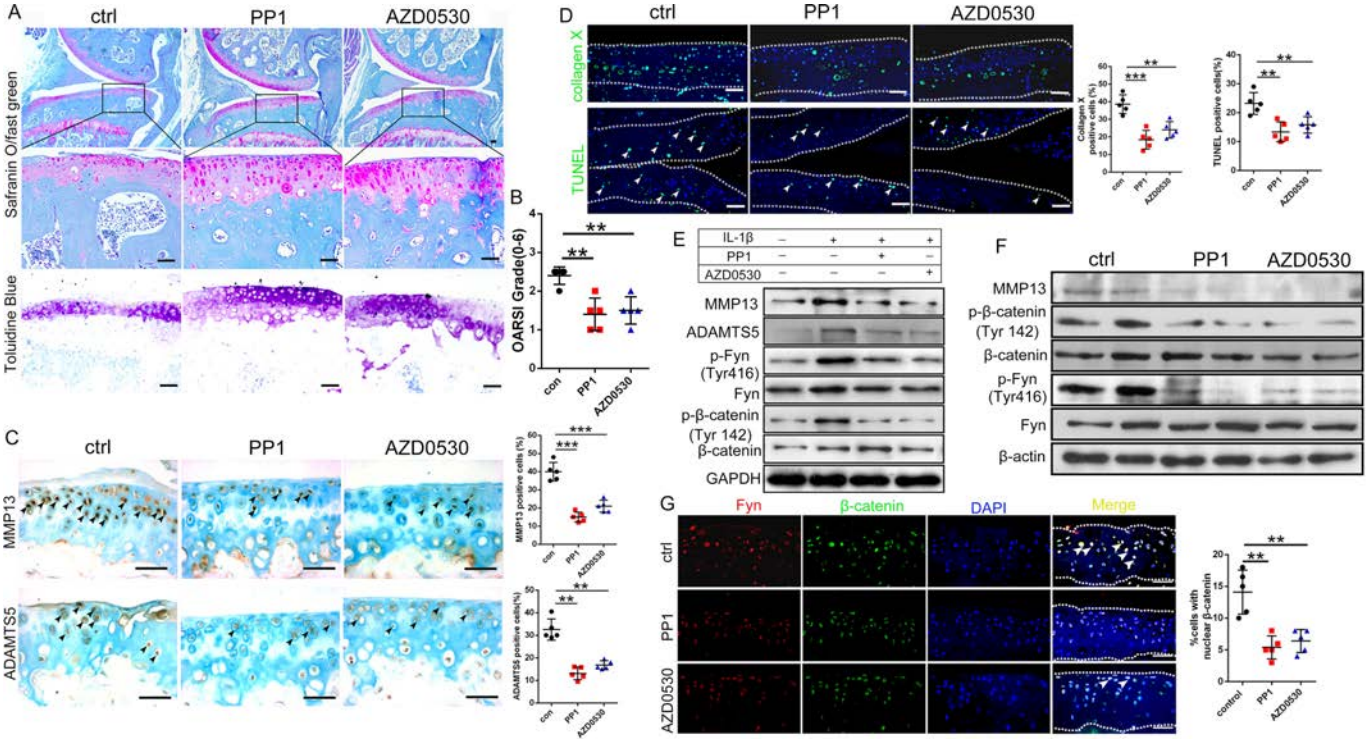

Figure 5 Fyn inhibitors AZD0530 and PP1 prevent surgically induced OA in mice by inhibiting β-catenin signalling. (A) Safranin O/Fast Green staining (upper), toluidine blue staining (lower) and Osteoarthritis Research Society International (OARSI) grades (right) (B) of joints from mice that underwent surgery to destabilise the medial meniscus (DMM mice) treated with vehicle, PP1 or AZD0530 for 4 weeks. n=5, **P<0.01. Scale bar, 50 µm. (C) Representative images of immunostaining (left) and quantification (right) of matrix metalloproteinase 13 (MMP13) (upper) and a disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS5) (lower) in joint cartilage from DMM mice treated with vehicle, PP1 or AZD0530 for 4 weeks. n=5, **P<0.01, ***P<0.001. Scale bar, 50 µm. (D) Representative images of immunofluorescence analysis (left) and quantification (right) of collagen X (upper) and terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) (lower) in joint cartilage from DMM mice treated with vehicle, PP1 or AZD0530 for 4 weeks. White arrowheads indicate positive cells. n=5. **P<0.01, ***P<0.001. Scale bar, 50 µm. (E) Immunoblotting analysis of MMP13, ADAMTS5, p-Fyn (Tyr416), Fyn, p-β-catenin (Tyr142), β-catenin and GAPDH in primary chondrocytes after incubation with vehicle, IL-1β, IL-1β plus PP1 or IL-1β plus AZD0530 for 24 hours. Data are representative of three independent experiments. (F) Immunoblotting analysis of the indicated proteins in articular chondrocytes from DMM mice treated with vehicle, PP1 or AZD0530 for 4 weeks. Data are representative of three independent experiments. (G) Representative images of immunofluorescence (left) for Fyn (red) and β-catenin (green) and quantification (right) of cells with nuclear β-catenin in articular chondrocytes from DMM mice treated with vehicle, PP1 or AZD0530 for 4 weeks. White arrows indicate β-catenin in the nuclei. n=5 per group. **P<0.01. Scale bar, 50 µm. All data are shown as means±SD. GADPH, glyceraldehyde-3-phosphate dehydrogenase .

demonstrate that Fyn activates the β-catenin pathway independent of Wnt signalling and enhances the nuclear translocation of β-catenin in articular cartilage chondrocytes during the development of OA.

We next investigated whether Fyn accumulation promotes OA development through the β-catenin pathway. ICG-001 is a selective low-molecular-weight inhibitor that antagonises β-catenin/TCF-mediated transcription.[22 23] After the articular injection of ICG-001 for 4 or 8 weeks, ICG-001 treatment markedly reduced cartilage damage, maintained the proteoglycan in the articular cartilage (online supplementary figure 6A) and reduced the arthritis grades during the development of OA (online supplementary figure 6B). The expression levels of MMP13 and ADAMTS5 also decreased correspondingly in the degenerated cartilage after ICG-001 injection (online supplementary figure 6C,D). Subsequently, we measured the effects of Fyn, the β-catenin inhibitor ICG-001 and the β-catenin activator SKL2001 on IL-1β-treated ATDC5 cells. Fyn overexpression reinforced the effects of IL-1β on the upregulation of MMP13 and ADAMTS5, whereas treatment with ICG-001 counteracted the Fyn-promoted expression of both enzymes induced by IL-1β (online supplementary figure 6E). SKL2001 upregulated the IL-1β-stimulated expression of MMP13 and ADAMTS5 in chondrocytes, whereas Fyn knockdown downregulated it (online supplementary figure 6F). These results demonstrate that the β-catenin pathway contributes to Fyn-stimulated MMP13 and ADAMTS5 expression in chondrocytes and the development of OA in mice.

Fyn inhibitors AZD0530 and PP1 inhibit β-catenin signalling and prevent OA development in mice

To investigate the potential use of Fyn as a therapeutic target for OA, we examined the effects of chemical inhibitors of Fyn on the pathogenesis and progression of OA. AZD0530 (also known as 'saracatinib') and PP1 are selective inhibitors of Src kinases (including Fyn), which were used in the treatment of cancer. These two compounds inhibited the kinase activity of Fyn and blocked Fyn phosphorylation (p-Fyn) in ATDC5 cells transfected with Fyn-encoding plasmids (online supplementary figure 7A,B). C57BL/6J mice treated with DMM surgery were orally administered AZD0530 or intraperitoneally injected with PP1 every day, and their joints were collected 4 weeks after surgery. The mice

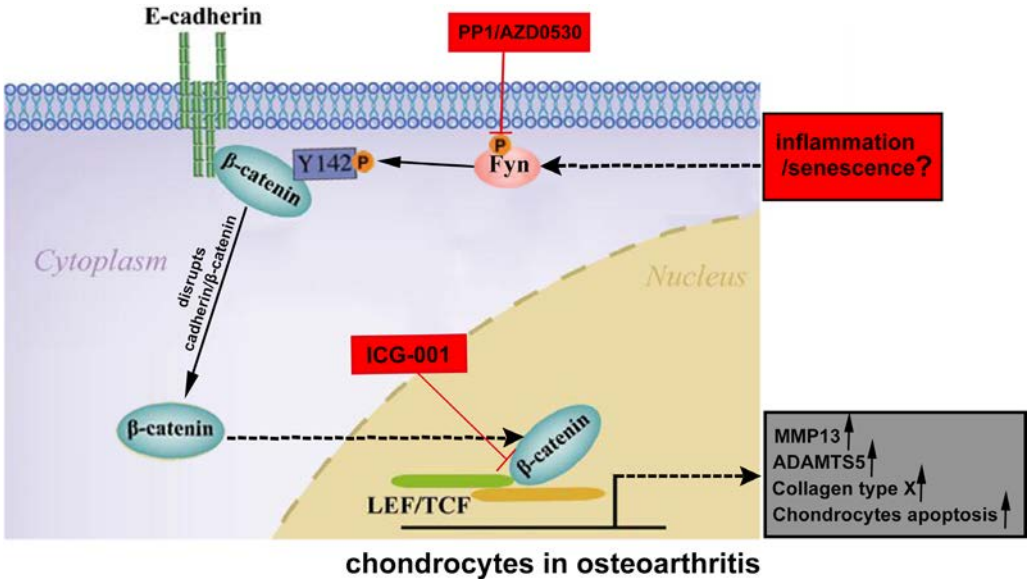

Figure 6 Proposed model for the role of Fyn in the development of OA. ADAMTS5, a disintegrin and metalloproteinase with thrombospondin motifs 5; LEF/TCF, lymphoid-enhancing factor/T-cell factor; MMP13, matrix metalloproteinase 13.

treated with AZD0530 or PP1 showed no significant adverse effects and no increase in mortality compared with the controls (body weight shown in online supplementary figure 7C). As expected, AZD0530 and PP1 significantly reduced p-Fyn level in the chondrocytes of the cartilage, confirming its inhibitory effect on Fyn (online supplementary figure 7D). Notably, the AZD0530 and PP1 treatments markedly reduced the destruction of the cartilage (figure 5A), OARSI grades (figure 5B) and synovial inflammation (online supplementary figure 7E) compared with those of the vehicle-treated mice. The levels of MMP13, ADAMTS5 and collagen X, the number of TUNEL-positive chondrocytes and osteocalcin expression in subchondral bone were also significantly reduced in the inhibitor-treated mice (figure 5C,D, online supplementary figure 7F). These results suggest that the inhibition of Fyn kinase activities prevents the development of post-traumatic OA in mice.

We next determined whether AZD0530 or PP1 ameliorates the progression of OA by reducing the phosphorylation of β-catenin. After stimulation with IL-1β, ATDC5 cells were treated with or without AZD0530 or PP1. As indicated in figure 5E, western blotting showed that the upregulation of MMP13 and ADAMTS5 caused by IL-1β stimulation was abolished by AZD0530 or PP1, and the increase in p-β-catenin (Tyr142) was also inhibited. We confirmed the reduced expression of p-β-catenin (Tyr142) with immunofluorescence staining (online supplementary figure 7G). We next measured the expression of p-β-catenin (Tyr142) in cartilage samples from mice with post-traumatic OA treated with or without AZD0530 or PP1. A western blotting analysis confirmed that the expressions of MMP13 and p-Fyn were lower and the expression of p-β-catenin (Tyr142) was significantly reduced in the AZD0530-treated and PP1-treated mice (figure 5F). We also noted that the colocalisation of Fyn and β-catenin in the nuclei of chondrocytes in OA was abolished by treatment with AZD0530 or PP1 (figure 5G). Incubation of human OA cartilage explants with AZD0530 or PP1 also decreased β-catenin expression in the nuclei of chondrocytes (online supplementary figure 7H). Collectively, these findings suggest that AZD0530 and PP1 prevent the development of OA by inhibiting the expression of Fyn-induced phosphorylation β-catenin.

DISCUSSION

There are currently no effective disease-modifying drugs approved for the treatment of OA. A comprehensive understanding of the pathogenesis in OA is essential for the development of specific and effective drugs to prevent and treat this disease.[24 25] The roles of SFKs in many diseases have been described, including cancer and neurological disorders, and they are therefore emerging as novel pharmacological targets.[26–28] However, to the best of our knowledge, the present study is the first to demonstrate the essential role of the SFK family member Fyn in cartilage degeneration and the development of OA. We found that Fyn accumulates in the degenerated and damaged articular cartilage of ageing mice, mice with experimental OA and humans with OA. We also found that Fyn deficiency protected mice against age-related and trauma-induced cartilage degradation and the development of OA. Importantly, the disruption of Fyn or its chemical inhibition with AZD0530 or PP1 effectively prevented the development of OA in mice, implying that Fyn is potentially a novel therapeutic target for OA, and that the use of drugs AZD0530 and PP1, which inhibit Fyn kinase activity, might be extended to the treatment of OA. We propose a model in which Fyn activates β-catenin signalling, and the accumulation of Fyn, and the tyrosine phosphorylation and nuclear translocation of β-catenin play vital roles in regulating the expression of matrix-degrading enzymes and the development of OA (figure 6). However, the mechanism underlying the accumulation of Fyn in OA and aged articular cartilage remains to be identified.

The activation of β-catenin in articular chondrocytes constitute a pathological mechanism for the conversion of normal articular chondrocytes into terminally differentiated chondrocytes during the development of OA, which is associated with the activation of chondrocyte-maturation genes and matrix degradation.[29] However, the mechanism of β-catenin activation is not fully understood. In the canonical WNT/β-catenin pathway, the activation of WNT signalling leads to the inhibition of GSK-3β activity and β-catenin degradation, resulting in the accumulation of cytoplasmic (signalling) β-catenin.[19] We screened for Fyn-interacting proteins and demonstrated that Fyn interacts with,

phosphorylates β-catenin at Tyr142 and stabilises it independent of Wnt signalling in chondrocytes. Previous studies have shown that tyrosine kinases can phosphorylate β-catenin, releasing it from the cadherin complex and leading to the accumulation of β-catenin in the cytoplasm, which represents an alternative mechanism by which β-catenin signalling is activated.[30] We also confirmed that Fyn phosphorylates β-catenin, thus releasing it from the cadherin complex in chondrocytes, leading to the accumulation and nuclear translocation of β-catenin and the activation of chondrocyte-maturation genes and matrix degradation, which promote the development of OA. This evidence suggests that Fyn activates β-catenin signalling via a Wnt-independent mechanism to promote the pathogenesis and progression of OA.

The established mechanisms that inhibit the development of OA largely depend on the inhibition of enzymes that catabolise cartilage.[2] We found that the deletion of Fyn inhibited the expression of cartilage-catabolising enzymes and attenuated OA in mice. Therefore, further investigation of the targeted inhibition of Fyn for the prevention and treatment of OA is warranted. AZD0530 (saracatinib) is an inhibitor of the SFKs, blocking Src with low (nanomolar) potency and also displaying activity against Fyn.[31] It was originally developed to treat various types of cancer, but was discontinued in phase II trials for its lack of efficacy. PP1 was identified as a high-potency inhibitor of Fyn and acts as a competitive inhibitor of ATP binding. Currently, both AZD0530 and PP1 are mainly under investigation for the treatment of solid tumours, lung cancer and Alzheimer's disease.[32] In this study, we have demonstrated that OA progression can be prevented by targeting Fyn with these two inhibitors. Moreover, the Fyn-stimulated accumulation and nuclear translocation of β-catenin is also inhibited by AZD0530 and PP1. OA-related catabolic enzymes and chondrocyte apoptosis in the articular cartilage decreased significantly after treatment with either inhibitor. This is the first report of the role of SFKs in OA, and the use of SFK and Fyn inhibitors may be successfully extended to its treatment.

In conclusion, in this study, we identified Fyn as a novel target for the prevention and treatment of OA. We also established links between Fyn, β-catenin and OA by demonstrating that the Fyn accumulated in articular chondrocytes during OA interacts directly with and phosphorylates β-catenin, promoting its nuclear translocation and inducing OA-related gene expression. An inhibitor of SFKs, AZD0530, and an inhibitor of Fyn, PP1, delay the development of OA and are therefore potential drugs for the treatment of this disease.

MATERIALS AND METHODS

Detailed experimental procedures are described in online supplementary materials and methods, including human and experimental OA, animals, histology, immunohistochemistry, cell line and primary chondrocyte culture, iTRAQ labelling and LC–MS/MS proteomics, siRNA and plasmid, antibodies, immunoblotting, immunoprecipitation, TUNEL staining, drug treatment and statistical analysis.

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Contributors KL: study design, data acquisition, data analysis, wrote the manuscript, DMM experiments. YueZ, YuwZ, WJ and JS: data analysis, discussion of results, confocal imaging and analysis. SX, QS and DC: data acquisition, histochemistry and western blotting analysis. BH, AL and ML: data acquisition. JS and YJ: statistical analyses. XB: study design, manuscript correction.

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Competing interests None declared.

Patient consent Obtained.

Ethics approval All animal experiments were approved by the Southern Medical University Animal Care and Use Committee (Guangzhou, China).

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EXTENDED REPORT

Differential ubiquitination in NETs regulates macrophage responses in systemic lupus erythematosus

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ABSTRACT

Objectives To assess if ubiquitinated proteins potentially present in neutrophil extracellular traps (NETs) can modify cellular responses and induce inflammatory mechanisms in patients with systemic lupus erythematosus (SLE) and healthy subjects.

Materials and methods We studied 74 subjects with SLE and 77 healthy controls. Neutrophils and low-density granulocytes were isolated, and NETs were induced. Ubiquitin content was quantified in NETs by western blot analysis, ELISA and immunofluorescence microscopy, while ubiquitination of NET proteins was assessed by immunoprecipitation. Monocyte-derived macrophages from SLE and controls were isolated and stimulated with NETs or ubiquitin. Calcium flux and cytokine synthesis were measured following these stimuli.

Results NETs contain ubiquitinated proteins, with a lower expression of polyubiquitinated proteins in subjects with SLE than in controls. Myeloperoxidase (MPO) is present in ubiquitinated form in NETs. Patients with SLE develop antiubiquitinated MPO antibodies, and titres positively correlate with Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) score ($P<0.01$), and negatively correlate with complement components ($P<0.01$). Stimulation of monocyte-derived macrophages with NETs or with ubiquitin led to enhanced calcium flux. In addition, stimulation with NETs led to enhanced cytokine (tumour necrosis factor-α and interleukin-10) production in macrophages from patients with SLE when compared with controls, which was hampered by inhibition of NET internalisation by macrophages.

Conclusion This is the first study to find ubiquitinated proteins in NETs, and evidence for adaptive immune responses directed towards ubiquitinated NET proteins in SLE. The distinct differences in ubiquitin species profile in NETs compared with healthy controls may contribute to dampened anti-inflammatory responses observed in SLE. These results also support a role for extracellular ubiquitin in inflammation in SLE.

INTRODUCTION

Neutrophil extracellular traps (NETs) are extracellular fibres primarily composed of nucleic acids bound to neutrophil granule-derived proteins.[1] The process of NET formation (NETosis) has recently been associated with the pathogenesis of systemic lupus erythematosus (SLE), since it causes the exposure of modified (oxidised) extracellular DNA.[2 3] Increased NET formation occurs in SLE, in particular mediated by low-density granulocytes (LDGs), a proinflammatory neutrophil subset increased in patients with lupus.[4] In addition, an impaired NET degradation has been described in SLE, promoting an imbalance that prolongs the half-life of NET components.[5 6] Macrophages, which have been found to be defective in phagocytosing autoantigens in patients with SLE,[7] are involved in NET clearance.[8] While NET degradation by macrophages has not been found to be proinflammatory in healthy subjects,[8] the process has not been studied in patients with SLE.

Post-translational modifications (PTMs) can alter protein structure and function. Different NET components, such as histones, are susceptible to undergo PTMs (ie, acetylation, citrullination).[9 10] Ubiquitination is a PTM that has typically been associated with protein degradation, but it can also impact many cellular processes by modifying protein function and gene transcription.[11–13] In patients with SLE, defects in ubiquitination have been proposed to promote loss of peripheral tolerance.[14] Furthermore, extracellular ubiquitin has been recently described as an immune system regulator in diverse conditions.[15–18] When extracellular ubiquitin binds its receptor, CXCR4,[19] a signalling cascade promotes numerous intracellular processes, including increases in calcium flux.[19 20]

Since NET proteins may be the target of different PTMs and ubiquitination is associated with immune tolerance, it is relevant to address whether NETs from patients with SLE are differentially ubiquitinated, in order to analyse the implications of this process in the immune dysregulation characteristic of this disease. Therefore, the aim of this study was to evaluate the potential role of ubiquitination in inflammatory responses triggered by NETs in patients with SLE and healthy controls.

METHODS

For full methods, see online supplementary text.

Patients and controls

We included 74 subjects with SLE, who fulfilled ≥4 American College of Rheumatology (ACR)[21] classification criteria and had different levels of disease activity according to the SLEDAI score,[22] as well as 77 healthy controls. All subjects signed informed consent.

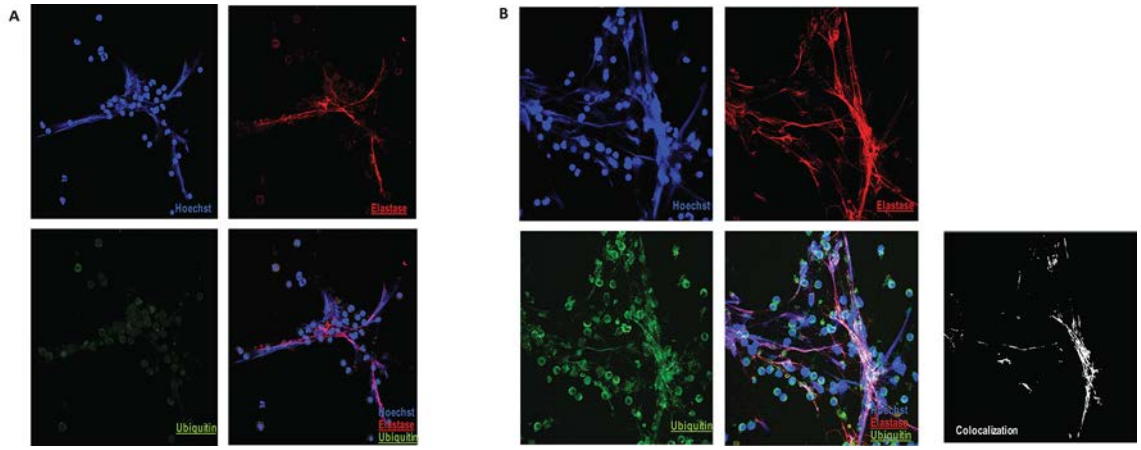


Figure 1 Ubiquitin is expressed in neutrophil extracellular traps (NETs) from patients with systemic lupus erythematosus (SLE) and healthy controls. Immunofluorescence of NETs from a representative patient with SLE (A) and a representative healthy control (B), analysed with confocal microscopy; red represents neutrophil elastase, blue represents DNA and green represents ubiquitin. Images positive for neutrophil elastase and nuclear stain, with a characteristic morphology, were classified as NETs. A representative image is shown, demonstrating colocalisation of ubiquitin with the other NET components (B); NIH ImageJ2 software analysis with the colocalisation RGB plugin was performed to find colocalised pixels in red, green and blue channels. Results are displayed in black and white; white represents the merged image of the three channels.

RESULTS

Most subjects were women (89%), with a mean age of 32±11 years. Fifty-nine subjects with SLE (78.6%) had a SLEDAI score ≥6. Most patients (64%) did not have any immunosuppressive treatment at the time of the blood draw. The most common disease activity at the time of inclusion was renal (68%). SLE-associated variables are shown in online supplementary table S1. Numbers and main characteristics of patients included for each experiment are shown in online supplementary table S2. Confirming previous results, when measured by Sytox green, lupus neutrophils had an increased NET synthesis compared with controls (P<0.001).

NETs contain ubiquitinated proteins and SLE NETs display lower K63-mediated polyubiquitination

Ubiquitin was detected in NETs of both patients with SLE and controls by immunofluorescence microscopy (figure 1). In order to confirm these results, an ELISA was performed to detect ubiquitin present in NETs. After analysing NETs from control normal-density granulocytes (NDGs) and lupus NDGs and LDGs, we found a lower ubiquitin concentration in NETs derived from SLE neutrophils compared with healthy controls (P<0.05). NETs from lupus LDGs had the lowest amounts of ubiquitin (P<0.01 vs healthy controls, P<0.05 vs SLE NDGs; figure 2), suggesting that NETs produced by cells with a higher proinflammatory capacity[3] have less ubiquitinated protein cargo.

Furthermore, ubiquitinated proteins, specifically polyubiquitinated through lysine 48 and lysine 63, were detected by western blot analysis in both NETs from subjects with SLE and healthy controls (figure 3). A differential ubiquitination pattern was found between subjects with SLE and healthy controls by western blot analysis, with a lower expression of K63 polyubiquitinated proteins in subjects with SLE (P<0.05), as shown in figure 3A. There was also a trend for significant differences in diminished expression of K48 polyubiquitinated proteins in lupus NETs compared with controls (P=0.065, figure 3B). We did not find a significant difference in the ubiquitin content in NETs (P=0.124) in patients under immunosuppressive treatment in comparison to those untreated. Overall, these results indicate that ubiquitinated proteins are detected in NETs and that lupus and control NETs display different ubiquitination patterns.

Antibody responses to ubiquitinated NET proteins develop in patients with SLE

After demonstrating the presence of ubiquitinated proteins in NETs, we searched for specific NET components that could be ubiquitin targets. We specifically focused on myeloperoxidase (MPO) because it is a major component of NETs,[23] and because it has an antigenic role in certain autoimmune diseases. Using immunoprecipitation assays, we detected ubiquitinated MPO in NETs from subjects with SLE and controls (see online supplementary figure S2).

In order to determine if adaptive immune responses develop to ubiquitinated MPO (Ub-MPO), sera from patients with SLE and healthy controls was analysed by ELISA to detect antibodies against Ub-MPO and non-Ub-MPO. For this assay, we included

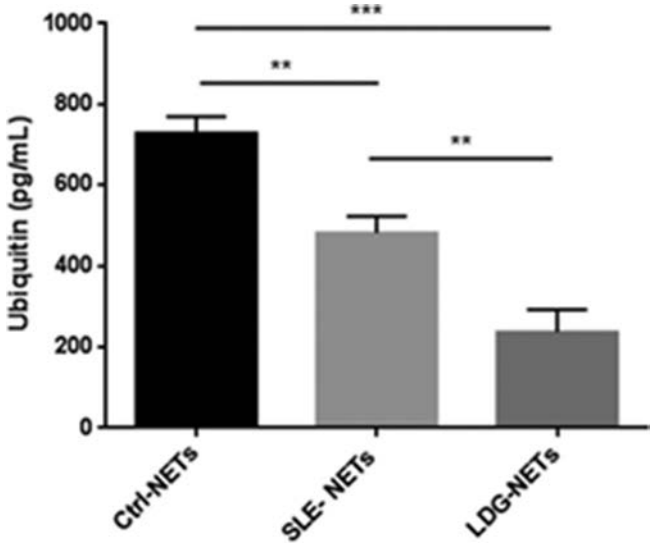


Figure 2 Differential ubiquitin concentration in neutrophil extracellular traps (NETs) from control and lupus normal-density granulocytes (NDGs) and lupus low-density granulocytes (LDGs). ELISA for total ubiquitin in NETs from healthy control neutrophils (Ctrl-NETs), lupus NDGs (systemic lupus erythematosus (SLE)-NETs) and lupus LDGs (LDG-NETs) (n=5 subjects per group). Bars represent mean±SEM of ubiquitin concentration in NETs from each group of subjects. **P<0.01, ***P<0.001.

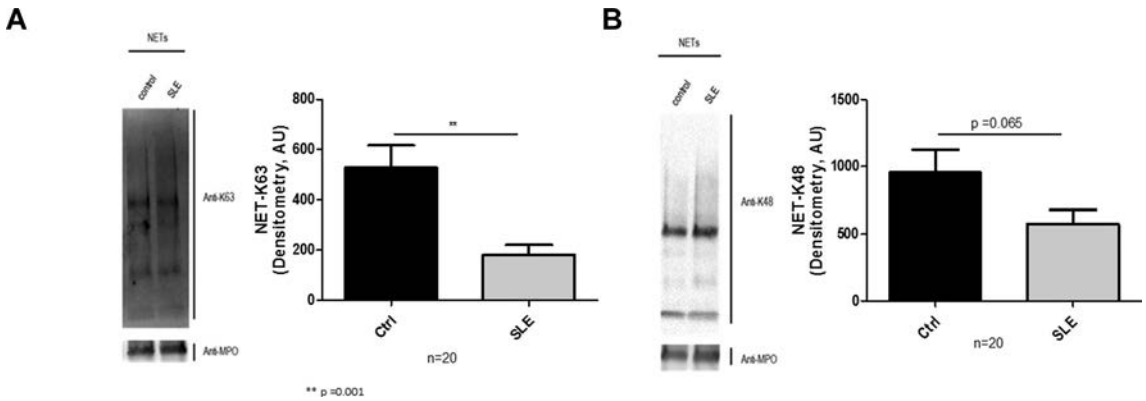


Figure 3 Neutrophil extracellular traps (NETs) from patients with systemic lupus erythematosus (SLE) have decrease in K63 polyubiquitin species. Western blot analysis of NETs from patients with SLE and controls was performed, and quantification was done by densitometry (n=20 subjects per group). Representative blots are included for both types of ubiquitin conjugation profile. Results are expressed as mean±SEM. There was less expression of K63-dependent (A) and K48-dependent (B) polyubiquitinated proteins in SLE NETs than in healthy control NETs. **P<0.01.

sera from 57 subjects with SLE and55 healthy controls. Considering the established cut-off value described in the 'Methods' section in online supplementary text, 21% of patients with SLE had antibodies against Ub-MPO, and 14% had antibodies against non-Ub-MPO, whereas only 3.6% and 1.8% of healthy controls had antibodies against both ubiquitinated and native MPO molecules, respectively. When compared with controls, patients with SLE displayed significantly increased titres of anti-Ub-MPO by a normalised optical density (OD) index (2.17 vs 1.26, P=0.0008), as well as antinative MPO antibodies (1.89 vs 1, P=0.001). Furthermore, when comparing antibodies against ubiquitinated and native MPO proteins, there was a higher concentration against Ub-MPO in subjects with SLE (2.17 vs 1.89, P<0.0001; figure 4).

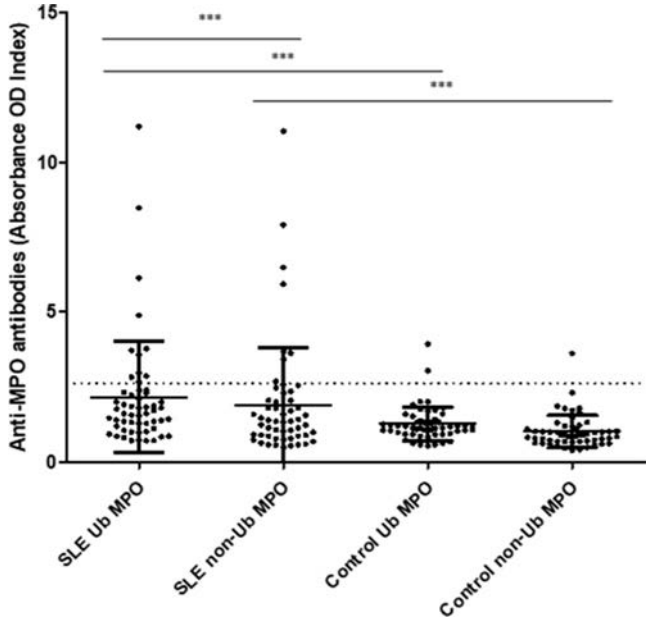


Figure 4 Serum samples from patients with systemic lupus erythematosus (SLE) contain antiubiquitinated myeloperoxidase (anti-Ub-MPO) antibodies. An in-house ELISA was performed to quantify antibodies against Ub-MPO (purified from human neutrophils) and native (non-ubiquitinated) MPO (recombinant protein) in serum from 57 patients with SLE and 55 healthy controls. Dots represent normalised values from individual subjects. The dotted line represents the established cut-off value. ***P≤0.001.

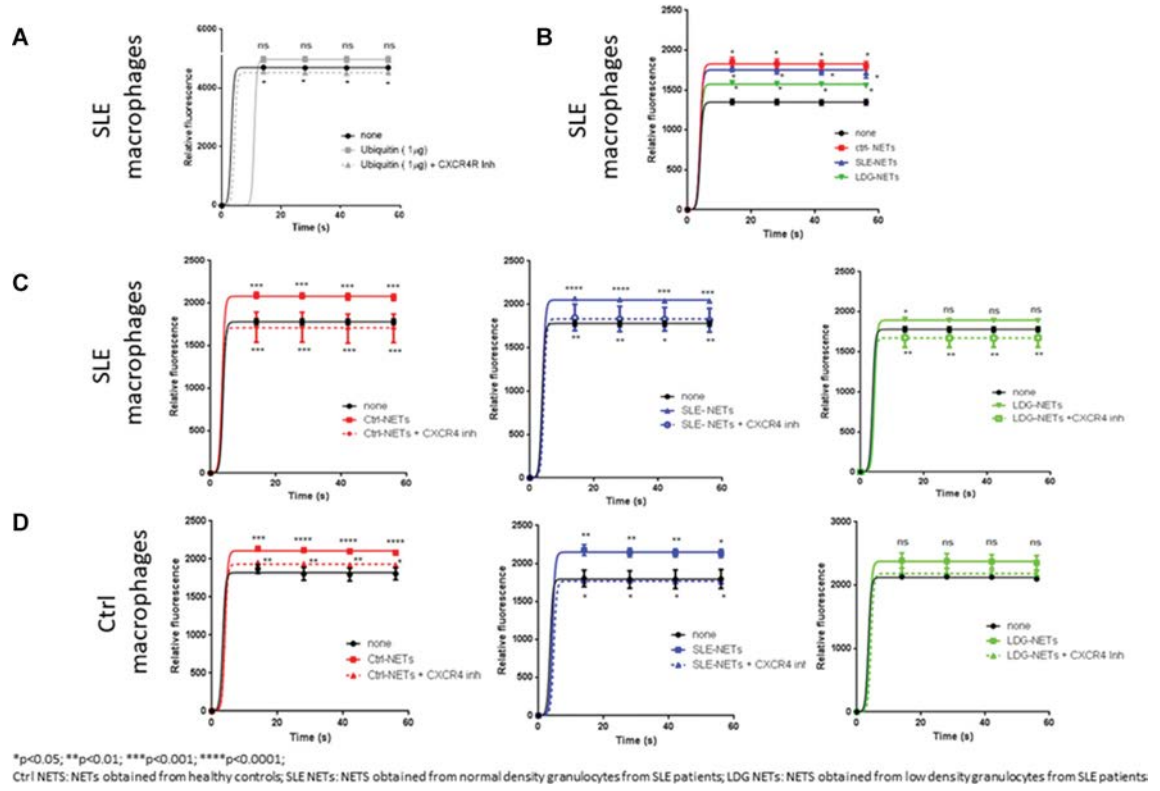
Anti-Ub-MPO antibody levels positively correlated with SLEDAI (r=0.44, P<0.01), and negatively with complement components (C3: r=−0.474, P<0.01; C4: r=−0.426, P<0.01), while no correlation with anti-dsDNA antibody levels was found (P=0.43). Moreover, patients with positive anti-Ub-MPO titres had a higher mean SLEDAI score (17.8 vs 9.58, P=0.022) and a higher frequency of vasculitis (P=0.03) compared with those with negative anti-Ub-MPO antibodies. Overall, these results suggest that patients with lupus, especially those with high disease activity, mount antibody responses to ubiquitinated NET components.

The activation of CXCR4, the receptor for extracellular ubiquitin, causes an increase in calcium flux

Extracellular ubiquitin is a natural CXCR4 agonist, and this axis is involved in various CXCR4-mediated cellular processes.[19] As NETs externalise ubiquitin, and subjects with SLE and controls display different ubiquitination patterns in NETs, we assessed the role of ubiquitin and NETs on macrophage responses in the presence and absence of a CXCR4 inhibitor. When macrophages were stimulated with recombinant extracellular ubiquitin, there was a trend for an increase in basal calcium flux, which significantly diminished in the presence of a CXCR4 inhibitor (AMD 3100) (figure 5A). When SLE and control macrophages were stimulated with NETs from control or SLE NDGs or from SLE LDGs, increased calcium flux was also triggered, and it similarly decreased in the presence of a CXCR4 inhibitor (figure 5C and D). These results suggest that there is activation of CXCR4 by ubiquitin in NETs. When the response from SLE macrophages was evaluated, the largest increase in calcium flux was observed with control NETs, while the lowest increase occurred with LDG NETs (figure 5B). These findings correlated with the ubiquitin concentration gradient found by ELISA in NETs. We did not find significant differences in calcium flux when macrophages were stimulated with NETs derived from neutrophils exposed to PYR-41 (E1 inhibitor). This finding could be related to inability to completely remove ubiquitin in NETs (see online supplementary figure S3). Overall, these results suggest that ubiquitinated proteins are one of the NET components able to modify calcium flux in macrophages.

Macrophages internalise NETs and this process is proinflammatory in SLE

Monocyte-derived macrophages from subjects with SLE and controls were cultured in the presence of NETs, and internalised



*p<0.05; **p<0.01; ***p<0.001; ****p<0.0001;
Ctrl NETS: NETs obtained from healthy controls; SLE NETs: NETS obtained from normal density granulocytes from SLE patients; LDG NETs: NETS obtained from low density granulocytes from SLE patients.

Figure 5 Extracellular ubiquitin and neutrophil extracellular traps (NETs) increase calcium flux in macrophages. Monocyte-derived macrophages from subjects with systemic lupus erythematosus (SLE) and healthy controls were obtained. They were stimulated with recombinant ubiquitin (1 µg) (A) or with 50 µg of NETs from healthy controls, lupus normal density granulocytes and lupus low-density granulocytes (LDGs) (B–D). In addition, a CXCR4 (extracellular ubiquitin receptor) inhibitor was used (A, C, D). Calcium flux was measured by Fluo-4 NW Calcium Assay Kit in all cases (five independent experiments). Calcium flux was enhanced by recombinant ubiquitin (A) and by NETs (B–D), and significantly decreased when a CXCR4 inhibitor was added. There was also a differential calcium influx in response to NETs from healthy controls, patients with SLE and LDGs. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001; ns, non-significant. Ctrl-NETs, NETs obtained from healthy controls; SLE-NETs, NETs obtained from normal density granulocytes from patients with SLE; LDG-NETs, NETs obtained from low-density granulocytes from patients with SLE.

NET components after 6 hours incubation (ie, as assessed by neutrophil elastase, which is not expressed by macrophages (figure 6A)). In order to address whether the internalisation of lupus NET components is a proinflammatory process in macrophages,[8] macrophage supernatants were examined for cytokine release (tumour necrosis factor (TNF)-α, interleukin (IL)-6 and IL-10) after exposure to NETs from subjects with SLE (n=18). In SLE macrophages, TNF-α and IL-10 release was enhanced following stimulation with lipopolysaccharide (LPS)-induced NETs, when compared with the effect observed in control macrophages (P<0.05; figure 6B-D). There was also a trend towards an increased IL-6 synthesis by SLE macrophages in response to NETs. This differential cytokine profile between patients with SLE and controls was not found after stimulation with spontaneous NETs or LPS alone (data not shown). Finally, we also used chloroquine, mainly as a toll like receptor (TLR) antagonist,[24 25] as well as a NET internalisation inhibitor.[26] We found that SLE macrophages that were pretreated with chloroquine showed a decrease in IL-6 and TNF-α synthesis, but not IL-10, after NET stimulation (P<0.01 and P<0.05, respectively; figure 6B-D). Besides, on CXCR4 inhibition, macrophages from controls had a decrease in TNF-α (P=0.027) and IL-10 (P=0.012) production (figure 6B-D). We found no significant effect of CXCR4 inhibition on cytokine production from lupus macrophages, which could be related to the dysregulation and higher expression of CXCR4 in patients with SLE.[27] These findings suggest that NET internalisation by macrophages is not a silent process, at least in lupus macrophages, and that extracellular ubiquitin-CXCR4 pathway may be partially implicated in cytokine induction triggered by NET internalisation. These results also suggest that chloroquine interferes with NET internalisation by lupus macrophages.

DISCUSSION

Several groups have proposed that NETs play pathogenic roles in SLE.[28 29] Therefore, characterising the role of specific NET modifications and their potential immunogenicity is fundamental in understanding how NETs can promote immune dysregulation and tissue damage. To our knowledge, we now describe for the first time that there are ubiquitinated proteins in NETs, as well as the ubiquitination of myeloperoxidase, one of the most abundant NET proteins.

We found polyubiquitinated proteins in NETs, which could have diverse implications in the immune system. Specifically, K63 ubiquitination is involved in DNA repair, signalling through nuclear factor-κB, and endosomal traffic regulation, all of which are related to the modulation of immune responses.[30] Also, we found decreased ubiquitination in NETs from subjects with SLE compared with controls. Indeed, LDG NETs had the lowest expression of ubiquitinated proteins. This could be related to the lower expression of ubiquitin ligases in SLE, as demonstrated with Cbl-b in lymphocytes.[14] Regarding the effect that this differential ubiquitin expression in NETs may have, extracellular ubiquitin has been associated with a predominantly

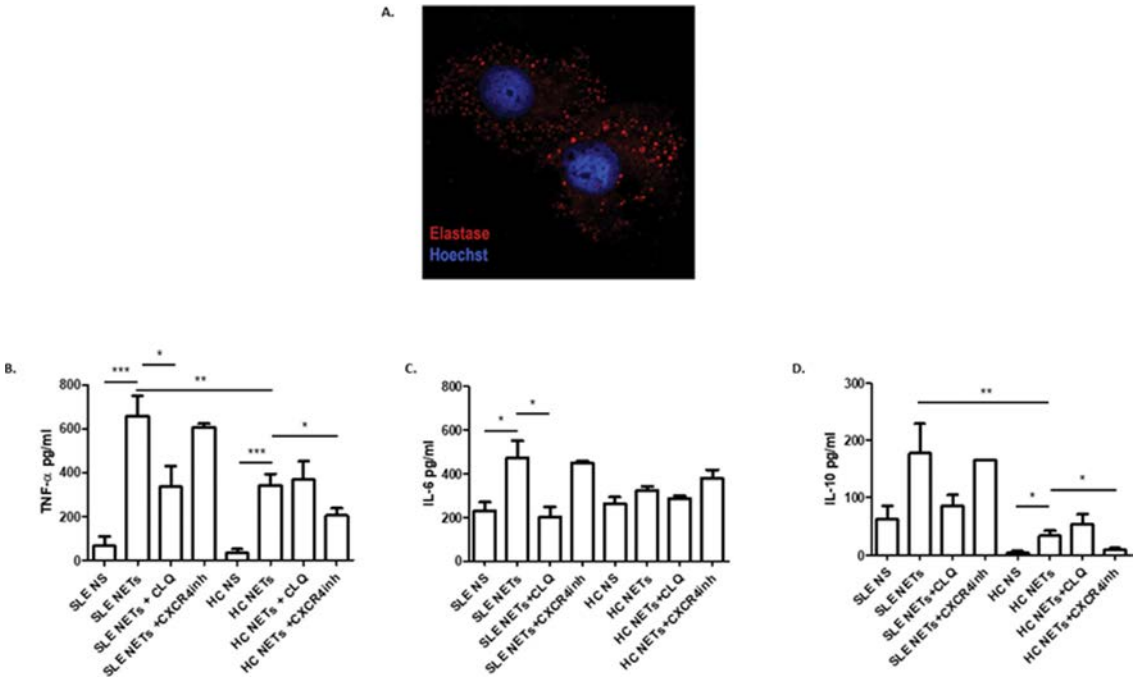


Figure 6 Systemic lupus erythematosus (SLE) macrophages internalise neutrophil extracellular traps (NETs) and synthesise cytokines in response to NET internalisation. Monocyte-derived macrophages from subjects with SLE and healthy subjects were incubated with 50 µg of normal-density granulocytes NETs from subjects with SLE for 6 hours (n=18). (A) Immunofluorescence was performed and analysed by confocal microscopy. Red represents neutrophil elastase and blue represents DNA. A representative image is shown, demonstrating internalisation of neutrophil elastase from NETs by macrophages. (B–D) Supernatants were obtained, and cytokine levels (tumour necrosis factor (TNF)-α, interleukin (IL)-6 and IL-10) were measured by ELISA. Boxes show the pooled data (mean and SEM) of each cytokine assessed in non-stimulated (NS) SLE and control macrophages, macrophages stimulated with NETs, macrophages stimulated with NETs after chloroquine pretreatment and macrophages stimulated with NETs after treatment with a CXCR4 inhibitor (AMD3100). There was a higher TNF-α and IL-10 synthesis in SLE macrophages compared with controls when they were stimulated with NETs. When SLE macrophages were pretreated with chloroquine, IL-6 and TNF-α release significantly decreased. After CXCR4 inhibition, there was a decrease in TNF-α and IL-10 synthesis in control macrophages. *P<0.05; **P<0.01; ***P<0.001.

anti-inflammatory and counter-regulatory response in sepsis and other diseases.[15 31] Therefore, its higher levels in healthy control NETs could constitute a regulatory mechanism to dampen the proinflammatory environment that initially leads to NETosis. This mechanism could be absent or deficient in patients with SLE. Furthermore, K63 ubiquitination has been found to play an important role in cellular resistance to oxidative stress.[32] The fact that NETs from patients with SLE have less K63 polyubiquitination could lead to a higher oxidative damage in the context of NETosis. This, in turn, could contribute to endothelial damage and the proatherogenic phenotype in patients with SLE.[33]

We also found that NETs contain ubiquitinated MPO. This enzyme seems to play a dual role in inflammation and autoimmunity. Particularly in SLE, it has been associated with enhanced atherosclerosis,[33] as well as with nephritis,[5 29] through the role it plays within NETs. However, a decreased renal inflammation has also been described in MPO deficient mice.[34] We found MPO might induce a humoral response in subjects with SLE, characterised by the production of more autoantibodies against both native and Ub-MPO molecules than healthy controls. The frequency of anti-native MPO antibodies in our study agrees with previous reports in SLE[35 36] and this is the first description of anti-Ub-MPO antibodies. Furthermore, patients with SLE had a higher concentration of anti-Ub-MPO antibodies in comparison to non-Ub-MPO. Since ubiquitin is a highly conserved and very abundant protein, it has been hypothesised that it is unlikely to elicit an immune response and could even protect its substrates from immune recognition.[37] However, by masking certain epitopes, it could expose other, more immunogenic, peptides. It has been reported in a murine model that T cells specific for the PTM variant of a self-antigen are able to escape central tolerance and could provide help to B cells and promote pathogenic autoimmune responses.[38] Also, specifically regarding MPO, ubiquitination could be involved in its dual function in immunity, since this PTM may influence its function, its enzymatic action and its oxidative capacity, both intracellularly and as a NET component. MPO ubiquitination could have a direct impact on tissue injury due to oxidative stress, and on the effect MPO has in other immune cells. Interestingly, MPO has been found to suppress dendritic cells, whereas in its enzymatically inactive form, it can activate lung macrophages.[34 39] Whether these interactions could be affected by ubiquitination remains to be defined.

We found a trend towards a higher macrophage calcium flux when we stimulated these cells with ubiquitin. This effect seems to be mediated by CXCR4, since its stimulation has been described to enhance calcium flux[19] in other biologic systems, and after its inhibition, calcium flux decreased significantly. When macrophages were stimulated with NETs, the highest calcium flux was observed with NETs derived from controls, which had the higher ubiquitin concentration, suggesting that this differential effect may be driven by ubiquitin. To dissect the precise role of ubiquitin from NETs in the enhanced calcium flux, we used NETs from PYR-41-exposed neutrophils, which is a E1-activating enzyme inhibitor.[40] We found no significant differences when comparing with non-PYR-41-exposed neutrophils. This could be secondary to the inability to attain complete removal of ubiquitin in NETs. Current available techniques are directed mainly at blocking the elongation of the ubiquitin chain, rather than removing the first

ubiquitin molecule conjugated to the protein substrate. Most of these techniques were developed for polyubiquitin chain removal in a single protein substrate and are not suitable for a mixture of proteins prone to ubiquitination, such as those present in NETs.[41] Therefore, we consider that the lack of significant differences in the calcium flux are related to the limitation of the current technology available for the assessment of ubiquitination in NETs and to the lack of knowledge of the ubiquitin code (E1, E2, E3 ligases, deubiquitination enzymes,[42] ubiquitin like proteins[43] and non-conventional ubiquitin linkages)[44] that guides ubiquitination during NETosis, a limitation of the present work. Overall, our findings partly support the role of ubiquitin in NETs as a trigger for calcium flux in macrophages, but do not allow us to conclude that it is the only NET component involved in this response. It will be relevant to determine if the concentration of ubiquitin in NETs makes them more or less immunogenic, which could be related to the specific ubiquitinated protein cargo and the lysine residue to which ubiquitin is anchored.

When SLE macrophages were stimulated with NETs, they were internalised and cytokine synthesis was documented. There was a higher synthesis of TNF-α and IL-10 by SLE macrophages compared with control macrophages after they were stimulated with LPS-induced NETs. This partially agrees with the findings by Farrera and Fadeel, who described a higher cytokine synthesis after stimulating with both LPS and NETs than with NETs alone.[8] These results with LPS may be secondary to a synergistic mechanism, which could be mediated by TLR4, a receptor that has been involved in SLE physiopathology.[25 45 46] Of note, pretreatment with chloroquine decreased TNF-α and IL-6 production in SLE macrophages. Chloroquine has been described to specifically alter macrophage IL-6 and TNF-α synthesis through TLR inhibition.[24] Macfarlane and Manzel found that antimalarials inhibit IL-6 synthesis after TLR-9 stimulation, but did not have an effect on LPS-induced responses,[24] which was confirmed by Cepika *et al*.[47] Furthermore, we recently found that in rheumatoid arthritis synovial fibroblasts can internalise NETs and increase cytokine synthesis, and that NET internalisation is inhibited by antimalarials.[26] This effect was not replicated in the case of IL-10, which could be partly explained by the other pathways involved in the synthesis of this cytokine,[48] and could also be related to its specific role in SLE. Although it is typically regarded as an anti-inflammatory cytokine, high levels of IL-10 have been described in lupus[49 50] and it has even been considered as a disease activity biomarker.[51] Finally, control macrophages displayed decreased TNF-α and IL-10 synthesis after CXCR4 inhibition, which could implicate the ubiquitin- CXCR4 pathway in this process, since ubiquitin has been found to regulate TNF-α and IL-10,[15] but not IL-6[52] synthesis. We did not find similar changes in lupus macrophages, but this could be related to the abnormally enhanced expression and dysregulation of CXCR4 that has been reported in patients with SLE,[27 53 54] which could have led to an incomplete inhibition of this pathway. Also, CXCR4 has a complex recycling process,[55] which could influence the effect of its inhibition in cytokine synthesis, without altering its effect on the initial calcium flux. Our data suggest that NET internalisation by lupus macrophages is not a silent process and may contribute to inflammation in this disease.

In summary, this is the first study that demonstrates the presence of polyubiquitinated proteins in NETs, with a differential profile between patients with SLE and healthy controls. MPO was found to be ubiquitinated in NETs and our data suggest it is a target of humoral responses in SLE. Furthermore, ubiquitin present in NETs is one of the components that regulates calcium flux in macrophages through CXCR4 signalling. Lupus macrophages synthesise inflammatory cytokines in response to NET internalisation, a process that could be partly mediated by TLR-4 and TLR-9. Overall, abnormalities in mechanisms involved in NET internalisation and the extracellular ubiquitination pathway could play important roles in the development of inflammatory responses in SLE.

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Contributors AB-V: study design, data acquisition (experiments), data analysis, manuscript preparation. DG-M: study design, data acquisition (experiments), data analysis, manuscript preparation. CC-R: study design, data acquisition (experiments), data analysis. JM-C: data acquisition (experiments), data analysis. JJT-R: data acquisition (experiments), data analysis. ZGM: study design and data acquisition (clinical data). SH: study design and data acquisition (clinical data). JA-V: study design, manuscript revision. MJK: study design, manuscript preparation and revision.

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Correction: *Cross-phenotype analysis of Immunochip data identifies KDM4C as a relevant locus for the development of systemic vasculitis*

Ortiz-Fernández L, Carmona FR, López-Mejías R, *et al*. Cross-phenotype analysis of Immunochip data identifies *KDM4C* as a relevant *locus* for the development of systemic vasculitis. *Ann of Rheum Dis* 2018;77:589–95.doi:10.1136/annrheumdis-2017-212372.

The additional author, Peter A Merkel, has been added to the author list. The correct order of authors is:

Lourdes Ortiz-Fernández, Francisco David Carmona, Raquel López-Mejías, Maria Francisca González-Escribano, Paul A Lyons, Ann W Morgan, Amr H Sawalha, Peter A Merkel, Kenneth G C Smith, Miguel A González-Gay, Javier Martín, Spanish GCA Study Group, UK GCA Consortium, Turkish Takayasu Study Group, Vasculitis Clinical Research Consortium, IgAV Study Group, AAV Study group.

This has been corrected online.

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Scheduled rituximab maintenance reduces relapse rate in eosinophilic granulomatosis with polyangiitis

Eosinophilic granulomatosis with polyangiitis (EGPA, Churg-Strauss syndrome) is an antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis with a frequently relapsing/refractory course.[1 2] Rituximab (RTX) is an established treatment in the other ANCA-associated vasculitides, but data on EGPA are scarce.[3 4] Mohammad[5] reported that RTX can be effective in EGPA, particularly in ANCA-positive patients.

1All patients but one (who stopped RTX for infusion reaction) completed RTX-induction. Of the remaining 20, 15 (75%) achieved remission (complete in 5, partial in 10) at month 3. Of the five patients who proved refractory to RTX, two were ANCA-positive and three ANCA-negative; major disease manifestations were asthma in four and mononeuritis multiplex in one. After RTX failure, they received high-dose oral prednisone (n=5), omalizumab (n=3) or intravenous immunoglobulins (n=1). The remission rates at 6 months and 12 months are shown in table 1. There was a significant reduction in BVAS, prednisone dose and eosinophil count between month 0 and subsequent time points (figure 1).

Of the 15 patients in remission at month 3, 5 (33%) relapsed within a median of 6 months (range 5–12). Relapses occurred only in patients not receiving scheduled RTX-maintenance; relapse-free survival was significantly shorter in this subgroup (figure 1). The two treatment regimen subgroups were comparable, given

Table 1 Main clinical and laboratory parameters in the study cohort at different time points

		Number of subjects	ANCA−	ANCA+	
Baseline data		**n=21**	**n=11**	**n=10**	**p Value**
Disease duration from diagnosis to first RTX, months (range)		21.5 (10–82)	24.5 (10–82)	16 (10–52)	ns
Indication for RTX	Refractory disease	12 (57.1%)	5 (45.4%)	7 (70%)	ns
	Relapsing disease	9 (42.8%)	6 (54.5%)	3 (30%)	ns
Median BVAS, score (range)		11 (2–24)	11 (2–16)	11 (2–24)	ns
Median EC, cells/uL (range)		1500 (300–7525)	1075 (300–10800)	3795 (820–15350)	ns
Median PDN dose, mg/d (range)		15 (5–75)	15 (7–75)	13.5 (5.0–25)	ns
Organ involvement	% renal	2 (10%)	0	2 (20%)	NC
	% PNS	15 (75%)	8 (80%)	7 (70%)	ns
	% heart	0	0	0	NC
	% asthma/lung	17 (85%)	9 (90%)	8 (80%)	ns
	% GI tract	6 (30%)	3 (30%)	3 (30%)	ns
	% skin	2 (10%)	2 (20%)	0	NC
	% ENT	3 (15%)	0	3 (30%)	NC
Concomitant IS		9 (45%)*	5 (50%)	4 (40%)	ns
Maintenance treatment		**n=15**	**n=5**	**n=10**	
Non-scheduled RTX (2011–2014)		6	2 (33%)	4 (66%)	ns
Scheduled RTX (2014–2017)		9	3 (33%)	6 (66%)	ns
At 6 months		**n=20**	**n=10**	**n=10**	
Overall response (CR+PR), n%		12 (60%)	4 (40%)	8 (80%)	ns
CR, n%		5 (25%)	2 (20%)	3 (30%)	ns
PR, n%		7 (35%)	2 (20%)	5 (50%)	ns
Median BVAS, score (range)		9 (0–15)	7.5 (0–15)	9 (0–12)	ns
Median EC, cells/uL (range)		450 (78–1000)	900 (100–1000)	390 (78–730)	ns
Median PDN dose, mg/d (range)		7 (2–25)	9.5 (5–25.0)	5.0 (2–20)	0.09
Concomitant IS		9 (45%)	7 (70%)	2 (20%)	0.07
At 12 months		**n=17**	**n=8**	**n=9**	
Overall response (CR+PR), n%		11 (64.7%)	4 (50%)	7 (77.7%)	ns
CR, n%		6 (35.3%)	2 (25%)	4 (44.4%)	ns
PR, n%		5 (29.3%)	2 (25%)	3 (33.3%)	ns
Median BVAS, score (range)		9 (0–15)	7 (0–15)	9 (0–15)	ns
Median EC, cells/uL (range)		475 (30–1040)	600 (30–1040)	170 (78–600)	0.05
Median PDN dose, mg/d (range)		6 (0–37.5)	6.5 (5–37.5)	5 (0–12.5)	ns
Concomitant IS		7 (41.2%)	5 (62.5%)	2 (22.2%)	ns

Categorical variables are presented as n (%), continuous variables as median (range). Differences in categorical variables were analysed using Fisher's exact test; while differences in continuous variables by the Mann–Whitney U exact test. Exact p values are shown only for p<0.1.

*At the time of the first rituximab infusion, five patients with refractory disease were also being treated with other immunosuppressants: two with azathioprine, two with ciclosporine, one with mycophenolate mofetil; among these, two were also on intravenous immunoglobulin therapy. Four patients with relapsing disease were treated with the following immunosuppressants: one with azathioprine, two with ciclosporine, one with methotrexate.

ANCA, antineutrophil cytoplasmic antibodies; BVAS, Birmingham Vasculitis Activity Score; CR, complete response; EC, eosinophil count; ENT, ear nose throat; GI, gastrointestinal; IS, immunosuppressants; ns, not significant; NC, not calculable; PDN, prednisone; PNS, peripheral nervous system; PR, partial response; RTX, rituximab.

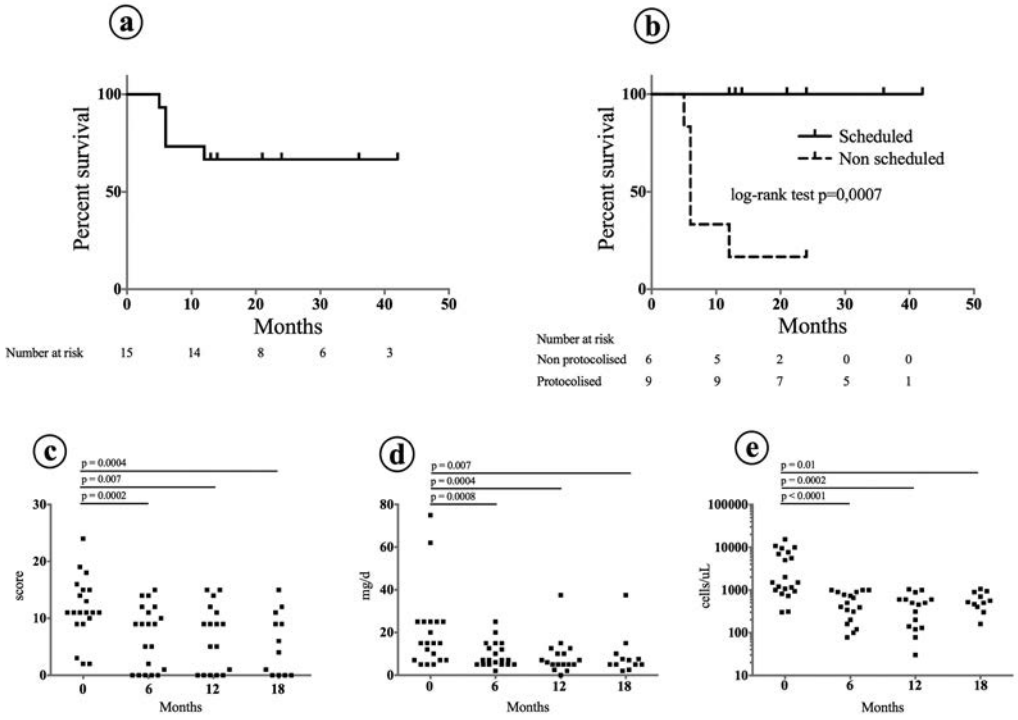

Figure 1 (a) Kaplan-Meier relapse-free survival estimate of the 15 patients with eosinophilic granulomatosis with polyangiitis (EGPA) who achieved remission after induction with rituximab. (b) Comparison of relapse-free survival between patients who received protocolised maintenance rituximab (continuous line) and patients who did not (dashed line). (c) Birmingham Vasculitis Activity Score, (d) prednisone dose (or equivalent) and (e) eosinophil counts of all patients 0, 6, 12 and 18 months after rituximab induction. Differences in continuous variables within groups were analysed using Wilcoxon matched-pairs signed-rank test. Differences in survival curves were analysed using the log-rank test. A p value of <0.05 was considered statistically significant for all analyses.

the similar proportions of ANCA-positive and ANCA-negative patients (table 1) and of major organ involvement frequencies (asthma/lung 100% vs 83.3%, neuropathy 88.9% vs 66.7%, gastrointestinal 33.3% vs 33.3% in the scheduled vs non-scheduled subgroups, all p values not significant).

No statistically significant differences in relapse rates or time-to relapse were observed between ANCA-positive and ANCA-negative patients (data not shown). However, a greater proportion of ANCA-positive patients were in remission at months 6 and 12, although the difference was not statistically significant.

Treatment-related adverse events included pneumonia in two patients (months 6 and 12, respectively), and bronchitis in one (month 6). Two patients died, one from arrhythmia (month 18), the other from brainstem astrocytoma (month 24). At last follow-up (median 24 months, range 6–42), of the 21 patients who initially received RTX, 19 are alive: 11 are in remission (9 while on RTX-maintenance), and 8 are stable while receiving prednisone and immunosuppressants.

Our data suggest that RTX is effective and well tolerated in refractory/relapsing EGPA. It rapidly induced remission and allowed glucocorticoid reduction in most patients. Scheduled RTX-maintenance significantly reduced relapse rate as compared with RTX given 'on demand' for relapse. This observation must be taken cautiously given the small sample size and the retrospective nature of the study, though it parallels the experience with other ANCA-associated vasculitides.[6 9]

Another biological agent, mepolizumab (antiinterleukin-5), has recently proved effective in reducing relapse rates and sparing glucocorticoids in EGPA;[10] however, half the trial cohort did not achieve remission, thus highlighting the need for patient-tailored treatments, taking into account the phenotypical diversity of EGPA.[1] RTX effectively maintained remission in our retrospective cohort. Hopefully the ad hoc prospective trial which is currently underway will confirm our findings (MAINRITSEG, clinicaltrials.gov: NCT03164473).

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Contributors GE, GMR and AV designed the study, collected data, analysed results and wrote the manuscript. MG performed statistical analysis. MLU, ES and DP contributed to clinical data and analysed the results. All authors revised the manuscript critically for important intellectual content and approved the final version.

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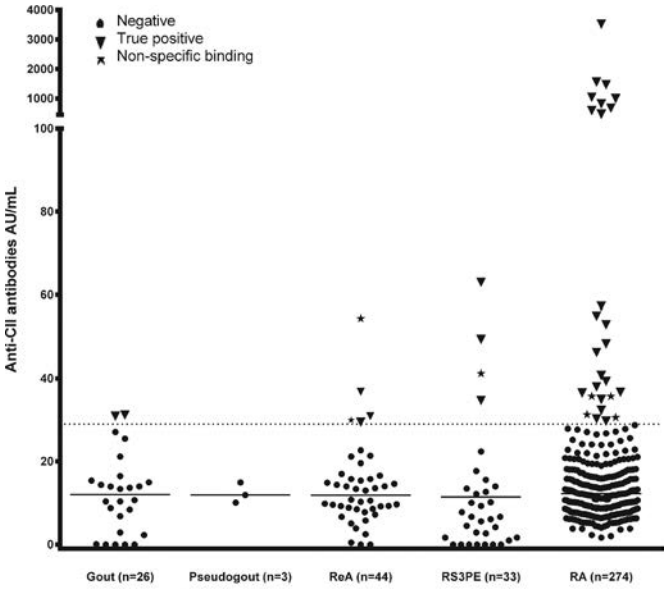

Figure 1 Levels of antibodies against native human collagen type II (CII). The dotted horizontal line represent the cut-off based on the 95th percentile among healthy controls (29 arbitrary units/mL). Values above the cut-off are divided into true positive samples (triangles) and samples with non-specific binding (stars) reacting with higher optical density (OD) levels in control wells coated only with bovine serum albumin. Only true positive values are reported as positive in table 1. Solid horizontal bars represent median values in each group. RA, rheumatoid arthritis; ReA, reactive arthritis; RS3PE, remitting seronegative symmetrical synovitis with pitting oedema. Data on the 274 RA patients were published previously.[4 5]

Antibodies against collagen type II are not a general marker of acute arthritis onset

The fibrillar protein collagen type II (CII) is essentially confined to hyaline cartilage in diarthrodial joints. Antibodies against CII (anti-CII) were previously described in 3%–27% of rheumatoid arthritis (RA) patients, and Kim *et al* described anti-CII to be associated with elevated levels of C reactive protein (CRP) and erythrocyte sedimentation rate (ESR) in a heterogeneous group of RA patients with 2–432 months of disease duration.[1] Contrary to anticitrullinated protein antibodies, anti-CII are not detected before RA onset.[2] We have shown that anti-CII levels are highest at the time of RA diagnosis and thereafter decline, and that elevated anti-CII levels at diagnosis associate with elevated CRP, ESR, swollen joint count, disease activity score and radiological destruction at the time of diagnosis but not later, thus representing an acute onset RA phenotype.[3–5] It is plausible that production of pro-inflammatory cytokines by macrophages

Table 1 Patient characteristics for the investigated patients. Not all patients had data on RF and ACPA

	Gouty arthritis	Pseudogout*	Reactive arthritis	RS3PE	All non-RA	RA (from ref 4)
Number	26	3	44	33	106	274
Female; number (%)	9 (34)	1 (33)	17 (39)	17 (52)	44 (42)	193 (70)
Age, years; median (IQR)	62 (48–69)	80 (74–80)	33 (27–47)	52 (28–72)	46 (30–66)	56 (46–70)
CRP; median (IQR)	7 (3–30)	52 (0–100)	13 (3–38)	5 (3–11)	7 (3–26)	14.5 (7–31)
ESR; median (IQR)	16 (10–37)	60 (17–77)	19 (8–63)	11 (6–26)	16 (8–39)	21 (12–40)
SJC (66 joint count); median (IQR)	3 (1.5–9.5)	11 (1–12)	2 (1–4)	3 (1–6)	2 (1–6)	9 (5–13)
TJC (66 joint count); median (IQR)	4 (1–6)	13 (1–17)	3 (2–5)	5 (2–10.5)	4 (2–6)	7 (4–12)
RF positive/total measured (%)	4/26 (15.4)	0/3 (0)	3/44 (6.8)	4/33 (12.1)	11/106 (10.4)	172/272 (63.2)
ACPA positive/total measured (%)	0/19 (0)	0/3 (0)	1/12 (8.3)	0/6 (0)	1/41 (2.4)	157/274 (57.3)
Anti-CII† positive/total measured (%)	2/26 (7.7)	0/3 (0)	3/44 (6.8)	3/33 (9.1)	8/106 (7.5)	24 (8.8)

*Patients presenting with an inflammatory arthritis of at least one joint in which calcium pyrophosphate crystals were identified in synovial fluid microscopy and in whom no alternative diagnosis was made.
†Samples yielding >29AU/mL of anti-CII and showing lower OD in bovine serum albumin blocked control wells without CII than in anti-CII coated and subsequently blocked wells.
ACPA, anti-citrullinated protein/peptide antibody; Anti-CII, antibodies against collagen type II; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; RA, rheumatoid arthritis; RF, rheumatoid factor; RS3PE, remitting seronegative symmetrical synovitis with pitting oedema; SJC, swollen joint count; TJC, tender joint count.

is stimulated by anti-CII-containing immune complexes (IC) in RA joints, as anti-CII-producing B cells are detectable in synovial fluid but not in the circulation.[6 7] Analogously, in rodents, collagen antibody-induced arthritis (CAIA) develops soon after injection of a defined blend of anti-CII antibodies.[8] Polymorphonuclear granulocytes (PMN) are central in CAIA pathogenesis, and we have recently shown that PMN induce chemokine production after stimulation with IC containing anti-CII from RA patients.[9]

In patients with very early synovitis with ≤3 months duration, anti-CII are not more common in patients developing RA than in patients developing other diagnoses during the following year.[10] Postpublication subgroup analysis showed that 3/11 (27%) of reactive arthritis and 2/7 (29%) of gouty arthritis patients had elevated anti-CII levels at the early synovitis stage (unpublished). As both reactive arthritis and gouty arthritis often have an acute arthritis onset, we hypothesised that high levels of anti-CII might not only be a marker of acute onset RA, but a marker of acute onset arthritis in general.

To investigate the hypothesis that high anti-CII levels might be a general marker of acute onset arthritides, we collected first visit sera from 26 patients with gouty arthritis, 3 patients with pseudogout, 44 patients with reactive arthritis and 33 patients with remitting seronegative symmetrical synovitis with pitting oedema (RS3PE), diagnoses that often start with acute onset arthritis. Patient characteristics are described in table 1. Antibodies against native human CII were measured with ELISA, and levels above the 95th percentile among controls were regarded as positive in agreement with our previous studies.[4–6] Serially followed internal laboratory controls showed that the analysis performance remained unaltered.[3 4]

Eight of the 106 (7.5%) first visit non-RA patients were anti-CII positive, very similar to our previously published data on early RA (6.6%–8.8%).[3 4] There was no significant difference between any diagnostic groups (table 1). Only moderately elevated anti-CII levels were found; no non-RA patients exhibited very high anti-CII levels comparable to the high outlier group that we have described in early RA patients (figure 1).[4 5]

We conclude that although high anti-CII levels are associated with acute onset in RA, this cannot be generalised to most patients with other acute onset arthritides.

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B-cell receptor sequencing of anti-citrullinated protein antibody (ACPA) IgG-expressing B cells indicates a selective advantage for the introduction of N-glycosylation sites during somatic hypermutation

The majority of patients with rheumatoid arthritis (RA) harbours IgG antibodies targeting citrullinated protein antigens (ACPA). Recently, we showed that >90% of ACPA-IgG in serum are glycosylated in the variable domain.[1] *N*-linked glycosylation requires a consensus sequence in the protein backbone (N-X-S/T (asparagine-X-serine/threonine), where X is any amino acid except proline), which is scarce in germline-encoded Ig variable region genes.[2 3] Accordingly, hyperglycosylation of ACPA-IgG requires either clonal expansion of B cells expressing B-cell receptors (BCR) containing germline-encoded *N*-glycosylation

sites or generation of *de novo* sites through somatic hypermutation (SHM).[4]

Here, we analysed the BCR repertoire of ACPA-expressing B cells to understand the molecular basis of this remarkable glycosylation. ACPA-expressing B cells were sorted as pools (10 cells per pool) from peripheral blood mononuclear cells of eight patients with ACPA-positive RA.[5] Anchoring reverse transcription of immunoglobulin sequences and amplification by nested (ARTISAN) PCR-based BCR sequencing[6] followed by full-length variable region IgG transcript analysis revealed high nucleotide mutation rates in 97 unique ACPA-IgG heavy chains (HC; mean ±SD: 52.86±16.73; figure 1A). 81% of these contained one or more *N*-glycosylation sites.

To replicate these findings and to acquire additional information on paired heavy and light chains (LC), Ig transcripts of 87 single cell-sorted ACPA-IgG clones (six donors) were analysed, again revealing high nucleotide mutation rates in the HC variable region (mean ±SD: 48.55±16.05; figure 1B). Significantly lower mutation rates were observed for 31 single cell-sorted tetanus toxoid (TT)-specific clones (mean ±SD: 25.15±18.92; figure 1B). TT-specific clones contained no *N*-glycosylation sites, in contrast to 79% of HC and 88% of paired HC/LC sequences from the ACPA-IgG clones. Additionally, both pool-sorted and single cell-sorted sequence analyses revealed similar, high nucleotide mutation rates for ACPA-LC (mean ±SD: 36.18±15.09 and mean ±SD: 34.51±16.79, respectively; data not shown). Furthermore, 59% of ACPA-LC contained one or more *N*-glycosylation sites compared with 4%–5% of healthy control LC.

Further analyses of HC revealed that all sites in pool-sorted and single cell-sorted ACPA-IgG clones were introduced by SHM; furthermore, the degree of SHM did not correlate with the frequency of sites (figure 1C and D). Moreover, no accumulation of N-P-S/T sites (chosen as reference due to its similarity to N-X-S/T) was observed in ACPA-IgG, in contrast to the *N*-glycosylation tripeptide N-X-S/T. In fact, no N-P-S/T sequences were identified by ACPA-IgG sequencing approach (pool-sorted/single cell-sorted). Finally, we observed a relative increase of sites in the complementarity-determining region (CDR) 1 and a relative absence in CDR3 compared with healthy controls (figure 2A). Together, these findings indicate that the remarkable frequency of *N*-glycosylation sites is not the result of random accumulation of mutations but of a selective process during maturation of ACPA-expressing B cells. Intriguingly, modelling of the spatial positioning of the sites revealed that most sites are located on the exterior of the antibody molecule (figure 2B–D).

In conclusion, we provide the first in-depth analysis of the presence of *N*-glycosylation sites in the variable region of ACPA-IgG. The distribution pattern of sites across the ACPA-IgG

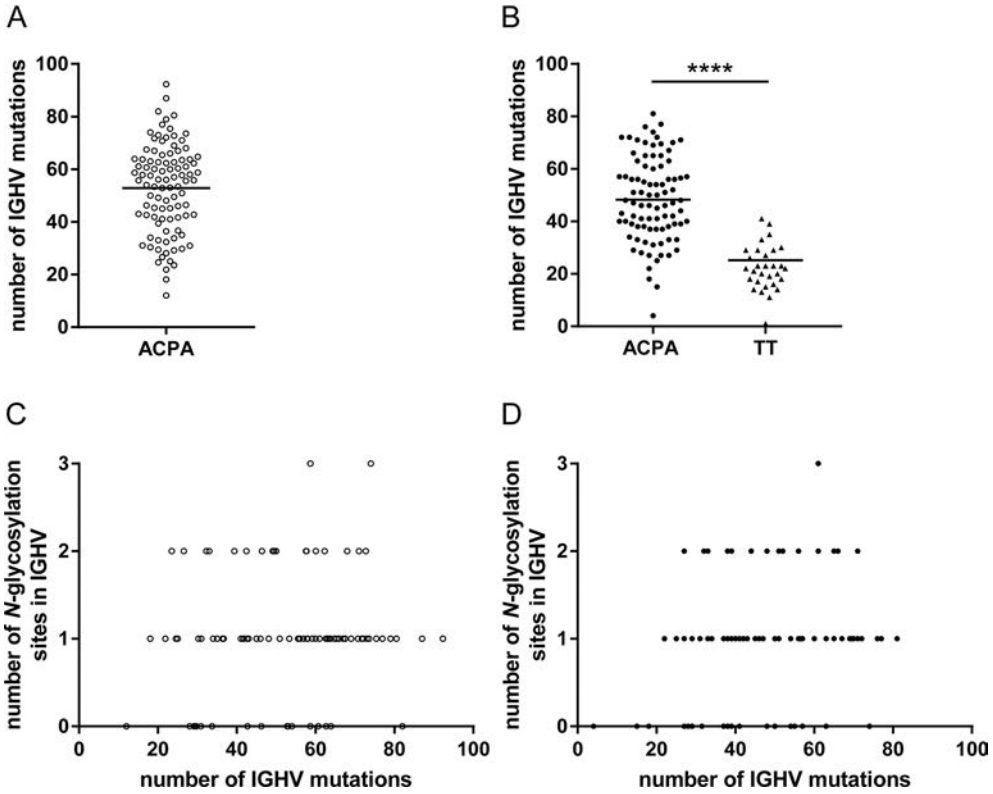

Figure 1 A high degree of somatic hypermutation in antibodies targeting citrullinated protein antigens (ACPA)-IgG clones which does not correlate with the frequency of *N*-glycosylation sites. Pool and single cells were sorted as described.[5] All independent clones are defined as identical V, D, J genes and complementarity-determining region 3. (A) Immunoglobulin heavy variable region (IGHV) mutations in 97 ACPA-IgG clones obtained with pool-sequencing (n=8 donors). (B) IGHV mutations in 87 ACPA-IgG clones that were positive in CCP2-ELISA compared with 31 tetanus toxoid (TT)-IgG clones obtained from sequencing of cultured single cells (n=8 and n=3 donors, respectively). *p* Value was calculated using Mann-Whitney *U* test for unpaired data (****$p<0.0001$). (C) Correlation of the number of IGHV mutations with the number of *N*-glycosylation sites of 97 ACPA-IgG clones. Non-parametric Spearman correlation, r=0.10, $p=0.32$. (D) Correlation of the number of IGHV mutations with the number of *N*-glycosylation sites of 87 ACPA-IgG clones. Non-parametric Spearman correlation, r=0.19, $p=0.071$. All *N*-glycosylation sites were introduced by somatic hypermutation. No association between IGHV-gene usage and number of *N*-glycosylation sites was observed using either method.

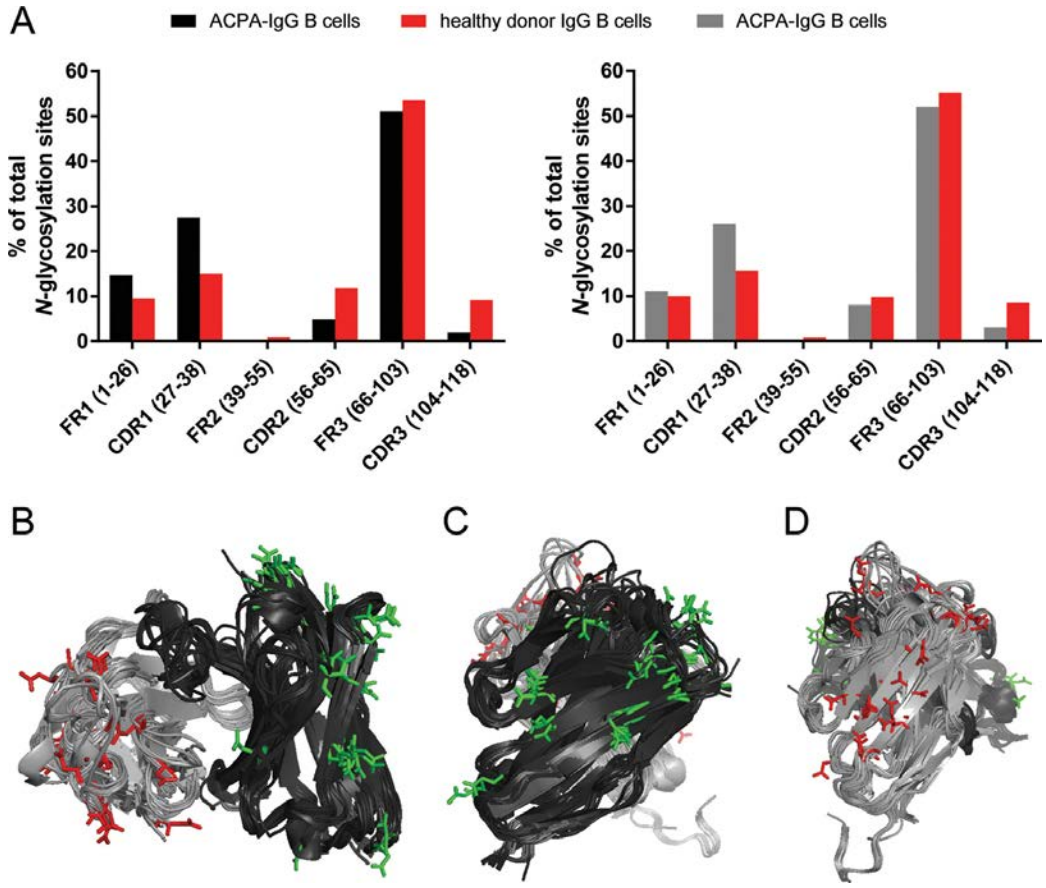

Figure 2 Distribution and spatial localisation of *N*-glycosylation sites in antibodies targeting citrullinated protein antigens (ACPA)-IgG clones. (A) Percentage of *N*-glycosylation sites located in framework (FR) 1, complementarity-determining region (CDR) 1, FR2, CDR2, FR3 and CDR3 of immunoglobulin heavy variable region (IGHV). Distribution of 102 sites in 97 ACPA-IgG clones obtained with pool-sequencing (left panel, black), 87 sites in 87 ACPA-IgG clones obtained with sequencing of cultured single cells (right panel, grey), both compared with 660 sites in 6724 IGHV sequences from 12 healthy donors (V-region matched, red). (B) Structural model of the top view on the antigen binding pocket of ACPA-IgG clones. (C) Front view of ACPA-IgG heavy chain structures containing *N*-glycosylation sites (asparagine residues coloured in green). (D) Front view of ACPA-IgG light chain structures containing *N*-glycosylation sites (asparagine residues coloured in red). All models contain variable regions of 58 ACPA-IgG clones with paired heavy and light chain sequences and had a confidence score of 100% with a sequence identity of 47.7%±7.96 and protein coverage of 98.55%±0.63.

variable domain and the spatial localisation of *N*-glycosylation sites on the exterior of the molecule suggest that their function in selection processes is not primarily related to antigen recognition. Our data favour the concept that introduction of *N*-glycosylation sites generates selective advantages which allow ACPA-expressing B cells to escape from classical selection mechanisms in germinal centres. This is in contrast to the selection of B cells against recall antigens, which is primarily driven by affinity for cognate antigens.[7] In fact, the overall low avidity of secreted polyclonal ACPA-IgG is in line with this hypothesis.[8] Possibly, ACPA-IgG variable domain glycans interact with glycan receptors in the vicinity of the BCR. These glycans are highly sialylated, suggesting siglecs as potential receptors.[2] Thus, these findings and considerations have important implications for understanding citrulline-specific immunity in RA.

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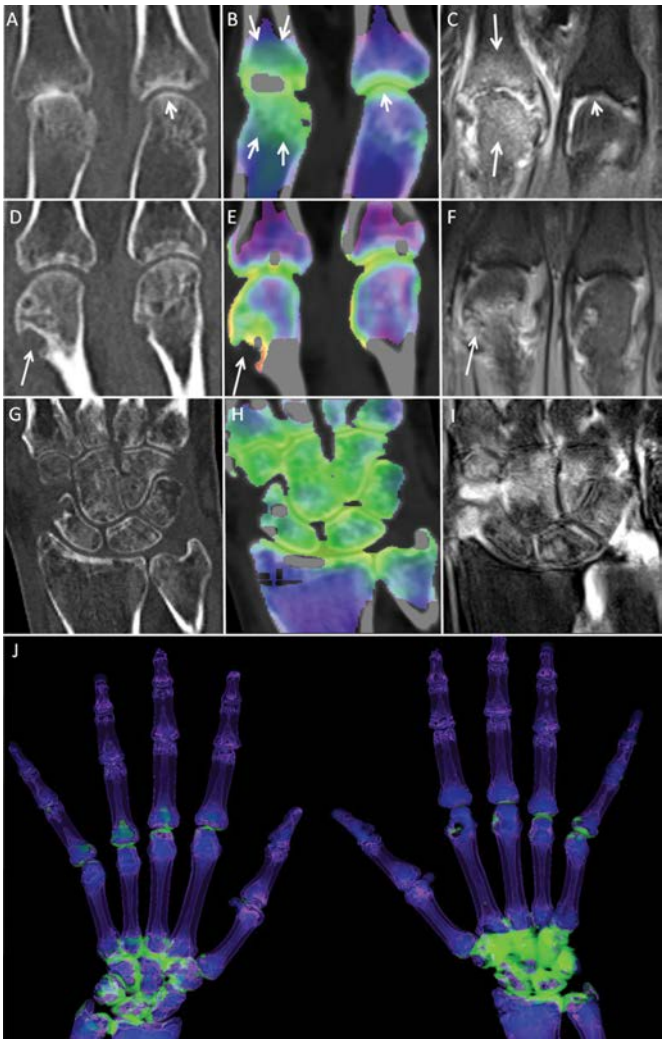

Figure 1 (A, B) Conventional and colour-coded DECT images from the same scan and (C) T2FS MRI of the second and third MCP joint in a 39-year-old woman with RA. MCP2 is normal with a smooth outline of cartilage and bone plate (short arrows) on the colour-coded maps and on MRI. BME of the bones of the MCP3 joint is seen as extensive and ill-defined green colour on the DECT image (arrows) and corresponds to BME on the MR image (long arrows). (D, E) Conventional and colour-coded DECT images and (F) T2FS MRI in a 43-year-old woman with RA showing BME (arrow) surrounding a large pocket erosion of head of the second metacarpal. (G, H) Conventional and colour-coded DECT and (I) T2FS MRI of the wrist showing extensive BME of the wrist in a 46-year-old woman with RA. (J) 3D DECT colour-coded images showing BME of the right carpal bones only. (B,E,H) The ability of DECT to detect BME in sclerotic areas (grey spots) is limited. BME, bone marrow oedema; BMEL, bone marrow oedema lesion; DECT, dual-energy CT; MCP, metacarpophalangeal joint; RA, rheumatoid arthritis; T2FS, T2-weighted fat saturated.

Dual-energy CT: a new imaging modality for bone marrow oedema in rheumatoid arthritis

Active joint inflammation is a key feature in rheumatoid arthritis (RA). Treatment guidelines for RA,[1] including 'Treat-to-Target' strategies,[2] stress the importance of abrogation of inflammation. MRI clearly demonstrates bone marrow oedema lesions (BMEL) as a sign of inflammation.[3] Dual-energy CT (DECT) scanners could provide a new approach to visualise BME.[4–7] We investigated if DECT could visualise BME of the hand and wrist in patients with active RA, with MRI-proven BME as a gold standard.

Institutional review board approval was obtained. Twenty consecutive patients with active clinical synovitis of a metacarpophalangeal, proximal interphalangeal or wrist joint provided written informed consent and were included; 9 men and 11 women with a mean age of 60.7 (±10.3) years. Thirteen patients were ACPA positive and 8 were ACPA and RF positive; mean tender joint count and swollen joint count were both 5.9. Mean DAS28 was 4.37 (±1.4). The mean disease duration was 6.6 (±6.1) years. Seventeen patients were treated with conventional disease-modifying antirheumatic drugs, 7 were also treated with biologicals.

DECT was performed on a dual-source CT, data were post-processed with SyngioVia 'Bone Marrow Oedema' application (see the online supplementary text). Images were presented as colour-coded maps. The presence of BMEL on MRI and DECT in the radius, ulna, each carpal bone, the head of metacarpals and the base of the proximal phalanges was scored in consensus by two radiologists (LJ, NH). Both imaging modalities were scored separately from each other. Agreement between DECT and MRI for detection of BME was calculated with κ-statistics

Table 1 Observed presence of BME on MRI and DECT per bone across the study group of 20 patients

		MRI		DECT		κ Value
		+	−	+	−	
MCP1	Head of MC	0	20	0	20	N/A
	Base of phalanx	0	20	0	20	N/A
MCP2	Head of MC	4	16	4	16	1
	Base of phalanx	3	17	3	17	1
MCP3	Head of MC	8	12	8	12	1
	Base of phalanx	3	17	3	17	1
MCP4	Head of MC	0	20	0	20	N/A
	Base of phalanx	0	20	1	19	0
MCP5	Head of MC	2	18	1	19	0.643
	Base of phalanx	1	19	1	19	1
Hamate bone		4	16	4	16	1
Capitate bone		5	15	6	14	0.875
Trapezoid bone		2	18	1	19	0.643
Trapezium		3	17	2	18	0.773
Scaphoid bone		7	13	5	15	0.765
Lunate bone		7	13	6	14	0.886
Triquetral bone		6	14	6	14	1
Pisiform bone		1	19	1	19	1
Radius		7	13	6	14	0.886
Ulna		4	16	5	15	0.857

Agreement between MRI and DECT for detection of BME is shown as κ-values.
BME, bone marrow oedema; DECT, dual-energy CT; MC, metacarpal; MCP, metacarpophalangeal joint; N/A, not applicable; κ, κ-value.

using SPSS V.25.0, κ-values 0.61–0.80 were considered 'good' and 0.81–1.00 'very good' agreement.[7]

The colour-coded DECT images showed blue colour in the cancellous bone with a well-defined rim of green colour overlaying the cartilage. BME was visualised as an ill-defined green colour overlaying the bone, indicating high fluid content (figure 1). The observed presence of BMEL on MRI and DECT and κ-values indicating 'very good' agreement for 70% of the bones are shown in table 1. The overall proportion of observations in agreement was Pa=0.98. Disagreement between DECT and MRI was found in 10 out of 400 assessed bones. In six bones, MRI depicted BMEL, whereas in four bones, DECT was positive.

BME is a sign of active inflammation and plays a key role in diagnosis, therapy guidance and follow-up in patients with RA. To our knowledge, this is the first prospective study applying DECT as a novel imaging technique for detection of BMEL in RA.[8] We found that the colour-coded DECT maps differentiate normal bone marrow from BMEL and corresponded well to BME on MRI. Overall, we found a strikingly good correlation between BMEL detection by MRI and DECT with Pa=0.98. DECT can evaluate BMEL, synovitis, tenosynovitis and bone erosions in RA in a single study at lower cost, higher accessibility and short acquisition time compared with MRI. If iodine contrast is admitted, CT shows synovitis and tenosynovitis equally compared with gadolinium-enhanced MRI.[9] A prominent advantage of using CT in RA is highly sensitive and reliable detectability of bone destruction since CT directly depicts the cortex. A limitation of DECT is its current poor spatial resolution for BMEL, which makes quantification difficult. DECT involved a negligible 0.03 mSv radiation dose in this study. Altered marrow composition due to degeneration, trauma, tumour or infection could also be detected by DECT, but this also applies to MRI.

In conclusion, this proof of concept study highlights that DECT is a novel technique that is feasible and enables to demonstrate BMEL in patients with RA at a lower cost and higher accessibility compared with MRI.

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Correction notice This article has been corrected since it published Online First. The shared contribution statement for Dirk Elewaut and Peggy Jacques has been added and the affiliations have been added to authors Filip Van den Bosch, Philippe Carron and Peggy Jacques.

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Correction: *EULAR/PreS standards and recommendations for the transitional care of young people with juvenile-onset rheumatic diseases*

Foster HE, Minden K, Clemente D, *et al*. EULAR/PreS standards and recommendations for the transitional care of young people with juvenile-onset rheumatic diseases. *Ann of Rheum Dis* 2017;76:639-46. doi:10.1136/annrheumdis-2016-210112.

The author's name, Erkan Demirkaya, has been corrected.

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Correction: *Stratification of knee osteoarthritis: two major patient subgroups identified by genome-wide expression analysis of articular cartilage*

Soul J, Dunn SL, Anand S, *et al*. Stratification of knee osteoarthritis: two major patient subgroups identified by genome-wide expression analysis of articular cartilage. *Ann of Rheum Dis* 2018;77:423–30. doi:10.1136/annrheumdis-2017-212603.

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Chondroitin sulfate for knee osteoarthritis

The apparent efficacy of chondroitin sulfate in treating knee osteoarthritis reported by Reginster *et al*[1] must be considered in the context of the literature on this subject. The results of trials of chondroitin and/or glucosamine for relief of pain caused by osteoarthritis of the knee and hip have been conflicting. Vlad *et al*[2] noted that the heterogeneity among trials of glucosamine was larger than expected by chance and examined the reasons for the difference. Trials funded by industry had large effect sizes, and independently funded trials had had no clinically relevant benefit compared with placebo. Potential explanations included a positive bias in industry-funded trials.

A meta-analysis of chondroitin and glucosamine studies found that trials that reported large effects on joint pain were often hampered by poor study quality and small sample sizes.[3] The authors concluded that large, methodologically sound trials found small or no effects, as did a meta-analysis of trials of chondroitin.[4] A trial of crystalline chondroitin sulfate combined with glucosamine sulfate found no superiority in reducing joint pain or functional impairment compared with placebo.[5]

None of these trials or meta-analyses, which noted consistent discrepancies between the outcomes of independent and industry-funded trials, was cited by Reginster *et al*. In light of the apparent positive bias in industry-funded trials, I believe that acceptance of the positive results of this industry-funded study should be withheld pending confirmation by independently funded trials.

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Some concerns and improvements from Turkey

First, thank you for this article which tries to answer a difficult clinical problem about anti-neutrophilic cytoplasmic antibody (ANCA)-associated vasculitis remission phase treatment.[1] We believe that the study will be a milestone in ANCA-associated vasculitis maintenance treatment paradigm. To improve clinical implications, we wanted to ask about some important points in the study.

As seen in the literature,[2] the Five Factor Score (FFS) is an easily applicable and valuable tool to predict relapse risk of ANCA-associated vasculitis. In the study, withdrawal of immunosuppression ($p<0.0001$) and ANCA positivity at randomisation ($p=0.017$) were the only predictors of relapse during follow-up. If FFS had been used, FFS' predictivity value of relapse risk would have been implicated. Also, the absence of organ (or system) involvements of included patients should be added to the limitations of the study, so the association between organ involvement(s) and risk of relapse has not been established, thus we know that specific organ involvements are important predictors of relapse.[3]

Another point of interest, as seen in the study, is that patients with eosinophilic granulomatosis with polyangiitis (EGPA) are absent. EGPA has a prevalence value close to other ANCA-associated vasculitis,[4 5] so we wonder why patients with EGPA are excluded from the study.

Also, we could not understand what 'Delay from diagnosis' parameter means in table 2. If it means the duration from diagnosis to randomisation point, sum of 3 months for induction phase and 18–24 months to remission phase is over 18–19 (mean) months. If it means the duration between diagnosis and involving in study, what treatments have patients been receiving within that duration? The aforementioned parameter must be described more clearly, we think.

The most curious point for us is which drug doses are used in remission phase before randomisation. There is no information about it. Is low-dose azathioprine regimen (1 mg/kg/day) used like postrandomisation phase or is optimum dose regimen[6] (2–3 mg/kg/day) used?

Thank you again for pioneering such a great work!

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Duration of maintenance therapy for ANCA-associated vasculitis: more questions than answers

Over the last decades, numerous randomised clinical trials and long-term observational studies were conducted in dozens or hundreds patients with antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitides (AAV) and provided a basis for an evidence-based treatment. With immunosuppressive therapy, up to 85%–90% of patients will achieve remission, though the disease can follow a relapsing course in a significant proportion of patients, and prolonged exposure to glucocorticoids and immunosuppressive agents may have devastating consequences. However, the attempts to shorten maintenance therapy can lead to recurrent exacerbations of AAV and an accumulation of irreversible organ damage.

In the recent randomised, prospective REMAIN (prolonged REmission-MAINtenance therapy in systemic vasculitis) study, conducted in 117 patients with AAV in stable remission after cyclophosphamide/prednisolone-based induction, Karras *et al* showed that prolonged maintenance therapy with azathioprine/prednisolone (up to 48 months from diagnosis) was relatively safe and more effective than withdrawal of azathioprine/prednisolone by 24 months[1]. Extension of immunosuppression was associated with a significantly lower risk both of any and major relapses (22% vs 63%, $p<0.0001$, and 14% vs 35%, $p<0.007$, respectively) and resulted in a better renal survival. Notably, almost all patients completed the prolonged follow-up. The results of the REMAIN study were satisfying to us since prolonged immunosuppression with low dose cyclophosphamide and currently azathioprine or metothrexate in combination with corticosteroids for many years remains a preferred treatment option for patients with AAV in our clinic.

However, there is the other side of the coin. A paradigm for treating patients with AAV has changed significantly over recent years. Currently, it is apparent that a significant proportion of patients can benefit from a shorter maintenance therapy. In the REMAIN study, major relapses occurred in 35% of patients in the withdrawal group. We can assume that at least two-thirds of patients did not require extended 4-year immunosuppression to prevent the recurrence or first appearance of at least one item of the Birmingham Vasculitis Activity Score (BVAS), which was indicative of threatened function of a vital organ attributable to active vasculitis. In fact, the number of overtreated patients was even higher given a residual risk of major relapse despite continued immunosuppressive treatment. Obviously, it means that we need strong predictors of relapse to personalise remission maintenance therapy and to avoid the risks associated with unnecessary extension of immunosuppression.

Unfortunately, the REMAIN study raises more questions than answers. Previous studies suggested that the risk of relapse is higher among patients with granulomatosis with polyangiitis (GPA) and/or circulating autoantibodies to proteinase 3 (PR3-ANCA) as opposed to patients with microscopic polyangiitis and/or autoantibodies to myeloperoxidase (MPO).[2] It is unknown whether disease type or ANCA type is a major determinant of clinical outcomes in patients with AAV. In Miloslavsky *et al* study, the risk of relapse correlated more closely with nosological diagnosis than with ANCA specificity.[3] These results were in contrast to the findings by other authors who showed that ANCA type is a stronger predictor of recurrent exacerbations of AAV.[4] A trend to increase the duration of therapy up to 36 months in patients who are PR3-ANCA positive was noted in the latest recommendations for the management of AAV published by the European League Against Rheumatism (EULAR) in conjunction with the European Renal Association—European Dialysis and Transplant Association (ERA-EDTA).[5] However, the members of an international task force recommended only that remission-maintenance therapy for AAV be continued for at least 24 months following induction of sustained remission and did not specify directly the indications for an extended immunosuppression. In the REMAIN study, ANCA specificity at diagnosis (PR3 vs MPO) or disease phenotype (GPA vs MPA) were not predictive of any relapse or major relapse. The unexpected finding was probably related to the limited statistical power of this study.

In the REMAIN study, persistent ANCA positivity at randomisation, that is, 2 years after initiation of immunosuppression, was independently associated with the risk of relapse and may suggest a possible benefit of continued immunosuppressive therapy. Similar data were obtained in the previous studies.[6 7] Persistence of ANCA is a frequent finding in patients with clinically stable remission of AAV. Autoantibodies persisted in 51% and 56% of patients in the continuation treatment and the withdrawal groups, respectively. Therefore, at least half of patients with AAV will require prolonged maintenance therapy based on this indication. ANCA testing as a means of predicting future relapse is a simple and attractive approach. However, its role remains controversial. This issue was addressed in the EULAR/ERA-EDTA recommendations, which stated that either persistence, fourfold rise or seroconversion from negative to positive ANCA indicated a higher risk of relapse in certain studies, while other studies did not confirm this association.[5] Of note, in the REMAIN study, relapse occurred in 29% of patients with negative ANCAs at randomisation, when assigned to azathioprine discontinuation. Therefore, recurrent exacerbations can develop in a significant proportion of patients with AAV who achieve ANCA-negativity after remission induction treatment.

Karras *et al* did not provide detailed information to precisely evaluate other factors known to influence relapse risk, for example, organ distribution or baseline renal function. Among them, previous cyclophosphamide exposure (total dose, intravenous/oral administration) may be particularly important given the results of the CYCLOPS study that showed a twofold increase in relapse risk after intravenous pulse cyclophosphamide for remission induction as opposed to daily oral cyclophosphamide.[8] In contrast to other predictors, remission induction treatment can be modified for achieving better long-term clinical outcomes. Therefore, we should probably try to understand where is the right balance between intensity of remission induction and maintenance therapy, for example, more aggressive starting treatment with more rapid withdrawal of immunosuppression or vice versa. In the REMAIN study, the price for a lower risk of relapses in the continuation treatment group was not high given the absence of statistical difference between the two groups in the prevalence or severity of adverse events. Cytopaenias and cardiovascular events were numerically more frequent among patients who continued immunosuppressive therapy after randomisation, but their numbers were small and insufficient for any definite conclusions, particularly regarding cardiovascular outcomes. Of note, the intensity of prolonged treatment in the REMAIN study was low, that is, 5–7.5 mg of prednisolone (this dose was gradually tapered) and 1 mg/kg of azathioprine. Less intensive therapy may improve its safety. However, it seems doubtful that it would provide adequate efficacy given a relatively high

residual frequency of exacerbations in the continuation group. The REMAIN study was conducted prior to widespread use of rituximab, and the authors avoided extrapolation its results to patients receiving B-cell-depleting agents as induction and/or maintenance therapy.

In summary, the REMAIN study contributes significantly to our understanding of treatment options in patients with AAV. A success of any study depends on its ability to achieve primary endpoint. Karras *et al* study confirmed a primary hypothesis and definitely showed that prolonged maintenance therapy with low-dose prednisolone and azathioprine reduces the frequency of relapse, when compared with withdrawal of immunosuppression 2 years after diagnosis. Who requires long-term treatment? In the nearest future, we should not expect a simple answer to this question. A decision to extend immunosuppression should be individual and should be based on a structured approach taking into account both established and probable predictors of relapse such as disease type, ANCA specificity, persistence of autoantibodies, organs involved, renal function, intensity and duration of induction therapy and so on. Almost all patients (96%) were enrolled in the REMAIN study after the first remission of newly diagnosed AAV. Therefore, the results of this study cannot be directly extrapolated to patients with recurrent disease who may be also candidates for a longer immunosuppressive therapy.

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Challenging judgement of a low-positive ACPA test in the context of individuals at risk of RA

We thank Bossuyt[1] for his letter and the comments related to our previous published study.[2] We agree that the calculated test likelihood ratios for low-positive anticitrullinated protein antibody (ACPA) reflect the relative uncommonness of low-titre ACPA in prevalent rheumatoid arthritis (RA) (despite a significant association between). However, in contrast, a low-ACPA titre result in the context of testing for future incident RA might represent an intermediate stage in disease development in individuals on their way to develop RA. We have preliminary unpublished data showing a significant association between low-ACPA titre and future incident RA in our cohort. Judgement of the ACPA test results is therefore more challenging in such context and probably additional information (such as presence or absence of the human leukocyte antigen (HLA)-shared epitope risk gene alleles) will improve the discrimination of individuals who will not and those who will continue to develop high ACPA titres and eventually RA. More studies addressing the additional benefit of a higher relative weight for high titre as compared with low-ACPA titre in the context of clinical manifest RA and future RA are needed.

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The challenge to interpret conflicting results and the need of a univocal definition for germinal centres in primary Sjögren's syndrome

We read with interest the article by Haacke *et al*[1] demonstrating that the presence of germinal centres (GCs) in minor salivary glands (MSG) of patients with primary Sjögren's syndrome (pSS) does not differ between subjects developing non-Hodgkin's lymphoma (NHL) and those who do not develop this complication. In recent years the prognostic role of MSG histopathology has been a matter of intense debate. The severity of the inflammatory infiltrate as defined by the focus score (FS) and/or by the presence of ectopic lymphoid structures (ELS) has been associated with a severe clinical picture characterised by extraglandular manifestations as well as NHL.[2–5] Although the calculation of FS is rather intuitive and reproducible,[6] making the comparison of different studies easy, discrepancies arise with regard to the assessment of ELS. The protocol provided by the Sjögren's International Clinical Collaborative Alliance is widely used[7]; however, it does not provide specific guidance regarding the GCs. On the contrary, in the recent standardisation of labial salivary gland histopathology in clinical trials in pSS, there was strong agreement that the presence of GC-like structures should be reported in routine practice.[8] H&E is considered sufficient to allow accurate detection of fully formed GC by experienced pathologists, in particular in secondary lymphoid organs with a well-distinguished light and dark zone segregation. However, in MSGs, this detection is more challenging and GC-like structures are often only appreciable as a lighter area within the follicular structure without the classical dark/light zone segregation. On this basis, additional immunostainings, including B-cell lymphoma (Bcl)-6, CD21 and activation-induced cytidine deaminase (AID), have been suggested to assess ELS, but without agreement on which staining protocol should be preferably used to identify ELS. Moreover, the combination of a double staining for CD3 and CD20 to identify B/T cell segregation would reduce the risk of GC-like structures overestimation compared with the detection of CD21 alone.[9 10] In our opinion, the lack of consensus and the difficulty to identify ELS, in association with the variability of pSS samples used in the literature (ie, parotid vs MSG samples), may explain the varying prevalence of GC-like structures in pSS biopsies,[11] and the different pathological consequences and clinical features associated with the presence of GC-like structures. In fact, the results of the present study, which assessed GCs through a combination of H&E and Bcl-6 staining and ruled out an association between ELS and lymphoma, are in striking contrast with those from Theander *et al*[5]who defined GCs based on H&E staining only and observed an association with the development of lymphoma. Bombardieri *et al*[12] indicated an increased incidence of GCs, determined by expression of AID and by presence of T cells, B cells and CD21^{+}follicular dendritic cells (FDC) networks, in diagnostic biopsies preceding NHL development. Likewise, in our cohort, the presence of GCs as defined by B/T cell segregation plus a positive CD21 staining in the B-cell area was associated with overall extraglandular involvement including lymphoma.[3] The availability of almost four different methods and the lack of harmonisation across different studies are a major pitfall that imposes to interpret conflicting results with caution. We envisage that the first step towards the harmonisation of ELS assessment, and therefore to the development of univocal recommendations to be used in clinical practice, is to explore the agreement/disagreement of the proposed procedures and their combination as well as the cost and time effectiveness in large cohorts of patients. This would allow to identify a standard and reproducible way to proceed in daily clinical practice as well as in future clinical trials.

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Standardisation of the detection of germinal centres in salivary gland biopsies of patients with primary Sjögren's syndrome is needed to assess their clinical relevance

We thank Alunno *et al*[1] for their comments, as a response to our recent publication[2] in which we describe that, in contrast to the prevailing view, germinal centres in diagnostic labial gland biopsies are not predictive for the development of mucosa-associated lymphoid tissue (MALT) lymphoma in parotid salivary glands in patients with (primary) Sjögren's syndrome (pSS). As we[2] and others also noted before,[3–6] Alunno *et al*[1] underpin in their comments the need for standardisation of the detection method of germinal centres in salivary gland biopsies in patients with pSS. Germinal centres are structures that arise in B-cell follicles of secondary lymphoid organs as a response to antigenic stimulation. In these structures, high-affinity memory B-cells are generated, as a consequence of an intimate interplay between B-cells, follicular helper T cells (T_{FH}) and immune-complex presenting follicular dendritic cells (FDCs). Germinal centres can also be found within B-cell areas of ectopic (tertiary) lymphoid tissue that develops in target tissues of various rheumatic autoimmune diseases, including pSS.[7] While detection in secondary lymphoid tissue is usually relatively simple, recognition of these structures in salivary gland biopsies of patients with pSS is generally more difficult. In particular in H&E stained sections, striated ducts infiltrated with lymphocytes (lymphoepithelial lesions), can erroneously be mistaken for germinal centres (see figure 1). As discussed before,[5] we proposed for this reason to use staining for transcription factor B-cell lymphoma (Bcl)-6 to identify germinal centres (figure 1). Bcl-6 is consistently expressed at high levels by germinal centre B-cells and is commonly applied by pathology laboratories for lymphoma subtyping. In our study,[2] we observed slightly more germinal centres in Bcl-6 stained sections of pSS salivary gland biopsies compared with H&E stained sections, which was due to the fact that also small germinal centres (defined as clusters of ≥ 5 Bcl-6^+ B-cells) could easily be detected. Larger cohorts of salivary gland biopsies are obviously needed to establish the higher sensitivity and specificity of Bcl-6 stained sections, compared with H&E salivary gland stained sections.

FDCs are not only essential for the generation and function of germinal centres but are, as CXCL13 producing cells, also critically involved in the maintenance of the spatial organisation of the ectopic lymphoid tissue into segregated B-cell and T-cell areas.[8] Indeed, the mere presence of FDC networks, as revealed by CD21 staining, in salivary gland lymphoid tissue of patients with pSS does not imply that germinal centres are also present.[9] Staining for (the long isoform of) CD21 is thus not appropriate for identification of germinal centres. Nevertheless, CD21 staining may be useful to discriminate unorganised infiltrates from organised ectopic lymphoid tissue. Besides proper detection of structures, we like to stress here that the proper nomenclature should be used in all histopathological studies and that ectopic lymphoid tissue is not equivalent with ectopic germinal centres. We propose the following levels of organisation of the infiltrating lymphoid cells: (1) unorganised lymphocytic focus (CD21− Bcl-6−), (2) organised lymphocytic focus (ectopic lymphoid tissue) without germinal centres (CD21+ Bcl-6−) and (3) organised lymphocytic focus (ectopic lymphoid tissue) with germinal centres (CD21+ Bcl-6+).

An important question is whether identification of germinal centres is clinically relevant and has an additional value over the already collected serological and clinical data. Germinal centres are observed in diagnostic (minor) salivary gland biopsies of approximately 25% of the patients with pSS.[10] Patients with germinal centres in their diagnostic biopsies seem to have more severe disease as witnessed by the presence of autoantibodies (rheumatoid factor, anti-SSA, anti-SSB), hypergammaglobulinaemia, increased levels of local and systemic proinflammatory cytokines and extraglandular manifestations.[10 11] Presence of germinal centres has also been associated with a higher risk of non-Hodgkin's lymphoma development.[12] This latter association has been used frequently to justify the evaluation of germinal

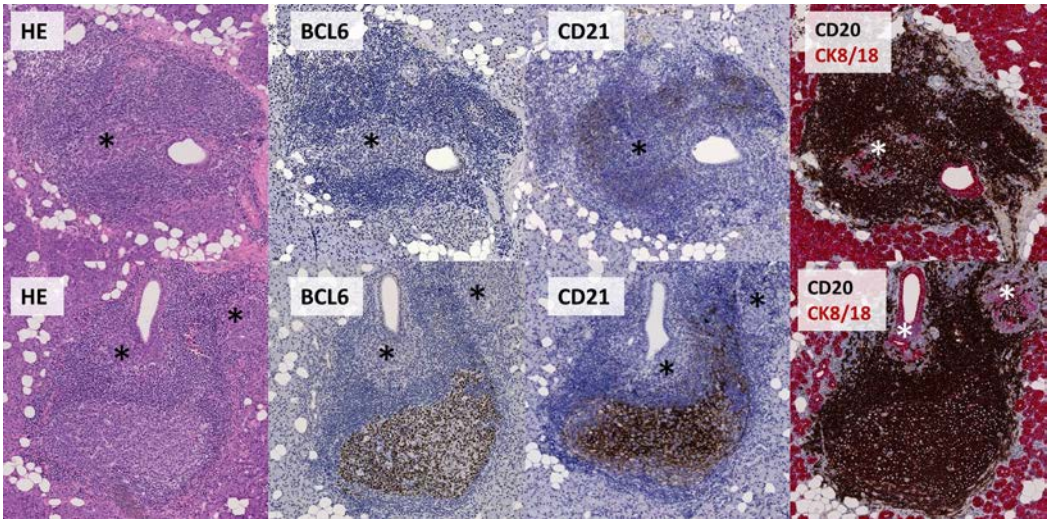

Figure 1 Histopathological evaluation of germinal centres in parotid salivary glands of pSS patients.[5] Consecutive formalin fixed-paraffin embedded parotid gland sections from two patients with pSS were stained for H&E, Bcl-6, CD21 and for CD20 (brown) plus cytokeratin (CK) 8+18 (red). The CK staining was used to identify epithelial cells. Lymphoepithelial lesions (composed of epithelial cells and lymphoid cells) are marked by an asterisk and can be mistaken for germinal centres in H&E stained sections. The H&E stained and the Bcl6 stained micrographs of the lower panels are from our reference.

centres in minor salivary gland biopsies. However, importantly, only one of the seven non-Hodgkin lymphomas observed in the pSS cohort of the study of Theander *et al*[12] was a parotid MALT lymphoma, that is, the type of lymphoma that is characteristically associated with pSS. In our retrospective study,[2] we could not demonstrate that the presence of well-defined germinal centres in diagnostic minor salivary gland biopsies (both H&E and Bcl-6-defined) was also predictive for the development of parotid MALT lymphoma. We agree with Alunno *et al*[1] that histopathological analysis of salivary gland biopsies from larger cohorts of patients scored using standardised protocols and with well-defined definitions are needed to assess the clinical relevance of the presence of germinal centres in salivary gland biopsies of patients with pSS.

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Large joint involvement at first presentation with RA, an unfavourable feature: results of a large longitudinal study with functioning and DMARD-free sustained remission as outcomes

With great interest we read the recently published letter by Rubbert-Roth *et al*.[1] In a post-hoc analysis of the U-Act-Early trial in 317 patients with very early rheumatoid arthritis (RA),[2] it was observed that patients with large joint involvement (LJI) at baseline had significantly more disease activity and more functional disability at baseline. In addition, while the disease activity score (DAS) was similar after 2 years of follow-up compared with patients without LJI, the difference in functional disability remained. Furthermore, patients without LJI had a higher chance of achieving drug-free remission. The authors concluded that equal weighting of joints in the DAS is contrasting an unequal impact on physical function.[1]

The idea of a weighted joint score is not new. A joint index that takes the joint surface into account, thus weighing large joints heavier than small joints, is the Lansbury index.[3] It has been reported that this index correlates better with C reactive protein (CRP) than joint indices that use unweighted scores with regard to joint size.[4] Furthermore, it has been shown that arthritis of large joints—the knee in particular—is associated with higher CRP levels at presentation with RA and a more destructive disease course.[5]

However, outcomes other than joint damage, such as functioning and the ability to stop therapy with disease-modifying antirheumatic drugs (DMARDs), are of increasing interest. With these insights in mind, we sought to validate the findings by Rubbert-Roth *et al* in RA-patients included in a large observational cohort study. The Leiden early arthritis clinic is a large population-based inception cohort of patients with newly diagnosed arthritis that started in 1993.[6] Patients included between 1993 and 2014 who fulfilled the 1987 criteria for RA within 1 year of follow-up were studied (n=1233). Patients were treated according to the insight of the treating rheumatologist, which differed according to the year of inclusion.[7]

We observed that LJI at baseline (n=506, defined as ≥1 swollen shoulder, elbow, knee and/or ankle joint) was associated with higher serological inflammatory markers, more swollen joints and more functional disability at baseline (table 1). Linear mixed models were then used to study the course of functional disability, as measured with the health assessment questionnaire (HAQ), and disease activity over a period of 4 years (after that there was limited data available). Both models were corrected for inclusion year, age and gender (figure 1A) and showed that patients with LJI had an average HAQ score that was 0.16 higher (95% CI 0.10 to 0.23) than that of patients without LJI, while the DAS score became similar after ~3 years (figure 1A). After correction for the DAS, the average HAQ score was 0.06 higher (95% CI −0.01 to 0.14). Furthermore, sustained DMARD-free remission (defined as persistent absence of synovitis for ≥1 year after cessation of DMARDs) was achieved more often by patients without LJI (figure 1B, p=0.001). This association remained significant also after correction for age, gender, inclusion year, anticitrullinated protein antibodies (ACPA) and DAS (HR 1.4, 95% CI 1.0 to 2.0, p=0.043).

Thus, although the absolute differences in HAQ for patients with/without LJI were somewhat smaller than that observed by Rubbert-Roth *et al*, our data validated that LJI at disease onset is important in a large longitudinal cohort of patients with RA.

Although (treat-to-target) therapy caused that initial differences in DAS disappeared over time, LJI at first presentation with RA is indeed an unfavourable predictor for important

Table 1 Baseline characteristics

	No LJI* (n=727)	LJI (n=506)	p Value
Age, mean (SD)	56 (16)	58 (15)	0.06
Female gender, n (%)	480 (66)	340 (67)	0.67
Symptom duration days, median (IQR)	123 (61–246)	131 (70–255)	0.47
ACPA positive, n (%)	377 (52)	256 (51)	0.66
RF positive, (%)	418 (58)	291 (58)	1.0
HAQ, mean (SD)	1.0 (0.7)	1.2 (0.7)	<0.001
DAS44, mean (SD)	3.0 (0.9)	3.7 (1.0)	<0.001
66-SJC, median (IQR)	6 (3–10)	10 (6–17)	<0.001
68-TJC, median (IQR)	9 (5–16)	14 (7–27)	<0.001
CRP (mg/L), median (IQR)	10 (4–22)	24 (11–53)	<0.001
ESR (mm/h), median (IQR)	25 (13–41)	40 (22–61)	<0.001

*LJI was defined as the presence of any shoulder, elbow, knee or ankle swelling at baseline.
ACPA, anticitrullinated protein antibodies; CRP, C reactive protein; DAS, disease activity score; ESR, erythrocyte sedimentation rate; HAQ, health assessment questionnaire; LJI, large joint involvement; RF, rheumatoid factor; SJC, swollen joint count; TJC, tender joint count.

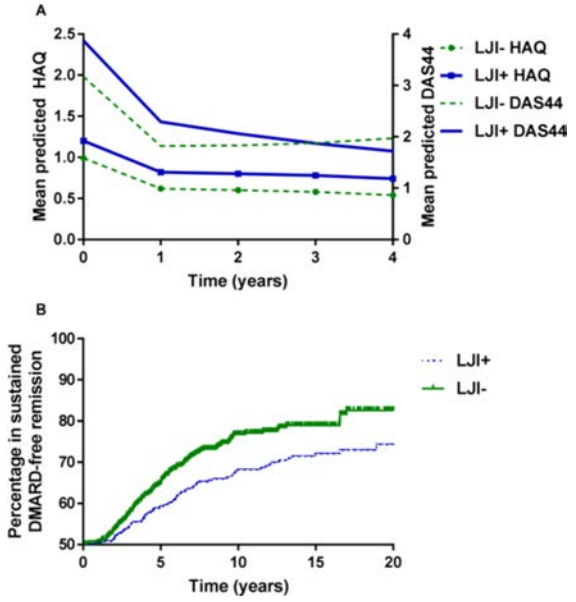

Figure 1 Predicted course of HAQ and DAS44 over time (A) and DMARD-free remission over time (B) in RA-patients with and without LJI at disease presentation. Panel A depicts the course of HAQ and DAS44 over time. The values are the predicted values of a linear mixed model analysis. Both models are corrected for age, gender and year of inclusion. Panel B depicts the amount of patients achieving sustained DMARD-free remission up to 20 years of follow-up. A log-rank test gave a p value of 0.001. A multivariable Cox regression analysis corrected for age, gender, ACPA, inclusion year and DAS gave a HR of 1.4 (95% CI 1.0 to 2.0, p=0.043) on achieving sustained DMARD-free remission in patients without LJI compared with those with LJI. ACPA, anticitrullinated protein antibodies; DAS, disease activity score; DMARD, disease-modifying antirheumatic drug; HAQ, health assessment questionnaire; LJI, large joint involvement; RA, rheumatoid arthritis.

long-term disease outcomes, such as functional ability and sustained DMARD-free remission.

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Contributors LEB drafted and approved the final manuscript and helped with the study design, data acquiring, data analysis and interpretation of the data. DMB helped with the data acquiring, data analysis, interpretation of the data and critically revised and approved the final manuscript. AvdHM designed the study, helped acquire and interpret the data and critically revised and approved the final manuscript.

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Alternative diagnoses in patients with chronic back pain not diagnosed with axial spondyloarthritis: data from the SPACE cohort

We recently published a study investigating whether the mere presence of multiple spondyloarthritis (SpA) features is sufficient for an axial spondyloarthritis (axSpA) diagnosis.[1] Patients (n=500) with chronic back pain (CBP) suspected of axSpA were stratified according to their number of SpA features into four subgroups: ≤1, 2, 3 and ≥4 SpA features based on medical history taking, physical examination and measurement of acute phase reactants, but before sacroiliac imaging and HLA-B27 testing. In total, 24% (38/159), 43% (62/143), 62% (49/79) and 85% (101/119) of CBP patients with ≤1, 2, 3 and ≥4 SpA features, respectively, were diagnosed with axSpA. In particular, HLA-B27 positivity and imaging findings highly suggestive of axSpA were strongly associated with an axSpA diagnosis. Although the likelihood of axSpA diagnosis increased with an increasing number of SpA features, not all patients with multiple SpA features were diagnosed as having axSpA.

In the News and Views section of *Nature Reviews Rheumatology*, Braun and Kiltz raised the question what diagnoses were made in patients with CBP who were *not* diagnosed with axSpA. This information was not provided in the manuscript.[2] The question is relevant since we agree with these authors that it is unlikely that a patient with 4 or more SpA features does not have SpA. This reasoning follows the modified Berlin algorithm in which patients with CBP and ≥4 SpA features are readily diagnosed with axSpA.[3]

In our study, rheumatologists were first asked to provide a diagnosis (ie, presence of axSpA: yes/no). In all patients, this question was answered with 250 patients diagnosed with axSpA after full diagnostic workup. Second, in case of 'no axSpA', rheumatologists were requested to provide a most likely alternative diagnosis.

In 76% (189/250) of patients who were not diagnosed as having axSpA, the alternative diagnosis for CBP was recorded in the study database. Across all subgroups based on the number of SpA features most common diagnoses were non-specific back pain, mechanical back pain, degenerative disc disease and (fibro)myalgia (table 1). Not surprisingly, in these patients, almost no positive imaging (sacroiliitis on either radiographs or MRI) was observed and HLA-B27 positivity was infrequent.[1] Especially in the 18 patients not diagnosed with axSpA, but with at least four SpA features, none of the patients had imaging abnormalities suggestive of axSpA on MRI or radiography, and only four of them were HLA-B27 positive. The most frequent SpA features in these patients were: inflammatory back pain (IBP) (16/18), a positive family history of SpA (16/18), a good response to non-steroidal anti-inflammatory drugs (11/18), elevated C reactive protein or erythrocyte sedimentation rate (ESR) (8/18) and enthesitis (8/18). Overall, these findings show that rheumatologists in clinical practice rightly dispute a diagnosis of axSpA even when there is a high number of SpA features, especially when imaging is normal and patients are negative for HLA-B27.

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Table 1 Alternative diagnoses in patients with CBP **not diagnosed with axSpA** (n=250) 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

Number of SpA features*	Alternative diagnoses	N (%)
0–1 (n=121)	Non-specific back pain	32 (26)
	Mechanical back pain	5 (4)
	IBP	4 (3)
	Degenerative disc disease/HNP	14 (12)
	Myalgia	17 (14)
	Fibromyalgia	8 (7)
	Hypermobility syndrome	3 (2)
	Other†	12 (10)
	Missing diagnosis	29 (24)
2 (n=81)	Non-specific back pain	15 (19)
	Mechanical back pain	8 (9)
	IBP	3 (4)
	Degenerative disc disease/HNP	9 (11)
	Myalgia	14 (17)
	Fibromyalgia	4 (5)
	Hypermobility syndrome	1 (1)
	Other‡	12 (15)
	Missing diagnosis	16 (20)
3 (n=30)	Non-specific back pain	6 (20)
	Mechanical back pain	3 (10)
	IBP	1 (3)
	Degenerative disc disease/HNP	3 (10)
	Myalgia	2 (7)
	Fibromyalgia	3 (10)
	Hypermobility syndrome	0 (0)
	Other§	2 (7)
	Missing diagnosis	10 (33)
≥4 (n=18)	Non-specific back pain	4 (22)
	Mechanical back pain	0 (0)
	IBP	1 (6)
	Degenerative disc disease/HNP	4 (22)
	Myalgia	1 (6)
	Fibromyalgia	0 (0)
	Hypermobility syndrome	1 (6)
	Other¶	1 (6)
	Missing diagnosis	6 (33)

*Total patient group: 0–1 SpA feature, n=159; 2 SpA features, n=143; 3 SpA features, n=79; ≥4 SpA features, n=119.
†Arthralgia, n=1; local pain syndrome, n=1; diffuse idiopathic skeletal hyperostosis, n=1; ileitis condensans, n=1; osteoarthritis, n=2; Tarlov cyst, n=1; undifferentiated arthritis, n=1; uterus myomatosus, n=1.
‡Arthritis, n=3; functional complaints, n=2; osteoarthritis, n=2; pelvic instability, n=1; uterus myomatosus, n=1; tendinopathy, n=2.
§Undifferentiated oligoarthritis, n=1; tendinopathy, n=1.
¶Osteoarthritis, n=1.
axSpA,axial spondyloarthritis; CBP, chronic back pain; HNP, herniated nucleus pulposus; SpA, spondyloarthritis.

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