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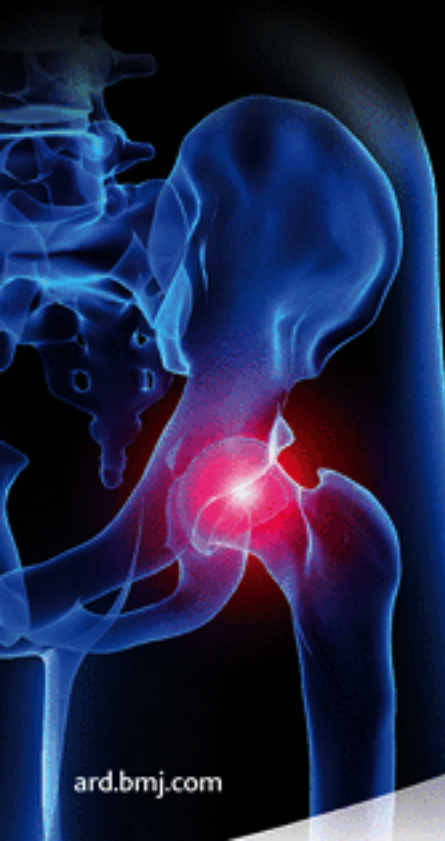

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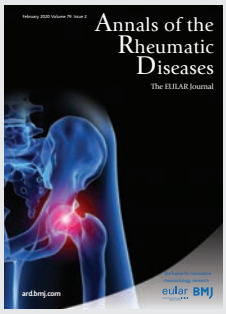

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Of mice, men and microbes: the impact of the microbiome on immune responses

Rheumatic diseases are a diverse group of conditions in which the host and the environment interact to drive inflammation and autoreactivity. In this dyadic model, the strongest environmental influence is infection, with bacteria and viruses likely to trigger disease in a host predisposed by genetics; infection can either non-specifically stimulate the immune system to heighten the propensity for autoreactivity or specifically stimulate B and T cell autoreactivity by molecular mimicry. For many years, this venerable model has powerfully influenced the design of experiments in both the human and animal systems as well as their interpretation.

While the 'one organism, one disease' mechanism may pertain to at least some rheumatic diseases (eg, rheumatic fever following *Streptococcus* infection), for most other conditions, pathogenesis is more complicated, at least in part because one of the major sources of foreign organisms—bacteria, viruses, fungi and others—resides in or around the host as the microbiome. The microbiome is a huge biomass, with the mammalian host containing as many prokaryotic as eukaryotic cells; the number is in the trillions. Many studies have therefore addressed whether the microbiome can influence the occurrence of not only rheumatic diseases but also metabolic, cardiovascular and neuropsychiatric conditions, among others.[1 2]

As shown recently in a provocative study by Rosshart *et al*[3], the host and the environment are not truly distinct, with data presented suggesting that the microbiome and the host have coevolved to produce an optimal balance of positive and negative effects. In their drive to study disease in a more reductionist way, investigators have likely disrupted this balance by breeding genetically inbred mice in very clean environments. Although a clean environment would seemingly reduce potential 'contaminating' influences of infection, it is very much unnatural and leads to a microbiome that itself can alter host responses and skew the mechanisms on pathogenesis in large and unpredictable ways.

The study by Rosshart and colleagues[3] represents an important step in understanding how the microbiome can influence disease by exploring a very novel model system called 'wildling' mice. The opposite of germ-free mice, wildling mice are the offspring of a pseudopregnant wild mouse dam which has received embryos from a C57BL/6 mouse by surgical transfer. This approach allows the development of a laboratory strain mouse with the microbiome of a wild mouse exposed to a natural environment. In this case, the wild mice were trapped in barns in the Washington DC area. Like laboratory mice, the wild mice were of the *Mus musculus domesticus* species.

In a series of elegant experiments, the investigators compared the immunological responses of conventional C57BL/6 mice, the wildling mice and wild mice as well as their microbiomes. Since gut is the largest and most accessible of the microbiomes, the investigators analysed it in the most detail. In addition to bacteria, the gut microbiome also contains viruses, fungi and, in some cases, multicellular organisms such as helminths, but the enumeration of bacteria is the most straightforward by sequencing ribosomal genes.[4] As the studies of Rosshart *et al*[3] showed, wildling mice significantly differ from laboratory mice in their microbiomes (gut, skin and vagina) and more closely resemble those of the wild mice in their size and diversity.

By the nature of this system, the relative contribution of genetics and the microbiome to shaping the immune system can be studied via a comparison between the immune cells of the laboratory, wildling and wild mice. Using mass spectroscopic techniques, Rosshart *et al* showed that the phenotypic properties of spleen immune cells appear to reflect primarily the microbiome, whereas the genome has a greater influence on the lymphocyte populations at sites such as the gut, skin and vagina. For peripheral blood, the pattern of gene expression in terms of transcriptional profile showed that wildling and wild mice are very similar despite genetic differences.

Wildling mice are not the only system that can help explore the impact of the microbiome on immune responses as well as disease. Studies of this kind go back many decades and began with the use of germ-free mice to elucidate the impact of infection (including colonisation) on various diseases. Other versions of this approach include treatment of mice with antibiotics; repopulation of germ-free mice with single or multiple organisms, including pathogens; and transfer of the microbiome of wild mice to conventional laboratory mice.[5–9]

While the microbiomes that develop in these different models vary, nevertheless, studies have clearly established that the microbiome can have a profound effect on immune responses. These effects are demonstrable in the response to infection, vaccination and development of autoimmunity in models such as the New Zealand mice.[1 2] In this regard, the perspective in evaluating these effects is important since the microbiome can promote host defence against infection as well as predispose to the occurrence of autoimmune and inflammatory diseases.

Other evidence for the impact of the microbiome on immune responses concerns aspects for the use of animal models to study diseases that have been troubling to investigators. The first concerns reproducibility of findings from different laboratories, which is now a major concern for both mechanistic and translational research. Unfortunately, studies addressing the same question can sometimes arrive at quite different conclusions about the role of particular cell types, for example, in a phenomenon. Among causes of irreproducibility or inconsistency of experimental results, issues of animal husbandry are always considered possible, with the nature of the microbiome high on the list.[4]

Another troubling aspect of animal research concerns the difficulty in translating results of studies with mice to patients. The greatest difficulties with translation have occurred in the setting of sepsis. While studies using agents such as tumour necrosis factor (TNF)-α blockers to treat sepsis appeared very promising in mice, clinical trials failed.[10 11] A failure to translate TNF-α blockers for sepsis is perhaps not surprising since, as shown in studies on gene transcription of immune cells, human and murine responses to challenges such as sepsis, trauma and burns show very little similarity. Of note, the responses of patients with these conditions can show common patterns of gene transcription.[12]

The divergent findings on the effects of TNF-α blockade in human and murine sepsis highlight a well-established fact: despite sharing of many genes, humans and mice have many differences in both innate and adaptive immune systems, the nature of their antibodies and even the composition of the blood in terms of different cells.[13] Humans and mice diverged between 65 and 75 million years ago in evolution and inhabit very different environments. In an obvious case, mice live close to the ground and have intimate contact with soil as its microbial components. In humans, on the other hand, the breathing apparatus is safely in the air, reducing a constant barrage of dirt and its abundance of foreign organisms.

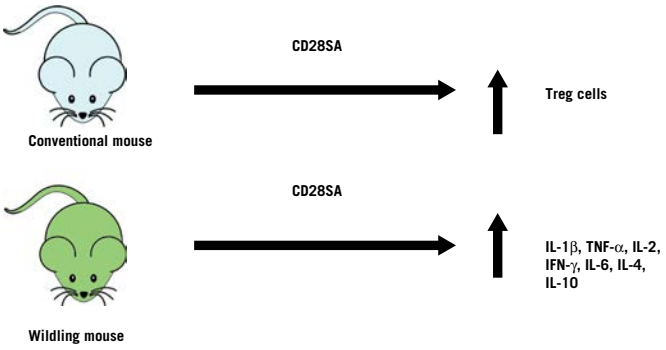

Figure 1 The response of laboratory and wildling mice to the administration of CD28SA. The figure illustrates the outcome of CD28SA treatment of conventional and laboratory mice. As the figure shows, while conventional mice showed a dramatic upregulation of Treg cell numbers at day 4, the wildling mice showed an increase in proinflammatory cytokine production at 2 hours (IL-1β, TNF-α, IL-2, IFN-γ), 6 hours (IL-6), 24 hours (IL-4) and 96 hours (IL-10). In contrast to the situation with conventional mice, the changes in Treg numbers of the wildling mice were not significant. The differences between cytokine levels of conventional and wildling mice were all significant at the time of sampling, which differed depending on the cytokine. Since CD28SA provoked cytokine storm in human subjects, the results of these experiments indicate that wildling mice provide much better prediction of the response of humans than conventional laboratory mice. CD28SA, CD28 superagonist antibody; IFN-γ, interferon gamma; IL, interleukin; TNF-α, tumour necrosis factor-alpha.

For the wildling model to have utility as a model for translation, it must display certain properties and most importantly predict responses of human subjects more accurately than those of conventional mice. The investigative team, therefore, conducted studies to explore the characteristics of the wildling mice as a model for translation, testing basic properties of the transferred wild microbiome, including its stability over time and its resiliency. These studies demonstrated that at least through the F5 generation, the gut microbiome of the wildling mice is stable, an important consideration in the intended use for translational research since their production takes time and skill. Other studies showed that a natural microbiome from wild mice is resilient and apparently better adapted than the microbiota of a conventional laboratory mouse.[3] In its own way, the microbiome of the laboratory mouse is dysbiotic, since the transition from the wild to the laboratory has represented a huge jolt to the system, upsetting millions of years of evolution.

With data demonstrating that wildling mice have favourable properties as laboratory models, the next experiments concerned the ability of these mice to predict responses to therapeutic interventions. For this purpose, the investigators explored two systems in which studies in mice did not predict eventual results with human subjects. The first system tested involved the CD28 superagonist antibody (CD28SA). In studies with mice, the CD28SA dramatically boosted the number of Treg cells and showed impressive efficacy in a variety of disease models, promising a new approach to control unwanted or deleterious immune responses.[14] The results in human trials, however, were totally divergent, leading to catastrophe.

In a phase I study, the CD28SA induced a cytokine storm that was near fatal in treated subjects.[15] Importantly, in the study by Rosshart *et al*, while the conventional laboratory mice showed the expected Treg cell induction with CD28SA treatment at day 4, the wildling mice did not increase this population but rather showed high levels of proinflammatory cytokines as well as interleukin (IL)-10 at various times after treatment depending on the cytokine. Figure 1 shows the differences in responses of these two types of mice. Importantly, for these responses, wildling mice showed much better prediction of the cytokine storm observed in the human subjects. Had the wildlings been used to screen for the effects of CD28SA in preclinical studies, the translation to humans would not have been contemplated and certainly no lives would have been placed in jeopardy.

The other model tested concerned the effects of TNF-α blockade on sepsis. Following the discovery of TNF-α as an important proinflammatory mediator, seminal experiments demonstrated that passive immunisation of mice with a polyclonal anti-TNF antiserum can prevent the shock syndrome induced by lipopolysaccharide (LPS) from *Escherichia coli*. Not surprisingly, clinical trials soon thereafter evaluated TNF-α blockade in humans as a treatment for sepsis (a condition with high morbidity and mortality), but these efforts were unsuccessful and the development programme curtailed.

The failure of TNF-α blockade in humans is often cited as a prime example of the limitations of animal models as a step towards translation. It is therefore important that, in the wildling mice, like humans and unlike conventional mice, TNF-α inhibition with either a monoclonal anti-TNF antibody or a TNFR:Fc fusion protein did not block shock induced by LPS administration. Taken in concert with the results of the study on the effects of CD28SA, these findings on sepsis suggest that wildling mice, because of the microbiome of the wild mice, show patterns of immune responsiveness more analogous to those of humans, pointing to an enhanced utility in translation studies.

Along with other studies on the microbiome, the paper by Rosshart *et al* shows the remarkable effects of the microbiome on the immune system and suggests that the mammalian organism is itself an environment or an ecosystem in which the host genome as well as the microbiome contribute to both physiology and pathophysiology. All of these 'omes' create the metagenome which has components of both eukaryotic and prokaryotic genomes.[4] Conceptually, the findings of the interaction of the host and the microbiome are fascinating since they reveal hitherto unappreciated facets of biology and should stimulate rethinking of fundamental questions on the nature of humans as mammals.

While the study of the microbiome promises a wealth of exciting new knowledge, operationally, the prospect of incorporating consideration of the role of the microbiome in all animal work is also intimidating and even terrifying for the experimentalist. As studies show, the composition of the microbiome (and hence its effects on the immune system) of mice is susceptible to a wide variety of factors, ranging from the diet to the temperature of the room to the nature of the bedding.[4 16] Determining the microbiome of a mouse or colony also requires special expertise as well as knowledge of many species whose names are unfamiliar to most investigators. Studies will have to determine which of the many differences between the microbiomes of conventional and wild mice are most relevant. How many bacterial, viral or fungal species are determining the differences in functional outcomes? Can mice with a standardised microbiome be created and, if so, what should its composition be?

The microbiome impacts on many physiological responses, and studies will need to determine whether changes in the microbiome important for immunological disease impact positively or negatively on cardiovascular or neuropsychiatric disease, for example. Genetics is also at play since genotype can affect the microbiome, as demonstrated in studies on the effects of human leukocyte antigen (HLA) molecules associated with rheumatoid

arthritis and spondyloarthropathy on the composition of the microbiomes by unaffected subjects.[17] The current studies assessed C57BL/6 strain mice. Whether other strains commonly used by immunologists (eg, BALB/c) would behave similarly as those of C57BL/6 background is unknown.

Even as the studies are elucidating the impact of the microbiome on immune function, the microbiome is also becoming a target of therapy, with faecal transplants for *Clostridium difficile* infection just the beginning. Making the microbiome a 'drug' will be a huge undertaking, with issues such as composition, pharmacokinetics and pharmacodynamics important considerations as this work goes forward.[18] While altering the microbiome could be contemplated to prevent or treat disease, the side effects are entirely unknown, and creating a microbiome to resist rheumatoid arthritis, for example, could predispose to other conditions in an unfavourable way.

In a more speculative vein, it is interesting to consider what would have happened to the field of rheumatology had mice with properties similar to those of the wildling mice been used to test the effects of anti-TNF on murine sepsis. Would investigators have concluded that monoclonal antibodies for inhibiting cytokine action in vivo are simply ineffective and the project dropped? Or would they have gone on to try TNF-α blockade in collagen-induced arthritis (CIA), undeterred by the failure with sepsis? Future experiments will have to address this and many other important questions as the most appropriate models for translation are developed and refined.

While many responses in mice show a striking effect of the microbiome, it is not unlikely that others are sufficiently hardwired and robust so that the effects of genetics, epigenetics and the metagenome are not determinative of outcome. The results with biological disease-modifying antirheumatic drugs to treat rheumatoid arthritis, spondyloarthropathy and psoriatic arthritis indicate that anticytokine agents can work widely among patients despite differences in genetics and no doubt the composition of their microbiomes.

For conditions such as rheumatoid arthritis, current animal models have been very informative and have been essential to the development pathway of many agents. Perhaps a model such as the CIA does not need much tinkering with respect to its microbiomes although, despite many successes, CIA has not always predicted results in humans. Thus, while antibodies to IL-17 performed well in CIA, the main benefits of anti-IL-17 therapy has occurred with psoriasis, psoriatic arthritis and ankylosing spondylitis and not rheumatoid arthritis.[19 20] Pending refinement of models for various facets of arthritis including synovitis as well as bone and cartilage destruction, it will be very important that scientific publications include information on animal care and husbandry (eg, light cycle, chow, bedding) so that, if differences between studies do occur, papers have sufficient detail in the methods section to identify possible contributory factors.[4 21]

John Donne, the poet, created some of the most memorable lines in the literature in his mediation that starts with 'No man is an island entire of itself'. As the elegant and exciting studies on the microbiome have shown, no person—man, woman or child—is an organism unto itself. Rather, each person progresses through life with trillions of companions in the microbiome that have participated in the same evolutionary pathway and seem to live in harmony most of the time. Future studies will determine whether the current relationship between humans and microbes can be made more harmonious and whether new animal systems can provide better prediction to promote translational research and develop new therapies.

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REFERENCES

1 Honda K, Littman DR. The microbiota in adaptive immune homeostasis and disease. *Nature* 2016;535:75–84.
2 Clemente JC, Manasson J, Scher JU. The role of the gut microbiome in systemic inflammatory disease. *BMJ* 2018;360:j5145.
3 Rosshart SP, Herz J, Vassallo BG, *et al*. Laboratory mice born to wild mice have natural microbiota and model human immune responses. *Science* 2019;365. doi:10.1126/science.aaw4361
4 Stappenbeck TS, Virgin HW. Accounting for reciprocal Host-microbiome interactions in experimental science. *Nature* 2016;534:191–9.
5 Beura LK, Hamilton SE, Bi K, *et al*. Normalizing the environment recapitulates adult human immune traits in laboratory mice. *Nature* 2016;532:512–6.
6 Gensollen T, Iyer SS, Kasper DL, *et al*. How colonization by microbiota in early life shapes the immune system. *Science* 2016;352:539–44.
7 Reese TA, Bi K, Kambal A, *et al*. Sequential infection with common pathogens promotes human-like immune gene expression and altered vaccine response. *Cell Host Microbe* 2016;19:713–9.
8 Rosshart SP, Vassallo BG, Angeletti D, *et al*. Wild mouse gut microbiota promotes host fitness and improves disease resistance. *Cell* 2017;171:1015–28.
9 Tao L, Reese TA. Making mouse models that reflect human immune responses. *Trends Immunol* 2017;38:181–93.
10 Beutler B, Milsark IW, Cerami AC. Passive immunization against cachectin/tumor necrosis factor protects mice from lethal effect of endotoxin. *Science* 1985;229:869–71.
11 Fisher CJ, Agosti JM, Opal SM, *et al*. Treatment of septic shock with the tumor necrosis factor receptor:Fc fusion protein. The Soluble TNF Receptor Sepsis Study Group. *N Engl J Med* 1996;334:1697–702.
12 Seok J, Warren HS, Cuenca AG, *et al*. Genomic responses in mouse models poorly mimic human inflammatory diseases. *Proc Natl Acad Sci U S A* 2013;110:3507–12.
13 Mestas J, Hughes CCW. Of mice and not men: differences between mouse and human immunology. *J Immunol* 2004;172:2731–8.
14 Hünig T, Dennehy K. Cd28 superagonists: mode of action and therapeutic potential. *Immunol Lett* 2005;100:21–8.
15 Suntharalingam G, Perry MR, Ward S, *et al*. Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412. *N Engl J Med* 2006;355:1018–28.
16 Carmody RN, Gerber GK, Luevano JM, *et al*. Diet dominates host genotype in shaping the murine gut microbiota. *Cell Host Microbe* 2015;17:72–84.
17 Asquith M, Sternes PR, Costello M-E, *et al*. Hla alleles associated with risk of ankylosing spondylitis and rheumatoid arthritis influence the gut microbiome. *Arthritis Rheumatol* 2019;71:1642–50.
18 Taroncher-Oldenburg G, Jones S, Blaser M, *et al*. Translating microbiome futures. *Nat Biotechnol* 2018;36:1037–42.
19 Lubberts E, Koenders MI, Oppers-Walgreen B, *et al*. Treatment with a neutralizing anti-murine interleukin-17 antibody after the onset of collagen-induced arthritis reduces joint inflammation, cartilage destruction, and bone erosion. *Arthritis Rheum* 2004;50:650–9.
20 Miossec P. Update on interleukin-17: a role in the pathogenesis of inflammatory arthritis and implication for clinical practice. *RMD Open* 2017;3:e000284.
21 Omary MB, Cohen DE, El-Omar EM, *et al*. Not all mice are the same: standardization of animal research data presentation. *Cell Mol Gastroenterol Hepatol* 2016;2:391–3.

CLINICAL SCIENCE

Shared decision making in routine clinical care of patients with rheumatoid arthritis: an assessment of audio-recorded consultations

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ABSTRACT

Objectives Although shared decision making (SDM) is advocated in rheumatoid arthritis (RA) treatment, it is largely unclear when, how and to what extent SDM is applied in routine clinical care of patients with RA. This study aimed to investigate the level of SDM in RA treatment from an observer perspective and to assess associations between the level of SDM and characteristics of the clinician, patient and consultation.

Methods The level of SDM was investigated by scoring audio-recordings of 168 routine consultations with unique patients with the observer patient involvement (OPTION) scale (scale 0–100, higher OPTION scores indicating higher levels of SDM). Associations between the level of SDM and characteristics of the clinician, patient and consultation were assessed using multilevel modelling. Statistical significance was set at p<0.05.

Results The mean OPTION score was 28.3 (SD=15.1). The multilevel model included four characteristics: clinician age, patient age, consultation duration and type of treatment decision. There were significant, positive associations between the level of SDM and the consultation duration (b=0.63, 95% CI 0.16 to 1.11), decision for stopping and/or starting medication (b=14.30, 95% CI 5.62 to 22.98), decision for adjusting medication doses (b=8.36, 95% CI 3.92 to 12.81) and decision for administering single dose glucocorticoids (b=15.03, 95% CI 9.12 to 20.93). Thus, a higher level of SDM was significantly associated with a longer consultation duration and the type of treatment decision. No other significant associations were found.

Conclusions Overall, the level of SDM in RA treatment leaves room for improvement. To foster SDM in routine clinical care, training programmes on patient-centred communication skills may be helpful.

Key messages

What is already known about this subject?

- Studies investigating clinicians' and patients' perceived level of shared decision making (SDM) in rheumatoid arthritis (RA) treatment suggest that SDM applied only to a limited extent.
- Empirical studies investigating the level of SDM in RA treatment from an observer perspective are needed to provide a more accurate and unbiased picture of current practice.

What does this study add?

- Using an observer-based measure, a low to moderate level of SDM in RA treatment was found.
- The efforts of clinicians to involve patients in decision making varied substantially and a higher level of SDM was significantly associated with a longer consultation duration and the type of treatment decision.

How might this impact on clinical practice or future developments?

- Overall, the level of SDM in RA treatment leaves room for improvement. Targeted strategies to support the application of SDM (eg, training programmes on patient-centred communication skills) are warranted in this era of patient-centred care.

INTRODUCTION

Disease-modifying antirheumatic drugs (DMARDs) are the cornerstone of rheumatoid arthritis (RA) treatment. A variety of DMARDs are available, each with different risks and benefits to consider. However, a substantial proportion of patients with RA do not adhere to DMARDs, resulting in increased disease activity and radiological progression.[1–4] It is increasingly recognised that patient preferences play an important role in adherence. Matching treatment to patient preferences has been shown to improve adherence.[5 6] Previous studies have shown that patient preferences for RA treatment vary widely and often differ from those of clinicians.[7–11] Therefore, it is important that clinicians and patients make treatment decisions together using the best available evidence and accounting for patient preferences.[12] Indeed, international guidelines for RA treatment state that all treatment decisions should be made through shared decision making (SDM) between clinicians and patients.[13 14] Although SDM is advocated in RA treatment, it is largely unknown when, how and to what extent SDM is applied in routine clinical care of patients with RA.

Previous studies have investigated clinicians' and patients' perceived level of SDM in RA treatment, suggesting that SDM is applied only to a limited extent.[15–18] In a study of 157 clinicians treating patients with RA, 27% of clinicians reported to apply SDM.[18] Likewise, in a study of 892 patients with RA, psoriatic arthritis and ankylosing

spondylitis, 43% of patients reported to perceive no involvement in decision making.[17] However, clinician-reported and patient-reported measures may not accurately reflect reality. Clinicians tend to overestimate their efforts to involve patients in decision making, while patients may not fully understand and identify SDM if they have not perceived it previously.[19–23] Consequently, they are likely to report how satisfied they were with the consultation in general instead of their perceived level of SDM. Although observer-based measures are considered more valid, research using these measures is lacking in the field of RA. Thus, empirical studies investigating the level of SDM in RA treatment from an observer perspective are needed to provide a more accurate and unbiased picture of current practice.

Finally, insight into factors associated with the level of SDM is of great value for the development of targeted strategies to support the application of SDM. Previously, studies in various clinical settings (eg, primary care, anaesthesiology and vascular surgery) have identified a number of factors associated with the level of SDM.[22 24–26] These factors were related to characteristics of the clinician (eg, sex), patient (eg, age) and consultation (eg, duration). However, results were inconsistent between studies and may not be generalised to RA treatment. Therefore, the aim of this study was twofold: first, to investigate the level of SDM in RA treatment from an observer perspective and second, to assess associations between the level of SDM and characteristics of the clinician, patient and consultation.

METHODS

Study design

This study had a cross-sectional design and was reported according to the Strengthening the Reporting of Observational Studies in Epidemiology statement.[27] This study used data that were collected, but not used, in a large, longitudinal study of van Heuckelum and colleagues.[28] In their study, van Heuckelum and colleagues explored the role of implicit and explicit attitudes towards synthetic DMARDs as possible target for improving medication adherence in RA treatment. This study, however, focused on SDM. We used data (eg, audio-recorded consultations) that were not previously analysed and/or published.

Participants

The study of van Heuckelum and colleagues was conducted in the two largest rheumatology centres in the Netherlands: Reade in Amsterdam and the Sint Maartenskliniek in Nijmegen. Recruitment took place between July 2016 and November 2017. First, all clinicians practicing as a rheumatologist or physician assistant in one of the two rheumatology centres were invited for participation. Thereafter, participating clinicians invited a consecutive series of patients after verifying their eligibility. Patients were eligible for participation if they were aged 18 years or older, diagnosed with RA by a rheumatologist and using at least one synthetic DMARD.

Data collection

Characteristics of the clinician and patient

Following recruitment, clinicians completed a short questionnaire on sociodemographic characteristics: rheumatology centre, sex, age, profession and work experience. Patients also completed a short questionnaire on sociodemographic characteristics: sex, age and educational level. Clinical characteristics (disease duration, presence of comorbidities, number of synthetic DMARDs in use, use of biologicals and use of glucocorticoids) were extracted from patients' medical records. All comorbidities registered in a patient's medical record were included, regardless of type and severity. Furthermore, patients' medication beliefs were assessed with the Beliefs about Medicines Questionnaire.[29] This 10-item questionnaire comprises two subscales, assessing patients' perceived need to take medication (necessity subscale) and their concerns about potential adverse consequences (concerns subscale). Scores range from 5 to 25 with higher scores indicating stronger beliefs. The health status of patients was assessed with the Dutch consensus version of the Health Assessment Questionnaire.[30] The range of scores is between 0 and 3 where 0 represents no disability and three complete disability.

Characteristics of the consultation

In addition, routine consultations were audio-recorded. Per patient, one audio-recording was made during a single consultation. Audio-recordings were checked for sound quality and completeness. Audio-recordings of poor sound quality and the ones not containing the full consultation were removed. The duration of each consultation was registered. The type of treatment decision that was made during the consultation was also registered. This study only took treatment decisions regarding synthetic DMARDs, biologicals and glucocorticoids into account. Treatment decisions were categorised into four types: stopping and/or starting medication, adjusting medication doses, administering single-dose glucocorticoids and continuing medication. The first two categories refer to changes to a patient's treatment. The third category refers to the additional administration of single-dose glucocorticoids. This type of medication was usually administered during or shortly after the consultation for short-term control of flares. The fourth category refers to the unchanged continuation of a patient's treatment. If multiple treatment decisions were made during the consultation, only one type of treatment decision was registered, following the above-mentioned sequence. For example, if it was decided to increase the total weekly dose of methotrexate and the patient also received an intra-articular injection of prednisone, the type of treatment decision was registered as adjusting medication doses.

Level of SDM

The level of SDM was investigated by scoring the audio-recorded consultations with the five-item observing patient involvement (OPTION) scale.[31] The OPTION scale is a reliable and valid measure, used across various clinical settings (eg, primary care, anaesthesiology and vascular surgery) to assess the efforts of clinicians to involve patients in decision making from an observer perspective.[22 24–26 32] This measure is based on a conceptual framework describing five core dimensions of SDM: justify deliberative work (item 1), justify deliberative work as a team (item 2), inform, describe options and exchange views (item 3), elicit preferences (item 4) and integrate preferences (item 5). Each item was scored on a five-point scale from 0 (no effort) to 4 (exemplary effort). Item scores were summed to obtain an overall score. The overall score, or OPTION score, was rescaled to lie between 0 and 100. Higher OPTION scores indicate higher levels of SDM. To ensure a reliable assessment, two observers (EM and FK) completed an online training provided by the developers of the OPTION scale.[33] Guided by a previous study of Stubenrouch and colleagues, the observers independently scored ten random consultations and percentages of absolute agreement and kappa values were calculated for each item to assess inter-rater reliability.[22] According to predetermined cut-offs of 80% (percentages of absolute agreement) or 0.6 (kappa values), an acceptable level of agreement was reached.[34 35] Therefore, the remaining consultations were divided

and scored by only one observer (EM scored 65% of consultations and FK 35%).

Data analysis

Descriptive statistics were computed for characteristics of the clinician, patient and consultation. Continuous variables were reported as means and SD or medians and IQR, depending on the normality of their distribution. Categorical variables were reported as frequencies and percentages. The mean OPTION score, range and mean item scores were reported. In addition, the median OPTION scores per clinician were reported and presented in box plots. The data had a hierarchical structure with patients (level-1) nested in clinicians (level-2). Therefore, multilevel modelling was used. To account for the number of events per variable, we first used univariate regression analyses to assess associations between the level of SDM and characteristics of the clinician (rheumatology centre, sex, age and work experience), patient (sex, age, educational level, disease duration, presence of comorbidities, medication necessity beliefs, medication concerns and health status) and consultation (duration and type of treatment decision). Thereafter, factors with a p value <0.2 were included in a multilevel model with random intercepts. Model fit was evaluated using likelihood ratio tests. Statistical significance was set at $p<0.05$. As for characteristics of the clinician, work experience was not included in the multilevel model because of multicollinearity with age ($r=0.9$). All analyses were performed using Stata V.13.

Ethical considerations

Clinicians and patients gave re-consent to use the data for the purposes of this study. Handling of the data complied with the General Data Protection Regulation (GDPR) and the Dutch act on the implementation of the GDPR.

Patient and public involvement

There was no patient and public involvement in this study.

RESULTS

Characteristics of the clinician, patient and consultation

One hundred and seventy-nine routine consultations with unique patients were audio-recorded. Eight audio-recordings were removed because of insufficient sound quality and/or incompleteness. In addition, three patients did not give reconsent to use the data for the purposes of this study. This brought the total number of audio-recordings to 168. Characteristics of the clinician, patient and consultation are shown in table 1.

Table 1 Characteristics of the clinician, patient and consultation

Clinician (n=22)	
Rheumatology centre, n (%)	
Reade	7 (32)
The Sint Maartenskliniek	15 (68)
Sex, n (%)	
Male	16 (73)
Female	6 (27)
Age in years, mean (SD)	48 (8.3)
Profession, n (%)	
Rheumatologist	20 (91)
Physician assistant	2 (9)
Work experience in years, mean (SD)	15.7 (9.1)
Patient (n=168)	
Rheumatology centre, n (%)	
Reade	47 (28)
The Sint Maartenskliniek	121 (72)
Sex, n (%)	
Male	52 (31)
Female	116 (69)
Age in years, mean (SD)	61.2 (11.4)
Educational level*†, n (%)	
Low	38 (23)
Medium	71 (43)
High	55 (34)
Disease duration in years, median (IQR)	9 (4–16.5)
Comorbidities, n (%)	
No	39 (23)
Yes	129 (77)
Number of synthetic DMARDs in use, n (%)	
1	136 (81)
2	26 (16)
3	6 (4)
Use of biologicals, n (%)	
No	116 (69)
Yes	52 (31)
Use of glucocorticoids, n (%)	
No	134 (80)
Yes	34 (20)
Medication beliefs, necessity subscale, mean (SD)	19.8 (3.5)
Medication beliefs, concerns subscale, mean (SD)	13.8 (3.9)
Functional status†, mean (SD)	1.7 (0.6)
Consultation (n=168)	
Duration in minutes, median (IQR)	9.0 (6.8–11.3)
Type of treatment decision, n (%)	
Stopping and/or starting medication	9 (5)
Adjusting medication doses	40 (24)
Administering single dose glucocorticoids	19 (11)
Continuing medication	100 (60)

*Educational level: low—up to and including lower technical and vocational training, medium—up to and including secondary technical and vocational training, high—up to and including higher vocational training and university.
†Missing values <3%.
DMARDs, disease-modifying antirheumatic drugs.

Level of SDM

The mean OPTION score was 28.3 (SD=15.1) (scale 0–100, higher OPTION scores indicating higher levels of SDM). The range of OPTION scores was between 0 and 75. Disregarding consultations in which the type of treatment decision was registered as continuing medication, the mean OPTION score was 35.9 (SD=14.6). On a scale from 0 to 4, the mean scores for item 1–5 were, respectively 1.0 (SD=1.0), 1.0 (SD=0.7), 0.8 (SD=0.9), 1.2 (SD=0.7) and 1.6 (SD=0.7). Quotes from the consultations that illustrate the efforts of clinicians to involve patients in decision making are shown in Box 1.

The number of consultations per clinician ranged from 1 to 18. The clinician with the lowest OPTION scores (seven consultations) had a median OPTION score of 5 (IQR=0–5). The clinician with the highest OPTION scores (six consultations) had a median OPTION score of 42.5 (IQR=30–55). Figure 1 shows the OPTION scores per clinician.

Associations between the level of SDM and characteristics of the clinician, patient and consultation

From the univariate regression analyses, four factors met the predefined selection criterion ($p<0.2$) and were included in the multilevel model: clinician age, patient age, consultation

Box 1 Quotes from the consultations

Item 1: justify deliberative work
For the health issue being discussed, the clinician draws attention to or confirms that alternate treatment or management options exist or that the need for a decision exists. If the patient rather than the clinician draws attention to the availability of options, the clinician responds by agreeing that the options need deliberation.
At this stage, we have two options. We either wait and see, right, if things get better by themselves. Because you said: overall, I feel quite good, but maybe I have just been doing too much. On the other hand, because there are so many inflamed joints at the moment, it may be a good thing to see if we try to get you moving again with an injection of prednisone, for example. (rheumatologist, male, 39 years old)
→Item score: 2

Item 2: justify deliberative work as a team
The clinician reassures the patient or re-affirms that the clinician will support the patient to become informed or deliberate about the options. If the patient states that they have sought or obtained information prior to the encounter, the clinician supports such a deliberation process.
Ultimately, I do think that it is important for you to decide what happens. Also, that you understand what we are suggesting so that you can also decide what is best. (rheumatologist, male, 45 years old)
→Item score: 3

Item 3: inform, describe options, and exchange views
The clinician gives information or checks understanding about the options that are considered reasonable (this can include taking no action), to support the patient in comparing alternatives. If the patient requests clarification, the clinician supports the process.
The only other thing we could also try… […] is to start you on a biologic agent after all, to see if we can manage your fatigue problem a little better. However, I do not think that this will provide you with much more benefit. (rheumatologist, female, 52 years old)
→Item score: 1

Item 4: elicit preferences
The clinician makes an effort to elicit the patient's preferences in response to the options that have been described. If the patient declares their preference(s), the clinician is supportive.
What is worrying you so much about methotrexate at the moment? (rheumatologist, male, 40 years old)
→Item score: 3

Item 5: integrate preference
The clinician makes an effort to integrate the patient's elicited preferences as decisions are made. If the patient indicates how best to integrate their preferences as decisions are made, the clinician makes an effort to do so.
I will reduce the dose to once every two weeks. I have no problem with that. […] Do you know what I mean? Apart from the fact that you do not look forward to it. Are you sure you understand that this is what I suggest? (rheumatologist, male, 59 years old)
→Item score: 0

duration and type of treatment decision. There were significant, positive associations between the level of SDM and the consultation duration (b=0.63, 95% CI 0.16 to 1.11, p=0.01),

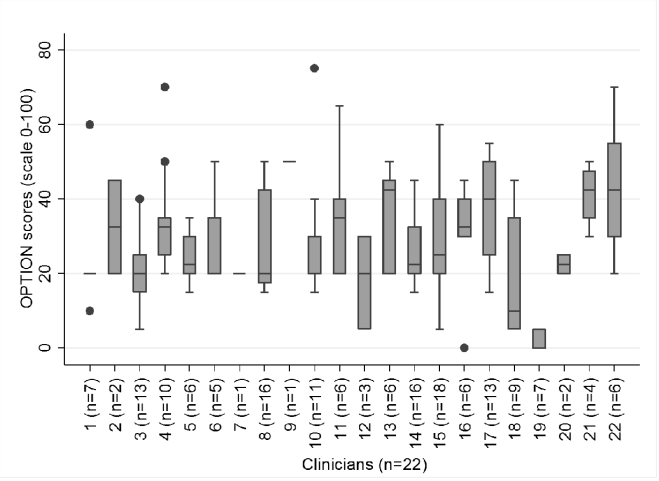

Figure 1 OPTION scores per clinician
The numbers on the x-axis refer to the participating clinicians with in parentheses the number of routine consultations with unique patients per clinician. Boxes represent values between the 25th and 75th percentiles, whiskers the upper and lower adjacent values and horizontal lines the medians. Outliers are shown as dots. Clinician 1 (n=7) had five OPTION scores of 20, one OPTION score of 10 and one OPTION score of 60.

decision for starting and/or stopping medication (b=14.3, 95% CI 5.62 to 22.98, p≤0.01), decision for adjusting medication doses (b=8.36, 95% CI 3.92 to 12.81, p≤0.01) and decision for administering single dose glucocorticoids (b=15.03, 95% CI 9.12 to 20.93, p≤0.01). Thus, a higher level of SDM was significantly associated with a longer consultation duration and the type of treatment decision. More specifically, if the consultation duration increased with 10 min, this led to an increase of 6 points on the OPTION scale. Also, compared with the decision to continue medication, the decisions to stop and/or start medication, adjust medication doses and administer single dose glucocorticoids led to an increase of respectively 14, 8 and 15 points on the OPTION scale. No other significant associations were found (table 2). The intraclass correlation indicated that 22% of the total variability in the level of SDM resided between clinicians.

DISCUSSION

This is the first study that used an observer-based measure to investigate the extent to which SDM is applied in routine clinical care of patients with RA. Using the OPTION scale, we found a low to moderate level of SDM in RA treatment. The efforts of

Table 2 Multilevel model (patients, level-1, nested in clinicians, level-2) of associations between the level of SDM and characteristics of the clinician, patient and consultation

Factor	B	SE	95% CI	P value
Clinician age	−0.34	0.2	−0.74 to 0.05	0.09
Patient age	−0.11	0.09	−0.28 to 0.06	0.2
Consultation duration	0.63	0.24	0.16 to 1.11	0.01
Type of treatment decision				
Continuing medication	Reference			
Stopping and/or starting medication	14.3	4.43	5.62 to 22.98	<0.01
Adjusting medication doses	8.36	2.27	3.92 to 12.81	<0.01
Administering single-dose glucocorticoids	15.03	3.01	9.12 to 20.93	<0.01

b, unstandardised coefficient; SDM, shared decision making.

clinicians to involve patients in decision making varied substantially. Furthermore, our results showed that a higher level of SDM was significantly associated with a longer consultation duration and the type of treatment decision.

This study supports the results of qualitative studies and surveys, suggesting that SDM is not current practice in RA treatment.[15–18] However, the level of SDM found in this study is higher than the level of SDM reported in most studies. A systematic review of 29 studies using the OPTION scale has shown that only 38% of studies reported a mean OPTION score ≥25.[24] The higher mean OPTION score reported in this study (28.3) may be explained by the clinical setting. It has been argued that SDM is particularly suitable for long-term treatment decisions in chronic care, relative to treatment decisions in acute care that are often urgent and irreversible.[36] Moreover, medical specialists and non-physicians have shown to involve patients in decision making more frequently than general practitioners.[24] In addition, SDM has been making headway in healthcare policy over the last decade. This may be reflected by equally increased levels of SDM found in current research.[26] Nevertheless, our results show that there is room for improvement. Indeed, a low to moderate level of SDM in RA treatment does not align with the fact that the vast majority of patients with RA prefer to be involved in decision making.[17 37]

In this study, the third item of the OPTION scale (inform, describe options and exchange views) scored lowest. This is in contrast to most studies in which the second item of the OPTION scale (justify deliberative work as a team) scored lowest.[24 38] Previous studies have shown that patients with RA express a strong need for full disclosure of all available treatment options and their associated risks and benefits.[39–41] Moreover, it has been shown that clinicians often underestimate or undervalue patients' information needs.[42] It is important that patients receive the information necessary to enable SDM. This may be achieved by the use of decision aids that help patients become well informed and prepared for decision making. Furthermore, our results showed that there was a substantial variety between the efforts of clinicians to involve patients in decision making. Training programmes on patient-centred communication skills have proven to be effective to help clinicians share treatment decisions with patients.[43] However, clinicians may perceive barriers that keep them from applying SDM.[44] Future research should focus on identifying barriers to the application of SDM in RA treatment as perceived by clinicians.

We found that a higher level of SDM was significantly associated with a longer consultation duration. Although the application of SDM may add time to the consultation, it is plausible that by applying SDM clinicians lay the groundwork for quicker follow-up care.[45] Furthermore, it should be noted that, in this study, adding 10 min to the consultation only led to a modest increase of the level of SDM (an increase of 6 points on the OPTION scale). Our results also showed that the level of SDM was significantly higher in consultations in which it was decided to make changes to a patient's treatment (stopping and/or starting medication, adjusting medication doses and administering single-dose glucocorticoids). This implies that SDM may be more suitable for some treatment decisions than others. Other researchers have also stated that the level of SDM will probably always differ, depending on the situation.[26] Moreover, a higher level of SDM is not necessarily better, for example, if a patient does not prefer to be involved in decision making. Therefore, it is important for clinicians to always be sensitive to the suitability of SDM in a particular situation.

There are several strengths and limitations of this study that need consideration. A strength was the use of an observer-based measure to assess the level of SDM in RA treatment. We had access to a large number of audio-recorded consultations (n=168), strengthening the ecological validity of our results. Audio-recording the consultations may have affected the behaviour of both clinicians and patients. However, at the time of the consultation neither physicians nor patients were aware that the audio-recordings would be used to investigate the level of SDM. As we used audio-recordings instead of video material, non-verbal behaviour could not be taken into account.[38] Furthermore, only a single consultation per patient was audio-recorded. Decision making may take place over multiple consultations. Therefore, it is possible that we have not always captured the full process. We were also limited by the availability of the data. Other factors, such as clinician–patient familiarity and patient health literacy, may also impact the level of SDM in RA treatment, but were not included in this study. Finally, the cross-sectional design of this study did not allow for conclusions about causality.

In summary, we found that a higher level of SDM was significantly associated with factors related to characteristics of the consultation, namely a longer duration and the type of treatment decision. No significant associations were found between the level of SDM and factors related to characteristics of the clinician and patient. Overall, the level of SDM in RA treatment leaves room for improvement. Targeted strategies to support the application of SDM are warranted in this era of patient-centred care. For example, training programmes on patient-centred communication skills may be helpful to foster SDM in routine clinical care. Nevertheless, clinicians should always be sensitive to the suitability of SDM in a particular situation.

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REFERENCES

1 Salt E, Frazier SK. Adherence to disease-modifying antirheumatic drugs in patients with rheumatoid arthritis: a narrative review of the literature. *Orthop Nurs* 2010;29:260–75.
2 van den Bemt BJF, Zwikker HE, van den Ende CHM. Medication adherence in patients with rheumatoid arthritis: a critical appraisal of the existing literature. *Expert Rev Clin Immunol* 2012;8:337–51.
3 Pasma A, van't Spijker A, Hazes JMW, *et al*. Factors associated with adherence to pharmaceutical treatment for rheumatoid arthritis patients: a systematic review. *Semin Arthritis Rheum* 2013;43:18–28.

4 Pasma A, Schenk CV, Timman R, *et al*. Non-adherence to disease-modifying antirheumatic drugs is associated with higher disease activity in early arthritis patients in the first year of the disease. *Arthritis Res Ther* 2015;17:281.
5 Barton J. Patient preferences and satisfaction in the treatment of rheumatoid arthritis with biologic therapy. *Patient Prefer Adherence* 2009;3:335–44.
6 Lofland J, Johnson P, Ingham M, *et al*. Shared decision-making for biologic treatment of autoimmune disease: influence on adherence, persistence, satisfaction, and health care costs. *Patient Prefer Adherence* 2017;11:947–58.
7 Fraenkel L, Bogardus ST, Concato J, *et al*. Patient preferences for treatment of rheumatoid arthritis. *Ann Rheum Dis* 2004;63:1372–8.
8 De Mits S, Lenaerts J, Vander Cruyssen B, *et al*. A nationwide survey on patient's versus Physician's evaluation of biological therapy in rheumatoid arthritis in relation to disease activity and route of administration: the Be-Raise study. *PLoS One* 2016;11:e0166607.
9 Hsiao B, Fraenkel L. Patient preferences for rheumatoid arthritis treatment. *Curr Opin Rheumatol* 2019;31:256–63.
10 Durand C, Eldoma M, Marshall DA, *et al*. Patient preferences for disease modifying anti-rheumatic drug treatment in rheumatoid arthritis: a systematic review. *J Rheumatol* 2019;46.
11 van Heuckelum M, Mathijssen EGE, Vervloet M, *et al*. Preferences of patients with rheumatoid arthritis regarding disease-modifying antirheumatic drugs: a discrete choice experiment. *Patient Pref Adher* 2019;13:1199–211.
12 Elwyn G, Frosch D, Thomson R, *et al*. Shared decision making: a model for clinical practice. *J Gen Intern Med* 2012;27:1361–7.
13 Singh JA, Saag KG, Bridges SL, *et al*. 2015 American College of rheumatology guideline for the treatment of rheumatoid arthritis. *Arthritis Rheumatol* 2016;68:1–26.
14 Smolen JS, Landewé R, Bijlsma J, *et al*. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2016 update. *Ann Rheum Dis* 2017;76:960–77.
15 Kjeken I, Dagfinrud H, Mowinckel P, *et al*. Rheumatology care: involvement in medical decisions, received information, satisfaction with care, and unmet health care needs in patients with rheumatoid arthritis and ankylosing spondylitis. *Arthritis Rheum* 2006;55:394–401.
16 Barton JL, Trupin L, Tonner C, *et al*. English language proficiency, health literacy, and trust in physician are associated with shared decision making in rheumatoid arthritis. *J Rheumatol* 2014;41:1290–7.
17 Nota I, Drossaert CHC, Taal E, *et al*. Patient participation in decisions about disease modifying anti-rheumatic drugs: a cross-sectional survey. *BMC Musculoskelet Disord* 2014;15:333.
18 Aoki A, Ohbu S. Japanese physicians' preferences for decision making in rheumatoid arthritis treatment. *Patient Prefer Adherence* 2016;10:107–13.
19 Santema TB, Stubenrouch FE, Koelemay MJW, *et al*. Shared decision making in vascular surgery: an exploratory study. *Eur J Vasc Endovasc Surg* 2016;51:587–93.
20 Barr PJ, Elwyn G. Measurement challenges in shared decision making: putting the 'patient' in patient-reported measures. *Health Expect* 2016;19:993–1001.
21 Joseph-Williams N, Lloyd A, Edwards A, *et al*. Implementing shared decision making in the NHS: lessons from the magic programme. *BMJ*;2017:j1744.
22 Stubenrouch FE, Mus EMK, Lut JW, *et al*. The current level of shared decision-making in anesthesiology: an exploratory study. *BMC Anesthesiol* 2017;17:95.
23 Geessink NH, Ofstad EH, Olde Rikkert MGM, *et al*. Shared decision-making in older patients with colorectal or pancreatic cancer: Determinants of patients' and observers' perceptions. *Patient Educ Couns* 2018;101:1767–74.
24 Couët N, Desroches S, Robitaille H, *et al*. Assessments of the extent to which health-care providers involve patients in decision making: a systematic review of studies using the option instrument. *Health Expect* 2015;18:542–61.
25 Menear M, Garvelink MM, Adekpedjou R, *et al*. Factors associated with shared decision making among primary care physicians: findings from a multicentre cross-sectional study. *Health Expect* 2018;21:212–21.
26 Meijers MC, Noordman J, Spreeuwenberg P, *et al*. Corrigendum: shared decision-making in general practice: an observational study comparing 2007 with 2015. *Fam Pract* 2019;36:529
27 von Elm E, Altman DG, Egger M, *et al*. The strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. *J Clin Epidemiol* 2008;61:344–9.
28 van Heuckelum M, Linn AJ, Vandeberg L, *et al*. Implicit and explicit attitudes towards disease-modifying antirheumatic drugs as possible target for improving medication adherence. *PLoS One* 2019;14:e0221290.
29 Horne R, Weinman J, Hankins M. The beliefs about medicines questionnaire: the development and evaluation of a new method for assessing the cognitive representation of medication. *Psychol Health* 1999;14:1–24.
30 Boers M, Jacobs JWG, van Vliet Vlieland TPM, *et al*. Consensus Dutch health assessment questionnaire. *Ann Rheum Dis* 2007;66:132–3.
31 Elwyn G, Tsulukidze M, Edwards A, *et al*. Using a 'talk' model of shared decision making to propose an observation-based measure: Observer OPTION5 Item. *Patient Educ Couns* 2013;93:265–71.
32 Barr PJ, O'Malley AJ, Tsulukidze M, *et al*. The psychometric properties of observer OPTION5, an observer measure of shared decision making. *Patient Educ Couns* 2015;98:970–6.
33 Elwyn G. Online, self-paced training for observer option 5. Available: http://www.glynelwyn.com/observer-option-5-2014.html [Accessed on 1 Feb 2018].
34 Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics* 1977;33:159–74.
35 McHugh ML. Interrater reliability: the kappa statistic. *Biochemia Medica* 2012;22:276–82.
36 Joosten EAG, DeFuentes-Merillas L, de Weert GH, *et al*. Systematic review of the effects of shared decision-making on patient satisfaction, treatment adherence and health status. *Psychother Psychosom* 2008;77:219–26.
37 Mahlich J, Sruamsiri R. Preference for shared decision-making in Japanese patients with rheumatoid arthritis. *Cogent Medicine* 2017;4:1.
38 Gionfriddo MR, Branda ME, Fernandez C, *et al*. Comparison of audio vs. audio + video for the rating of shared decision making in oncology using the observer OPTION5 instrument: an exploratory analysis. *BMC Health Serv Res* 2018;18:522.
39 Fraenkel L, Bogardus S, Concato J, *et al*. Preference for disclosure of information among patients with rheumatoid arthritis. *Arthritis Rheum* 2001;45:136–9.
40 Neame R, Hammond A, Deighton C. Need for information and for involvement in decision making among patients with rheumatoid arthritis: a questionnaire survey. *Arthritis Rheum* 2005;53:249–55.
41 Mathijssen EGE, Vriezekolk JE, Eijsbouts AMM, *et al*. Support needs for medication use and the suitability of eHealth technologies to address these needs: a focus group study of older patients with rheumatoid arthritis. *Patient Prefer Adherence* 2018;12:349–58.
42 Kinnersley P, Edwards AGK, Hood K, *et al*. Interventions before consultations for helping patients address their information needs. *Cochrane Database Syst Rev* 2007;56:CD004565.
43 Diouf NT, Menear M, Robitaille H, *et al*. Training health professionals in shared decision making: update of an international environmental scan. *Patient Educ Couns* 2016;99:1753–8.
44 Légaré F, Ratté S, Gravel K, *et al*. Barriers and facilitators to implementing shared decision-making in clinical practice: update of a systematic review of health professionals' perceptions. *Patient Educ Couns* 2008;73:526–35.
45 Légaré F, Thompson-Leduc P. Twelve myths about shared decision making. *Patient Educ Couns* 2014;96:281–6.

CLINICAL SCIENCE

Efficacy and safety of ixekizumab through 52 weeks in two phase 3, randomised, controlled clinical trials in patients with active radiographic axial spondyloarthritis (COAST-V and COAST-W)

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ABSTRACT

Objectives To investigate the efficacy and safety of ixekizumab for up to 52 weeks in two phase 3 studies of patients with active radiographic axial spondyloarthritis (r-axSpA) who were biological disease-modifying antirheumatic drug (bDMARD)-naive (COAST-V) or tumour necrosis factor inhibitor (TNFi)-experienced (COAST-W).

Methods Adults with active r-axSpA were randomised 1:1:1:1 (n=341) to 80 mg ixekizumab every 2 (IXE Q2W) or 4 weeks (IXE Q4W), placebo (PBO) or 40 mg adalimumab Q2W (ADA) in COAST-V and 1:1:1 (n=316) to IXE Q2W, IXE Q4W or PBO in COAST-W. At week 16, patients receiving ixekizumab continued their assigned treatment; patients receiving PBO or ADA were rerandomised 1:1 to IXE Q2W or IXE Q4W (PBO/IXE, ADA/IXE) through week 52.

Results In COAST-V, Assessment of SpondyloArthritis international Society 40 (ASAS40) responses rates (intent-to-treat population, non-responder imputation) at weeks 16 and 52 were 48% and 53% (IXE Q4W); 52% and 51% (IXE Q2W); 36% and 51% (ADA/IXE); 19% and 47% (PBO/IXE). Corresponding ASAS40 response rates in COAST-W were 25% and 34% (IXE Q4W); 31% and 31% (IXE Q2W); 14% and 39% (PBO/IXE). Both ixekizumab regimens sustained improvements in disease activity, physical function, objective markers of inflammation, QoL, health status and overall function up to 52 weeks. Safety through 52 weeks of ixekizumab was consistent with safety through 16 weeks.

Conclusion The significant efficacy demonstrated with ixekizumab at week 16 was sustained for up to 52 weeks in bDMARD-naive and TNFi-experienced patients. bDMARD-naive patients initially treated with ADA demonstrated further numerical improvements after switching to ixekizumab. Safety findings were consistent with the known safety profile of ixekizumab.

Trial registration number NCT02696785/NCT02696798.

Key messages

What is already known about this subject?

- Ixekizumab was superior to placebo at week 16 for treating the signs and symptoms of active radiographic axial spondyloarthritis (r-axSpA) in two phase 3 trials in patients who were biological disease-modifying antirheumatic drug (bDMARD)-naive (COAST-V) or tumour necrosis factor inhibitor (TNFi)-experienced (prior inadequate response or intolerance to one or two TNFi; COAST-W).

What does this study add?

- The significant improvements observed with ixekizumab at week 16 were sustained for up to 52 weeks in bDMARD-naive (COAST-V) and TNFi-experienced patients (COAST-W); results were similar between two ixekizumab regimens (80 mg every 2 or every 4 weeks) across endpoints. In COAST-V, bDMARD-naive patients who were initially treated with adalimumab for 16 weeks demonstrated further numerical improvements through week 52 after switching to ixekizumab.
- COAST-W is the first phase 3 study of a bDMARD in an exclusively TNFi-experienced patient population and provides robust information on the long-term efficacy and safety of ixekizumab in this population.
- Adverse events were consistent with the known safety profile of ixekizumab.

How might this impact on clinical practice or future developments?

- The findings from COAST-V and COAST-W suggest that ixekizumab could be a longer-term treatment option for patients with axSpA, regardless of prior experience with TNFi. The additional numeric improvement in adalimumab-treated patients after switching to ixekizumab is of particular interest and deserves further exploration.

INTRODUCTION

Axial spondyloarthritis (axSpA) is a chronic inflammatory condition comprising non-radiographic

axSpA and radiographic axSpA (r-axSpA). The latter, also known as ankylosing spondylitis (AS), is characterised by inflammatory back pain and radiographic evidence of damage to the sacroiliac joint.[1] These manifestations, and peripheral musculoskeletal and extra-articular signs and symptoms, may contribute to limited mobility, progressive disability and decreased quality of life (QoL).[2 3] Biological disease-modifying antirheumatic drugs (bDMARDs), including tumour necrosis factor inhibitors (TNFi)[4 5] and an interleukin (IL)-17A antagonist,[5] are recommended for managing patients with axSpA who do not respond to or tolerate non-steroidal anti-inflammatory drugs (NSAIDs). However, up to 40% of patients fail to achieve satisfactory disease control with TNFi,[6] and treatment with TNFi may be contraindicated in other patients.[7]

The IL-17 signalling pathway plays a key role in the pathogenesis of axSpA.[8 9] Ixekizumab, a high-affinity monoclonal antibody that selectively targets IL-17A, is approved for treating active psoriatic arthritis and moderate-to-severe plaque psoriasis and has demonstrated efficacy in two phase 3 trials in patients with r-axSpA who were bDMARD-naive (COAST-V) or TNFi-experienced (prior inadequate response or intolerance to TNFi; COAST-W).[10 11] In both studies, ixekizumab resulted in significantly greater improvement versus placebo (PBO) at week 16 for measures of disease activity (including the primary endpoint of Assessment of SpondyloArthritis international Society 40 response (ASAS40)), function, QoL and spinal inflammation.

Here, we evaluated the sustainability of improvements observed at week 16 for treatment of r-axSpA with ixekizumab 80 mg every 4 or 2 weeks (IXE Q4W or IXE Q2W) up to week 52 in COAST-V and COAST-W. We also evaluated the safety of ixekizumab for up to 52 weeks, with a specific focus on overall safety, including events of special interest such as injection site reactions (ISRs) and candidiasis, and extra-articular manifestations such as inflammatory bowel disease (IBD), anterior uveitis (AU) and psoriasis.

MATERIALS AND METHODS

Study design

COAST-V[10] and COAST-W[11] are phase 3, multicentre, randomised, double-blind, active-controlled (COAST-V only) and PBO-controlled, 52-week trials, followed by an optional 2-year extension study.

All patients provided written informed consent.

Patient and public involvement

Patients were not involved in the design or conduct of the study, development of outcomes or dissemination of study results.

Patients

Patient eligibility criteria have been described previously.[10 11] Patients were to be ≥18 years of age, have an established diagnosis of r-axSpA and meet ASAS criteria (with central reading of radiographic sacroiliitis).[12] Patients in COAST-W were required to have discontinued one or two TNFi because of intolerance or inadequate response; COAST-V only included bDMARD-naive patients.

Treatment protocol

Study procedures for COAST-V and COAST-W have been described elsewhere.[10 11] Patients in COAST-V were randomised 1:1:1:1 to PBO, adalimumab 40 mg (ADA) Q2W, IXE Q2W or IXE Q4W. ADA represents an active reference group; the study was not powered to test equivalence/non-inferiority of the active treatment groups to each other, including ixekizumab versus ADA. Patients in COAST-W were randomised 1:1:1 to PBO, IXE Q2W or IXE Q4W. In both trials, patients assigned ixekizumab were further randomised 1:1 to a 160 mg or 80 mg starting dose.

Patients completing week 16 entered a dose double-blind extended treatment period (ETP; weeks 16 to 52). During this period, patients originally randomised to PBO or ADA (COAST-V only) were rerandomised 1:1 to IXE Q2W or IXE Q4W (160 mg starting dose for patients switching from PBO, 80 mg starting dose for patients switching from ADA). Patients originally randomised to IXE Q2W or IXE Q4W continued these regimens.

Assessments

Efficacy

Efficacy assessments were made at weeks 20, 24, 28, 32, 36, 44 and 52 in the ETP, except where specified below.

Categorical efficacy endpoints assessed included the proportion of patients achieving ASAS40,[13] ASAS20, ASAS partial remission, Ankylosing Spondylitis Disease Activity Score (ASDAS)[14] low disease activity (score <2.1), ASDAS inactive disease (score <1.3), ASDAS clinically important improvement (≥1.1 change from baseline), ASDAS major improvement (≥2.0 change from baseline or reached a minimal ASDAS score of 0.6361) and ≥50% improvement in Bath Ankylosing Spondylitis Disease Activity Index (BASDAI50).[15] Continuous endpoints included changes from baseline in ASDAS, BASDAI, Bath Ankylosing Spondylitis Functional Index (BASFI),[16] Medical Outcomes Study Short Form 36 (SF-36) health survey Physical Component Score (PCS),[17] ASAS Health Index (ASAS HI),[18 19] Spondyloarthritis Research Consortium of Canada (SPARCC) MRI[20] of the spine and sacroiliac joint (the latter in COAST-V only) and serum C reactive protein (CRP) concentrations. After week 16, SF-36 PCS and ASAS HI assessments were performed at weeks 36 and 52; MRI assessments were performed at week 16 in both studies and at week 52 in COAST-V only. MRI from baseline, week 16 and week 52 were read in a single campaign. Concomitant NSAID use and ASAS-NSAID[21] scores were assessed.

Safety

Safety assessments included the evaluation of adverse events (AEs; per the Medical Dictionary for Regulatory Activities) and treatment-emergent antidrug antibodies (TE-ADAs).[10 11] Cerebrocardiovascular events and suspected IBD were adjudicated by an independent clinical event committee. All IBD events were adjudicated by an external committee following EPIMAD criteria.[22]

At every visit, patients were evaluated for any symptoms of AU; AU events were confirmed by an ophthalmologist. Psoriasis and IBD were not proactively evaluated, but new onset or flares were recorded as AEs.

Statistical analysis

Efficacy analyses through 52 weeks were performed on the intent-to-treat population (ITT; IXE Q4W, IXE Q2W), which included all patients initially randomised to ixekizumab, and the ETP population, which included all patients who received ≥1 dose of ixekizumab during the ETP. Considering the consistent performance of the IXE Q4W and IXE Q2W regimens, data for patients in the ETP who were initially randomised to PBO or ADA were analysed as single groups (PBO/IXE or ADA/IXE), regardless of which ixekizumab dose they received during the ETP. Safety analyses were performed on the ETP population and on the all ixekizumab exposure safety population (IXE Q4W, IXE Q2W), which included all patients who received ≥1 dose of ixekizumab at any time during the 52-week study period.

No between-treatment group comparisons were made for ETP data. For primary analyses of the ITT and ETP populations, the most conservative approach was followed, where missing data

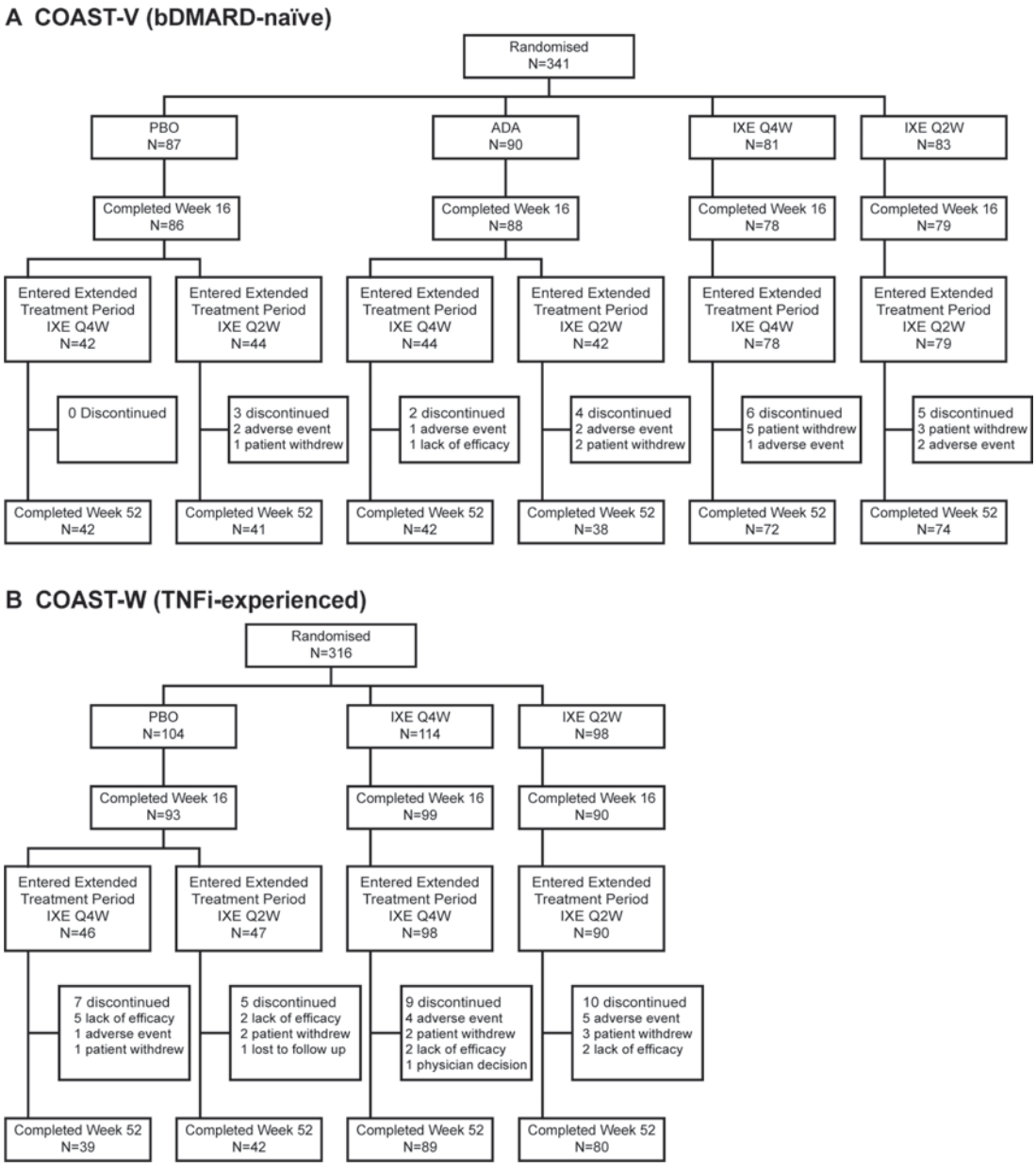

Figure 1 Patient disposition: (A) COAST-V; (B) COAST-W. ADA, adalimumab; bDMARD, biological disease-modifying antirheumatic drug; extended treatment period, dose double-blind extended treatment period; IXE Q4W, ixekizumab 80 mg every 4 weeks; IXE Q2W, ixekizumab 80 mg every 2 weeks; PBO, placebo; TNFi, tumour necrosis factor inhibitor.

were imputed using non-responder imputation (NRI) for categorical variables and modified baseline observation carried forward (mBOCF, a more stringent method of analysis than last observation carried forward) for continuous variables. For secondary analyses of ITT data, missing data were imputed using modified NRI for categorical variables and multiple imputation for continuous variables. ITT data were also analysed as observed. SF-36 PCS data are reported as t-scores, based on 2009 US general population norms.

Statistical analyses were performed using SAS V.9.2 or higher (SAS Institute).

RESULTS

Patients

The majority of patients in COAST-V (309/329; 93.9%) and COAST-W (250/281; 89.0%) who entered the ETP completed week 52 (figure 1). Of the patients initially randomised to ixekizumab, 146/164 (89.0%) in COAST-V and 169/212 (79.7%) in COAST-W completed week 52. The most common reason for discontinuation was patient withdrawal (n=11; 3.3%) in COAST-V and lack of efficacy (n=11; 3.9%) in COAST-W.

Demographics and baseline clinical characteristics for the ETP populations were similar between treatment groups within each study (online supplementary table S1) and similar to those of the ITT populations.[10 11] Baseline and historical peripheral/extra-articular manifestations of axSpA are summarised in online supplementary table S2.

Efficacy

Among patients continuously treated with ixekizumab, week 16 ASAS40 response rates were sustained for up to 52 weeks (figure 2, table 1). Week 52 ASAS40 response rates were 53.1% (IXE Q4W) and 50.6% (IXE Q2W) in COAST-V and 34.2% (IXE Q4W) and 30.6% (IXE Q2W) in COAST-W. Patients randomised to PBO and rerandomised to ixekizumab at week 16 (PBO/IXE) showed

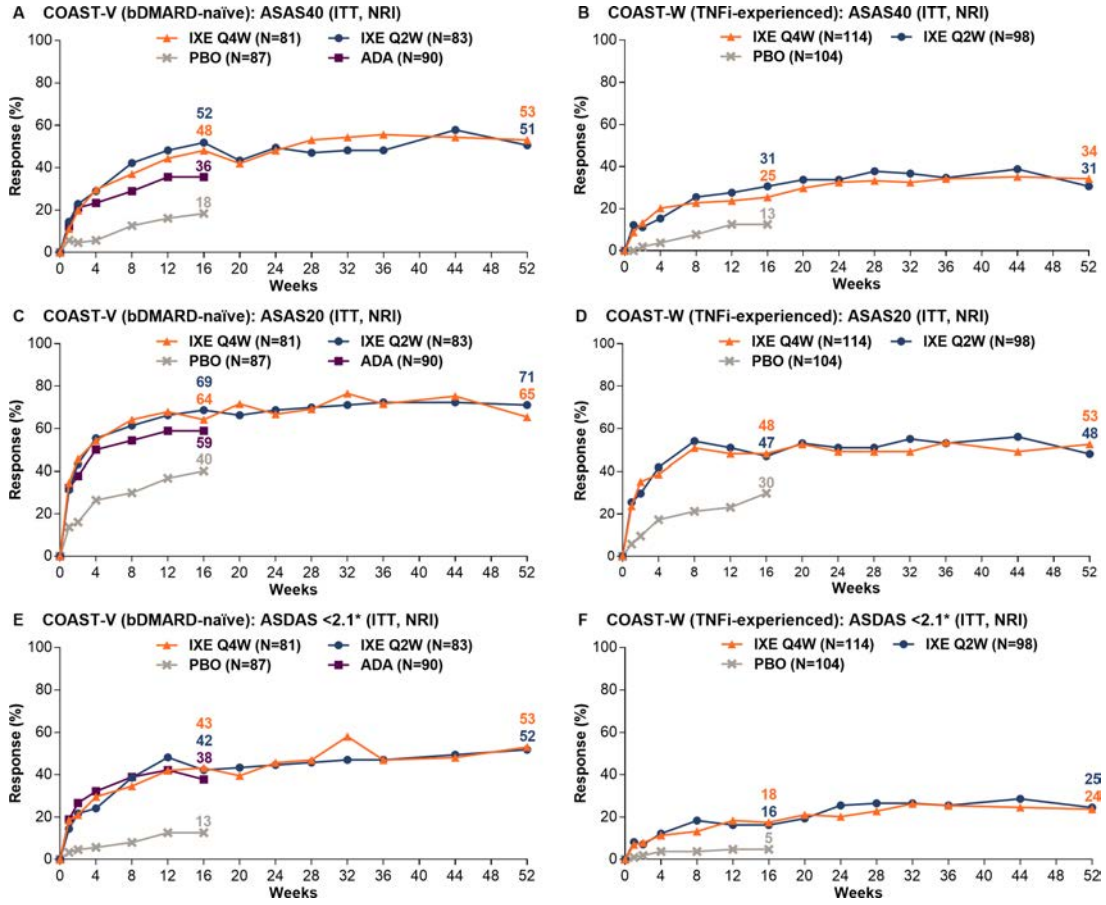

Figure 2 Proportion of patients achieving ASAS40, ASAS20 and ASDAS <2.1 responses through 52 weeks in COAST-V (A, C, E) and COAST-W (B, D, F). ITT population. Missing data were imputed using NRI. ADA represents an active reference group; the study was not powered to test equivalence or non-inferiority of the active treatment groups to each other, including ixekizumab versus ADA. *ASDAS <2.1 indicates low disease activity. ADA, adalimumab; ASAS, Assessment of SpondyloArthritis international Society; ASDAS, Ankylosing Spondylitis Disease Activity Score; bDMARD, biological disease-modifying antirheumatic drug; ITT, intent to treat; IXE Q4W, ixekizumab 80 mg every 4 weeks; IXE Q2W, ixekizumab 80 mg every 2 weeks; NRI, non-responder imputation; PBO, placebo; TNFi, tumour necrosis factor inhibitor.

rapid improvement in ASAS40 response rates after switching to ixekizumab (figure 3, table 2); week 52 response rates (46.5% in COAST-V, 38.7% in COAST-W) were numerically similar to those in patients initially randomised to ixekizumab. In COAST-V, patients randomised to ADA showed further numerical improvements in ASAS40 response rates (36.0% at week 16, 51.2% at week 52) after switching to ixekizumab (figure 3A, table 2); week 52 response rates were numerically similar to those in patients initially randomised to ixekizumab. Among ADA/IXE patients who were ASAS40 non-responders at week 16, but ASAS40 responders at week 52, 47.4% were ASAS20 non-responders and 52.6% were ASAS20 responders at week 16.

Consistent with the ASAS40 findings, week 16 improvements in ASAS20 response rates were sustained for up to 52 weeks among patients continuously treated with ixekizumab in both studies (figure 2C and D). Week 16 improvements in other measures of disease activity were sustained for up to 52 weeks, including changes from baseline in ASDAS and BASDAI, and achievement of ASAS partial remission, ASDAS improvement categories (low disease activity (figure 2), inactive disease, clinically important improvement and major improvement) and BASDAI50 (table 1). Improvements in patient function at week 16 (change from baseline in BASFI) were sustained for up to 52 weeks in patients continuously treated with ixekizumab, as were improvements in measures of QoL (change from baseline in SF-36 PCS) and health functioning (change from baseline in ASAS HI) (table 1). Week 16 improvements in spinal MRI and objective inflammation were sustained for up to 52 weeks per SPARCC spine and sacroiliac joint scores (assessed beyond week 16 in COAST-V only) and changes from baseline in CRP (table 1).

In COAST-V, patients randomised to ADA showed further numerical improvements in most efficacy endpoints on switching to ixekizumab (table 2).

Results from secondary analyses (online supplementary table S3 and S4) were consistent with the primary analyses (NRI and mBOCF).

Concomitant NSAID and ASAS-NSAID findings are summarised in online supplementary table S5.

Safety

ETP population (weeks 16 to 52)

Overall, 201 (61.1%) patients in COAST-V and 179 (63.7%) patients in COAST-W reported treatment-emergent adverse events (TEAEs) during the ETP (table 3). Most TEAEs were of mild or moderate severity. Eight (2.4%) patients in COAST-V and 10 (3.6%) patients in COAST-W discontinued treatment because of an AE. The most common TEAEs were nasopharyngitis, ISRs and upper respiratory tract infection. Serious adverse events (SAEs) occurred in 18 (5.5%) patients in COAST-V and 9 (3.2%) patients in COAST-W; the frequency of SAEs was similar between ixekizumab regimens. The only SAE reported by more than one patient was bradycardia (n=2 patients; neither SAE

Table 1 Weeks 16* and 52 efficacy endpoints for patients treated continuously with ixekizumab: COAST-V and COAST-W (ITT population: NRI, modified baseline observation carried forward)

	COAST-V (bDMARD-naive)				COAST-W (TNFi-experienced)			
	IXE Q4W (n=81)		IXE Q2W (n=83)		IXE Q4W (n=114)		IXE Q2W (n=98)	
Patients achieving response, n (%)								
NRI	Week 16	Week 52	Week 16	Week 52	Week 16	Week 52	Week 16	Week 52
ASAS40	39 (48.1)	43 (53.1)	43 (51.8)	42 (50.6)	29 (25.4)	39 (34.2)	30 (30.6)	30 (30.6)
ASAS20	52 (64.2)	53 (65.4)	57 (68.7)	59 (71.1)	55 (48.2)	60 (52.6)	46 (46.9)	47 (48.0)
ASAS partial remission	12 (14.8)	22 (27.2)	12 (14.5)	20 (24.1)	7 (6.1)	13 (11.4)	5 (5.1)	8 (8.2)
ASDAS clinically important improvement	50 (61.7)	51 (63.0)	50 (60.2)	51 (61.4)	51 (44.7)	53 (46.5)	48 (49.0)	44 (44.9)
ASDAS major improvement	24 (29.6)	30 (37.0)	19 (22.9)	29 (34.9)	18 (15.8)	27 (23.7)	21 (21.4)	26 (26.5)
ASDAS <2.1 (low disease activity)	35 (43.2)	43 (53.1)	35 (42.2)	43 (51.8)	20 (17.5)	27 (23.7)	16 (16.3)	24 (24.5)
ASDAS <1.3 (inactive disease)	13 (16.0)	18 (22.2)	9 (10.8)	16 (19.3)	4 (3.5)	10 (8.8)	5 (5.1)	4 (4.1)
BASDAI50	34 (42.0)	40 (49.4)	36 (43.4)	37 (44.6)	25 (21.9)	31 (27.2)	23 (23.5)	27 (27.6)
Mean change from baseline (SD)								
mBOCF†	Week 16	Week 52	Week 16	Week 52	Week 16	Week 52	Week 16	Week 52
ASDAS	−1.4 (1.2)	−1.6 (1.1)	−1.4 (1.0)	−1.6 (1.0)	−1.1 (1.0)	−1.2 (1.1)	−1.2 (1.1)	−1.3 (1.2)
BASDAI	−3.0 (2.4)	−3.3 (2.5)	−2.7 (2.1)	−3.1 (2.3)	−2.1 (2.0)	−2.4 (2.4)	−2.1 (2.3)	−2.4 (2.3)
BASFI	−2.4 (2.3)	−2.8 (2.5)	−2.5 (2.2)	−2.8 (2.4)	−1.6 (2.1)	−2.1 (2.5)	−1.9 (2.3)	−2.1 (2.3)
SF-36 PCS‡	7.6 (8.4)	8.3 (9.5)	7.8 (7.0)	8.1 (7.5)	6.3 (7.5)	6.5 (8.5)	6.0 (7.7)	7.1 (7.6)
ASAS Health Index	−2.3 (3.3)	−2.7 (3.3)	−2.8 (3.2)	−3.3 (3.6)	−2.0 (3.1)	−2.3 (3.7)	−1.8 (3.9)	−2.5 (3.5)
SPARCC MRI spine score§	−8.9 (16.2)	−8.8 (17.3)	−8.7 (16.5)	−8.5 (15.9)	−3.2 (8.3)	NA	−5.1 (11.9)	NA
SPARCC MRI sacroiliac joint score¶	−3.4 (7.6)	−3.3 (8.7)	−4.1 (7.3)	−4.2 (7.5)	NA	NA	NA	NA
CRP, mg/L	−6.8 (16.7)	−9.2 (12.4)	−8.4 (15.7)	−9.6 (14.5)	−11.5 (30.1)	−10.4 (31.1)	−10.3 (19.3)	−10.0 (18.5)

*Except for ASAS partial remission (both studies), ASDAS clinically important improvement (both studies), ASDAS major improvement (both studies), ASDAS <1.3 (COAST-W) and BASDAI50 (COAST-W), all week 16 data have been previously reported.[10 11]

†For patients who discontinued study drug because of an adverse event, the baseline observation was carried forward to the corresponding time point for evaluation. For patients discontinuing study drug for any other reason, the last non-missing observation before discontinuation was carried forward to the corresponding time point for evaluation.

‡SF-36 PCS data are reported as t-scores, based on 2009 US general population norms.

§Observed data only (not assessed after week 16 in COAST-W). COAST-V: week 16, n=78 (IXE Q4W) and n=74 (IXE Q2W); week 52, n=72 (IXE Q4W) and n=68 (IXE Q2W). COAST-W: week 16, n=49 (IXE Q4W) and n=45 (IXE Q2W).

¶Observed data only (not assessed in COAST-W). COAST-V: week 16, n=78 (IXE Q4W) and n=75 (IXE Q2W); week 52, n=72 (IXE Q4W) and n=69 (IXE Q2W).

ASAS, Assessment of SpondyloArthritis international Society; ASDAS, Ankylosing Spondylitis Disease Activity Score; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; bDMARD, biological disease-modifying antirheumatic drug; CRP, C reactive protein; ITT, intent to treat; IXE Q2W, ixekizumab 80 mg every 2 weeks; IXE Q4W, ixekizumab 80 mg every 4 weeks; mBOCF, modified baseline observation carried forward; NA, not applicable; NRI, non-responder imputation; SF-36 PCS, Medical Outcomes Study 36-item Short-Form Health Survey Physical Component Score; SPARCC, Spondyloarthritis Research Consortium of Canada; TNFi, tumour necrosis factor inhibitor.

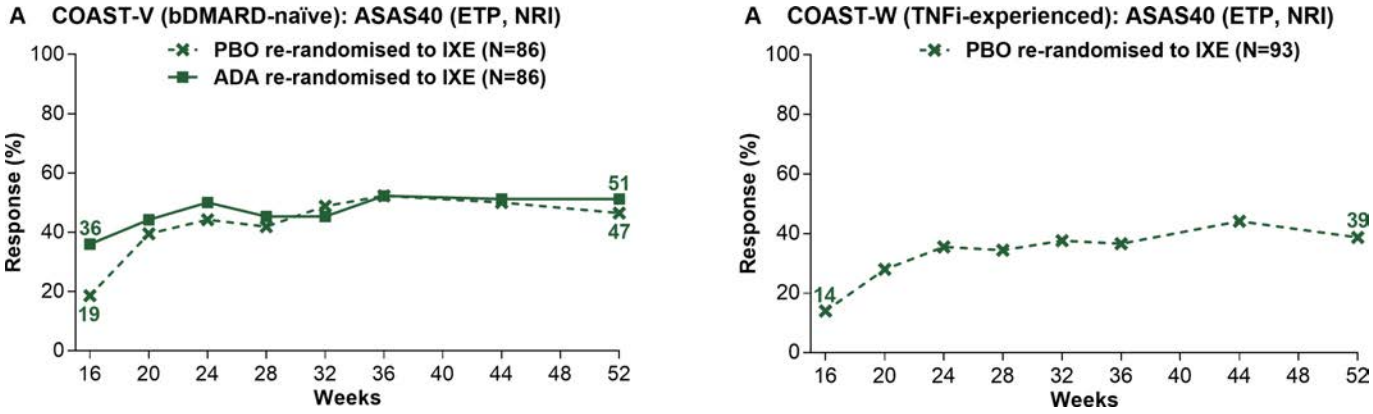

Figure 3 Proportion of patients initially randomised to PBO or ADA achieving ASAS40 responses on treatment with ixekizumab from week 16 through week 52 in COAST-V (A) and COAST-W (B). ETP population. Missing data were imputed using NRI. ADA, adalimumab; ASAS, Assessment of SpondyloArthritis international Society; bDMARD, biological disease-modifying antirheumatic drug; ETP, dose double-blind extended treatment period; IXE, ixekizumab; NRI, non-responder imputation; PBO, placebo; TNFi, tumour necrosis factor inhibitor.

Table 2 Week 16* and 52 efficacy endpoints for PBO and ADA patients rerandomised to ixekizumab at week 16: COAST-V and COAST-W (ETP population: NRI, modified baseline observation carried forward)

	COAST-V (bDMARD-naive)				COAST-W (TNFi-experienced)	
	PBO/IXE (n=86)		ADA/IXE (n=86)		PBO/IXE (n=93)	
Patients achieving response, n (%)						
NRI	Week 16	Week 52	Week 16	Week 52	Week 16	Week 52
ASAS40	16 (18.6)	40 (46.5)	31 (36.0)	44 (51.2)	13 (14.0)	36 (38.7)
ASAS20	35 (40.7)	58 (67.4)	52 (60.5)	58 (67.4)	31 (33.3)	50 (53.8)
ASAS partial remission	7 (8.1)	16 (18.6)	13 (15.1)	18 (20.9)	1 (1.1)	9 (9.7)
ASDAS clinically important improvement	20 (23.3)	55 (64.0)	48 (55.8)	55 (64.0)	18 (19.4)	49 (52.7)
ASDAS major improvement	4 (4.7)	27 (31.4)	21 (24.4)	28 (32.6)	4 (4.3)	25 (26.9)
ASDAS<2.1 (low disease activity)	11 (12.8)	35 (40.7)	33 (38.4)	41 (47.7)	5 (5.4)	27 (29.0)
ASDAS<1.3 (inactive disease)	2 (2.3)	14 (16.3)	14 (16.3)	15 (17.4)	1 (1.1)	6 (6.5)
BASDAI50	15 (17.4)	40 (46.5)	28 (32.6)	39 (45.3)	10 (10.8)	35 (37.6)
Mean change from baseline (SD)						
mBOCF†	Week 16	Week 52	Week 16	Week 52	Week 16	Week 52
ASDAS	−0.6 (0.8)	−1.6 (1.0)	−1.3 (1.2)	−1.5 (1.1)	−0.2 (1.1)	−1.4 (1.3)
BASDAI	−1.5 (1.7)	−2.9 (2.1)	−2.4 (2.3)	−3.0 (2.3)	−1.0 (2.1)	−2.7 (2.6)
BASFI	−1.3 (1.8)	−2.4 (2.2)	−2.2 (2.2)	−2.7 (2.3)	−0.7 (2.1)	−2.2 (2.7)
SF-36 PCS‡	4.2 (6.3)	7.7 (8.0)	6.6 (7.2)	7.7 (8.0)	1.0 (7.2)	6.2 (8.7)
ASAS Health Index	−1.4 (2.5)	−2.5 (3.3)	−2.4 (3.1)	−2.9 (3.6)	−0.9 (3.2)	−2.4 (3.6)
SPARCC MRI spine score§	−1.1 (5.9)	−8.5 (14.6)	−12.6 (21.4)	−13.9 (21.2)	NA	NA
SPARCC MRI sacroiliac joint score¶	0.76 (5.4)	−2.7 (6.2)	−2.8 (8.4)	−3.0 (9.0)	NA	NA
CRP, mg/L	−1.0 (22.9)	−11.2 (22.3)	−8.4 (17.3)	−9.4 (17.0)	6.8 (29.9)	−9.7 (25.8

*Except for ASAS partial remission (both studies), ASDAS clinically important improvement (both studies), ASDAS major improvement (both studies), ASDAS <1.3 (COAST-W) and BASDAI50 (COAST-W), all week 16 data have been previously reported.[10 11]

† For patients who discontinued study drug because of an adverse event, the baseline observation was carried forward to the corresponding timepoint for evaluation. For patients discontinuing study drug for any other reason, the last non-missing observation before discontinuation was carried forward to the corresponding time point for evaluation.

‡ SF-36PCS data are reported as t-scores, based on 2009 US general population norms.

§ Observed data only (not assessed after week 16 in COAST-W). COAST-V: week 16, n=81 (PBO/IXE) and n=80 (ADA/IXE); week 52, n=76 (PBO/IXE) and n=76 (ADA/IXE). COAST-W: week 16, n=49 (IXE Q4W) and n=45 (IXE Q2W).

¶ Observed data only (not assessed in COAST-W). COAST-V: week 16, n=81 (PBO/IXE) and n=80 (ADA/IXE); week 52, n=76 (PBO/IXE) and n=76 (ADA/IXE).

ADA, adalimumab; ASAS, Assessment of SpondyloArthritis international Society; ASDAS, Ankylosing Spondylitis Disease Activity Score; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; bDMARD, biological disease-modifying antirheumatic drug; CRP, C reactive protein; ETP, dose double-blind extended treatment period; mBOCF, modified baseline observation carried forward; NA, not applicable; NRI, non-responder imputation;PBO, placebo; SF-36 PCS, Medical Outcomes Study 36-item Short-Form Health Survey Physical Component Score; IXE Q2W, ixekizumab 80 mg every 2 weeks; IXE Q4W, ixekizumab 80 mg every 4 weeks; TNFi, tumour necrosis factor inhibitor.

Table 3 Safety summary: COAST-V and COAST-W (ETP population (weeks 16 to 52) and all ixekizumab exposure safety population (weeks 0 to 52))

	COAST-V (bDMARD-naive) ETP population (weeks 16–52)				COAST-W (TNFi-experienced) ETP population (weeks 16–52)			COAST-V+COAST W All ixekizumab exposure safety population (weeks 0–52)	
	PBO/ IXE (n=86) n (%)	ADA/ IXE (n=86) n (%)	IXE Q4W/ IXE Q4W (n=78) n (%)	IXE Q2W/ IXE Q2W (n=79) n (%)	PBO/ IXE (n=93) n (%)	IXE Q4W/ IXE Q4W (n=98) n (%)	IXE Q2W/ IXE Q2W (n=90) n (%)	Total IXE Q4W (n=327) n (%) (IR*)	Total IXE Q2W (n=314) n (%) (IR*)
Exposure, patient-years	58.5	51.7	51.9	53.2	59.6	64.1	58.2	259.4	250.8
Any TEAE	57 (66.3)	50 (58.1)	50 (64.1)	44 (55.7)	52 (55.9)	69 (70.4)	58 (64.4)	234 (71.6) (90.2)	217 (69.1) (86.5)
Mild	31 (36.0)	32 (37.2)	34 (43.6)	28 (35.4)	21 (22.6)	30 (30.6)	24 (26.7)	115 (35.2) (44.3)	97 (30.9) (38.7)
Moderate	22 (25.6)	15 (17.4)	13 (16.7)	13 (16.5)	23 (24.7)	33 (33.7)	30 (33.3)	101 (30.9) (38.9)	98 (31.2) (39.1)
Severe	4 (4.7)	3 (3.5)	3 (3.8)	3 (3.8)	8 (8.6)	6 (6.1)	4 (4.4)	18 (5.5) (6.9)	22 (7.0) (8.8)
Discontinuation due to AE	2 (2.3)	3 (3.5)	1 (1.3)	2 (2.5)	1 (1.1)	4 (4.1)	5 (5.6)	17 (5.2) (6.6)	17 (5.4) (6.8)
SAEs	4 (4.7)	7 (8.1)	4 (5.1)	3 (3.8)	6 (6.5)	2 (2.0)	1 (1.1)	17 (5.2) (6.6)	19 (6.1) (7.6)
Death	0	0	0	0	0	0	0	0	1 (0.3) (0.4)
Most common TEAEs†									
Nasopharyngitis	17 (19.8)	7 (8.1)	8 (10.3)	7 (8.9)	3 (3.2)	3 (3.1)	4 (4.4)	37 (11.3) (14.3)	25 (8.0) (10.0)
Injection site reaction	8 (9.3)	8 (9.3)	3 (3.8)	6 (7.6)	3 (3.2)	2 (2.0)	5 (5.6)	13 (4.0) (5.0)	30 (9.6) (12.0)
Upper respiratory tract infection	4 (4.7)	4 (4.7)	4 (5.1)	8 (10.1)	5 (5.4)	4 (4.1)	8 (8.9)	29 (8.9) (11.2)	27 (8.6) (10.8)
AEs of special interest									
Grade 3 or 4 neutropenia	0	0	0	0	0	0	0	1 (0.3) (0.4)	0
Infections	34 (39.5)	19 (22.1)	25 (32.1)	25 (31.6)	32 (34.4)	29 (29.6)	33 (36.7)	134 (41.0) (51.7)	118 (37.6) (47.1)
Serious infections	1 (1.2)	1 (1.2)	0	1 (1.3)	2 (2.2)	0	1 (1.1)	3 (0.9) (1.2)	7 (2.2) (2.8)
Candida infection	2 (2.3)	0	0	0	0	2 (2.0)	0	4 (1.2) (1.5)	1 (0.3) (0.4)
Injection site reactions	15 (17.4)	13 (15.1)	5 (6.4)	9 (11.4)	8 (8.6)	3 (3.1)	7 (7.8)	30 (9.2) (11.6)	54 (17.2) (21.5)
Allergic reactions/ hypersensitivities	4 (4.7)	4 (4.7)	4 (5.1)	2 (2.5)	2 (2.2)	6 (6.1)	4 (4.4)	20 (6.1) (7.7)	20 (6.4) (8.0)
Potential anaphylaxis	0	1 (1.2)	0	0	0	0	0	0	1 (0.3) (0.4)
Hepatic	6 (7.0)	1 (1.2)	3 (3.8)	4 (5.1)	4 (4.3)	2 (2.0)	2 (2.2)	16 (4.9) (6.2)	13 (4.1) (5.2)
Cerebrocardiovascular events‡, adjudicated	1 (1.2)	0	0	0	1 (1.1)	1 (1.0)	0	3 (0.9) (1.2)	3 (1.0) (1.2)
MACE	0	0	0	0	1 (1.1)	0	0	0	1 (0.3) (0.4)
Malignancies	0	1 (1.2)	0	0	0	0	0	2 (0.6) (0.8)	0
Anterior uveitis	2 (2.3)	2 (2.3)	1 (1.3)	1 (1.3)	2 (2.2)	4 (4.1)	5 (5.6)	9 (2.8) (3.5)	11 (3.5) (4.4)
Depression	0	0	0	0	0	1 (1.0)	1 (1.1)	1 (0.3) (0.4)	2 (0.6) (0.8)
Crohn's disease	1 (1.2)	1 (1.2)	0	0	0	0	0	2 (0.6) (0.8)	2 (0.6) (0.8)
Ulcerative colitis	1 (1.2)	0	0	0	0	0	0	2 (0.6) (0.8)	0
IBD not otherwise specified	0	0	1 (1.3)	0	0	0	0	2 (0.6) (0.8)	0
Psoriasis	0	0	0	0	0	3 (3.1)	1 (1.1)	4 (1.2) (1.5)	1 (0.3) (0.4)

*IR calculated per 100 patient-years.
†Defined as events reported by ≥5% of all patients in either of the two studies in the ETP population.
‡Cerebrocardiovascular events included death, cardiac ischaemic events including myocardial infarction and hospitalisation for unstable angina, hospitalisation for heart failure, serious arrhythmia, resuscitated sudden death, cardiogenic shock, coronary revascularisation procedure, stroke/transient ischaemic attack, peripheral revascularisation procedure and peripheral arterial event and hospitalisation for hypertension.
ADA, adalimumab; AE, adverse event; bDMARD, biological disease-modifying antirheumatic drug; ETP, dose double-blind extended treatment period; IBD, inflammatory bowel disease; IR, incidence rate; IXE, IXE Q4W and IXE Q2W combined; MACE, major adverse cerebrocardiovascular events; PBO, placebo; IXE Q2W, ixekizumab 80 mg every 2 weeks; IXE Q4W, ixekizumab 80 mg every 4 weeks; SAE, serious adverse event; TEAE, treatment-emergent adverse event; TNFi, tumour necrosis factor inhibitor.

was considered related to treatment). There were no deaths during the ETP in either study.

Malignancy (bladder cancer) was reported by one patient (ADA/IXE) in COAST-V; the event was rated severe and led to study discontinuation. Depression was reported by two patients in COAST-W (both continued treatment); there were no events of suicide or attempted suicide in the ETP (one event of suicide occurred during the placebo-controlled period in a patient (IXE Q2W) with a history of depression).[11] There were no events of grade 3/4 neutropenia in either study.

Cerebrocardiovascular events were reported by one patient in COAST-V and two patients in COAST-W. One patient (PBO/IXE) in COAST-W reported a major adverse cerebrocardiovascular event of acute myocardial infarction; the event was severe, resolved and did not lead to study nor treatment discontinuation. Allergic reactions/hypersensitivities were reported by 14 (4.3%) patients in COAST-V and 12 (4.3%) patients in COAST-W.

Infections were reported by 103 (31.3%) patients in COAST-V and 94 (33.5%) patients in COAST-W; most were mild or moderate in severity. Serious infections were reported by three patients (cellulitis, pneumonia and tonsillitis; all n=1 patient) in COAST-V and three patients (gastroenteritis, pneumonia and sinusitis; all n=1 patient) in COAST-W; one of these patients discontinued the study. *Candida* infection was reported by two patients (oesophageal candidiasis and fungal oesophagitis; both n=1 patient) in COAST-V and two patients (oesophageal

candidiasis and oral candidiasis; both n=1 patient) in COAST-W (all were mild or moderate in severity); one of these patients discontinued the study. Three *Candida* infection events resolved and the other was resolving at the time of patient discontinuation. ISRs were reported by 42 (12.8%) patients in COAST-V and 18 (6.4%) patients in COAST-W. Most were mild or moderate in severity; two were severe. One patient discontinued study drug due to an ISR.

AU was reported by 17 patients, 6 (1.8%) in COAST-V and 11 (3.9%) in COAST-W; none were SAEs and 14 had a history of AU. One patient in COAST-W (IXE Q4W/IXE Q4W) discontinued the study because of AU.

In COAST-V, two patients (with no prior diagnosis) reported Crohn's disease and two patients with a prior diagnosis of ulcerative colitis reported a flare (online supplementary table S6). All events were mild or moderate in severity; one patient discontinued treatment. All events, except one, were adjudicated as 'probable'; one event of Crohn's disease (ADA/IXE) was adjudicated as 'definitive'. There were no events of Crohn's disease or ulcerative colitis in COAST-W.

All ixekizumab exposure safety population (weeks 0 to 52)

During the 52-week study periods of COAST-V and COAST-W (n=641), the pooled exposure-adjusted incidence rate per 100 patient years (EAIR) of serious infections was 2.0 among patients treated with ixekizumab (table 3). Pooled EAIRs of *Candida* infection and grade 3/4 neutropenia were 1.0 and 0.2, respectively. Corresponding EAIRs for Crohn's disease, ulcerative colitis and IBD not otherwise specified (NOS) were 0.8, 0.4 and 0.4, respectively (total IBD EAIR: 1.6). The EAIR for AU was 3.9; 15/20 (75%) patients had a history of AU and 14/20 (70%) patients were from COAST-W. The pooled EAIR for psoriasis was 1.0. One patient had a major adverse cerebrocardiovascular event (acute myocardial infarction) and two malignancies were reported (acute promyelocytic leukaemia and bladder cancer).

Fewer ISRs were reported with IXE Q4W (9.2%) versus IXE Q2W (17.2%). The number of patients reporting an ISR decreased over time. Specifically, 6.4%, 3.8% and 3.4% of patients on IXE Q4W and 14.3%, 8.6% and 5.2% of patients on IXE Q2W reported an ISR from weeks 0–12, weeks 12–24 and weeks 24–36, respectively. Few patients (IXE Q4W ≤1%; IXE Q2W approximately 3%) reported an ISR beyond week 36.

Treatment-emergent antidrug antibodies

TE-ADAs were detected during the 52-week study period in 23 (6.9%) patients in COAST-V and 27 (8.9%) patients in COAST-W. Most TE-ADA-positive patients had low titres (COAST-V, 18 (78%); COAST-W, 23 (85%)) and four patients (COAST-V, 1 (0.3%); COAST-W, 3 (1.0%)) were neutralising antibody positive. There were no associations between TE-ADA positivity and ASAS40 response, ISRs or allergic reaction/hypersensitivity events.

DISCUSSION

In COAST-V and COAST-W, the significant improvements observed at week 16[10 11] were sustained for up to 52 weeks with ixekizumab treatment as measured by ASAS40 responses and other efficacy outcomes assessing disease activity, function, objective inflammation, QoL, health status and overall functioning. The results for IXE Q4W and IXE Q2W were similar across endpoints. ASAS40 response rates in patients rerandomised from PBO rapidly increased to levels consistent with those seen with continuous ixekizumab treatment. Patients rerandomised from ADA to ixekizumab at week 16 achieved numerically greater response rates for ASAS40 and other efficacy outcomes at week 52 than at week 16. Collectively, the data from COAST-V and COAST-W demonstrate that ixekizumab is an effective treatment in patients with active r-axSpA who are bDMARD-naive or TNFi-experienced.

In general, treatment responses were numerically smaller in TNFi-experienced (COAST-W) versus bDMARD-naive (COAST-V) patients, reflecting a more difficult to treat population with prior treatment failure and more long-standing disease.

Currently approved biological therapies for axSpA include several TNFi and one IL-17A antagonist. Although only head-to-head trials can fully assess the relative efficacy and safety of different treatments, the week 52 ASAS40 findings reported herein are consistent with those reported for TNFi in patients who were bDMARD-naive[23–26] and for secukinumab in subgroups of patients who were bDMARD-naive or had previously failed TNFi treatment.[27]

The safety profile of ixekizumab during the ETP (week 16 to 52) in both COAST-V and COAST-W is consistent with that observed during weeks 0 to 16.[10 11] Discontinuation due to AEs was <4% in both studies, whereas <6% of patients reported SAEs. Most infections and ISRs were mild or moderate in severity and did not result in study discontinuation. ISRs were more frequent with IXE Q2W than IXE Q4W. Furthermore, ISRs were most frequently reported during the first 4 weeks of treatment and decreased in frequency over time. During the 52-week study period, pooled EAIRs for Crohn's disease, ulcerative colitis, IBD NOS, *Candida* infection and grade 3/4 neutropenia were ≤1 event/100 patient-years among patients treated with ixekizumab. Among patients who reported IBD events, most had a prior diagnosis of IBD or a gastrointestinal history potentially indicative of IBD. Fewer IBD events were reported with IXE Q2W versus IXE Q4W, and there was no apparent relationship between the length of ixekizumab exposure and IBD. Previous reports have indicated that the EAIR for AU in patients with AS ranges from 2.6 to 3.5 for patients treated with TNFi.[28] The EAIR of AU reported herein is at the upper limit of this range, primarily driven by patients from the TNF-experienced population. All but one patient were HLA-B27 positive, with the majority having a history of AU.

An important strength of these analyses is use of the most conservative methods of missing data imputation (NRI and mBOCF) for the primary analyses. Furthermore, as COAST-V and COAST-W exclusively enrolled bDMARD-naive and TNFi-experienced patients, respectively, both studies were fully powered for analyses in these populations. Notably, patients in COAST-W had very active disease (baseline ASDAS >4) and more than 30% had failed two prior TNFi. Another strength is the use of objective measures of inflammation, including MRI at week 52 (COAST-V only); to date, ixekizumab is the only IL-17A antagonist for which short-term and long-term MRI clinical trial data are available. The ETP results are limited by the lack of any placebo or active control comparators.

In conclusion, ixekizumab provided sustained and clinically meaningful improvement in the signs and symptoms of active r-axSpA for up to 52 weeks in COAST-V and COAST-W, with a high rate of completion. The safety findings were consistent with the known safety profile of ixekizumab. These findings suggest that ixekizumab could be a treatment option for axSpA in patients who are bDMARD-naive or who have had a prior inadequate response or intolerance to TNFi.

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REFERENCES

1 Sieper J, Poddubnyy D. Axial spondyloarthritis. *Lancet* 2017;390:73–84.
2 Gao X, Wendling D, Botteman MF, *et al*. Clinical and economic burden of extra-articular manifestations in ankylosing spondylitis patients treated with anti-tumor necrosis factor agents. *J Med Econ* 2012;15:1054–63.
3 Boonen A, van der Linden SM. The burden of ankylosing spondylitis. *J Rheumatol Suppl* 2006;78:4–11.
4 Ward MM, Deodhar A, Akl EA, *et al*. American College of Rheumatology/Spondylitis association of America/Spondyloarthritis research and treatment network 2015 recommendations for the treatment of ankylosing spondylitis and nonradiographic axial spondyloarthritis. *Arthritis Care Res* 2016;68:151–66.
5 van der Heijde D, Ramiro S, Landewé R, *et al*. 2016 update of the ASAS-EULAR management recommendations for axial spondyloarthritis. *Ann Rheum Dis* 2017;76:978–91.
6 Sepriano A, Regel A, van der Heijde D, *et al*. Efficacy and safety of biological and targeted-synthetic DMARDs: a systematic literature review informing the 2016 update of the ASAS/EULAR recommendations for the management of axial spondyloarthritis. *RMD Open* 2017;3:e000396.
7 D'Angelo S, Carriero A, Gilio M, *et al*. Safety of treatment options for spondyloarthritis: a narrative review. *Expert Opin Drug Saf* 2018;17:475–86.
8 Kenna TJ, Davidson SI, Duan R, *et al*. Enrichment of circulating interleukin-17-secreting interleukin-23 receptor-positive γ/δ T cells in patients with active ankylosing spondylitis. *Arthritis Rheum* 2012;64:1420–9.
9 Shen H, Goodall JC, Hill Gaston JS. Frequency and phenotype of peripheral blood Th17 cells in ankylosing spondylitis and rheumatoid arthritis. *Arthritis Rheum* 2009;60:1647–56.
10 van der Heijde D, Cheng-Chung Wei J, Dougados M, *et al*. Ixekizumab, an interleukin-17A antagonist in the treatment of ankylosing spondylitis or radiographic axial spondyloarthritis in patients previously untreated with biological disease-modifying anti-rheumatic drugs (COAST-V): 16 week results of a phase 3 randomised, double-blind, active-controlled and placebo-controlled trial. *Lancet* 2018;392:2441–51.
11 Deodhar A, Poddubnyy D, Pacheco-Tena C, *et al*. Efficacy and safety of ixekizumab in the treatment of radiographic axial spondyloarthritis: sixteen-week results rrom a phase III randomized, double-blind, placebo-controlled trial in patients with prior inadequate response to or intolerance of tumor necrosis factor inhibitors. *Arthritis Rheumatol* 2019;71:599–611.
12 Rudwaleit M, van der Heijde D, Landewé R, *et al*. The development of Assessment of Spondyloarthritis international Society classification criteria for axial spondyloarthritis (part II): validation and final selection. *Ann Rheum Dis* 2009;68:777–83.
13 Sieper J, Rudwaleit M, Baraliakos X, *et al*. The Assessment of Spondyloarthritis international Society (ASAS) handbook: a guide to assess spondyloarthritis. *Ann Rheum Dis* 2009;68 Suppl 2:ii1–44.
14 Machado PMMC, Landewé RBM, van der Heijde DM. Endorsement of definitions of disease activity states and improvement scores for the Ankylosing Spondylitis Disease Activity Score: results from OMERACT 10. *J Rheumatol* 2011;38:1502–6.
15 Garrett S, Jenkinson T, Kennedy LG, *et al*. A new approach to defining disease status in ankylosing spondylitis: the Bath Ankylosing Spondylitis Disease Activity Index. *J Rheumatol* 1994;21:2286–91.

16 Calin A, Garrett S, Whitelock H, *et al*. A new approach to defining functional ability in ankylosing spondylitis: the development of the Bath Ankylosing Spondylitis Functional Index. *J Rheumatol* 1994;21:2281–5.
17 Ware JE, Snow KK, Kosinski M, *et al*. *Sf-36 health survey: manual and interpretation guide*. 2nd ed. Lincoln, RI: QualityMetric, 2000.
18 Kiltz U, van der Heijde D, Boonen A, *et al*. The ASAS Health Index (ASAS HI) - a new tool to assess the health status of patients with spondyloarthritis. *Clin Exp Rheumatol* 2014;32:S-105-8.
19 Kiltz U, van der Heijde D, Boonen A, *et al*. Development of a health index in patients with ankylosing spondylitis (ASAS HI): final result of a global initiative based on the ICF guided by ASAS. *Ann Rheum Dis* 2015;74:830–5.
20 Maksymowych WP, Inman RD, Salonen D, *et al*. Spondyloarthritis Research Consortium of Canada magnetic resonance imaging index for assessment of spinal inflammation in ankylosing spondylitis. *Arthritis Rheum* 2005;53:502–9.
21 Dougados M, Simon P, Braun J, *et al*. ASAS recommendations for collecting, analysing and reporting NSAID intake in clinical trials/epidemiological studies in axial spondyloarthritis. *Ann Rheum Dis* 2011;70:249–51.
22 Gower-Rousseau C, Salomez JL, Dupas JL, *et al*. Incidence of inflammatory bowel disease in northern France (1988-1990). *Gut* 1994;35:1433–8.
23 van der Heijde D, Schiff MH, Sieper J, *et al*. Adalimumab effectiveness for the treatment of ankylosing spondylitis is maintained for up to 2 years: long-term results from the ATLAS trial. *Ann Rheum Dis* 2009;68:922–9.
24 Davis JC, van der Heijde DM, Braun J, *et al*. Sustained durability and tolerability of etanercept in ankylosing spondylitis for 96 weeks. *Ann Rheum Dis* 2005;64:1557–62.
25 Braun J, Deodhar A, Inman RD, *et al*. Golimumab administered subcutaneously every 4 weeks in ankylosing spondylitis: 104-week results of the GO-RAISE study. *Ann Rheum Dis* 2012;71:661–7.
26 Sieper J, Landewé R, Rudwaleit M, *et al*. Effect of certolizumab pegol over ninety-six weeks in patients with axial spondyloarthritis: results from a phase III randomized trial. *Arthritis Rheumatol* 2015;67:668–77.
27 Sieper J, Deodhar A, Marzo-Ortega H, *et al*. Secukinumab efficacy in anti-TNF-naive and anti-TNF-experienced subjects with active ankylosing spondylitis: results from the MEASURE 2 study. *Ann Rheum Dis* 2017;76:571–92.
28 Deodhar A, Miceli-Richard C, Baraliakos X, *et al*. Low incidence of both new-onset and flares of uveitis in secukinumab-treated patients with ankylosing spondylitis: clinical trial and post-marketing safety analysis. *Ann Rheum Dis* 2018;77(Suppl 2):999.

EPIDEMIOLOGICAL SCIENCE

Frequency of MRI changes suggestive of axial spondyloarthritis in the axial skeleton in a large population-based cohort of individuals aged <45 years

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ABSTRACT

Objective To investigate the frequency of bone marrow oedema (BME) and fatty lesions (FL) suggestive of axial spondyloarthritis (axSpA) on MRI of the spine and sacroiliac joints (SIJ) in a general population sample.

Methods As part of a community-based cohort project (Study of Health in Pomerania), volunteers underwent spinal (sagittal T1/T2) and SIJ (semicoronal short tau inversion recovery) MRI examinations. Two calibrated readers evaluated the images to detect BME in SIJ and vertebral corners (VC) and FL in VC suggestive of axSpA using Assessment of SpondyloArthritis international Society definitions.

Results MRIs of 793 volunteers (49.4% males, mean age 37.3±6.3 years, 8.4% human leucocyte antigen-B27+) aged <45 years were evaluated. SIJ BME was seen in 136 (17.2%), VC BME in 218 (27.5%) and FL in 645 (81.4%) volunteers. SIJ BME in ≥1, ≥3 and ≥5 SIJ quadrants was seen in 136 (17.2%), 7 (0.9%) and 1 (0.1%) volunteers, respectively. In VC, BME≥1, ≥3 and ≥5 lesions were seen in 218 (27.5%), 38 (4.8%) and 6 (0.8%) volunteers, respectively, while FL≥1, ≥3 and ≥5 were seen in 645 (81.3%), 351 (44.3%) and 185 (23.3%) volunteers, respectively. Logistic regression analysis showed that BME and FL in VC were related to increasing age: OR 1.33, 95% CI 1.02 to 1.72, and OR 1.73, 95% CI 1.32 to 2.27, per decade increase, respectively.

Conclusions In this large population-based study, a high frequency of inflammatory and fatty MRI lesions suggestive of axSpA was found, especially in the spine. This indicates a limited value of such MRI findings for diagnosis and classification of axSpA. The increasing frequency with age suggests that mechanical factors could play a role.

Key messages

What is already known about this subject?
- The sensitivity and specificity of the Assessment of SpondyloArthritis international Society criteria for a 'positive' MRI for the classification of axial spondyloarthritis (axSpA) have been challenged due to high sensitivity with relatively low specificity.

What does this study add?
- There seems to be a relatively high frequency of inflammatory and fatty spinal/inflammatory sacroiliac joints (SIJ) and spinal MRI lesions suggestive of axSpA in the general population.
- Such MRI changes tend to occur more frequently in patients of higher age groups, suggesting an influence of a mechanical factor and potential development of osteoarthritis.

How might this impact on clinical practice or future developments?
- Caution is needed to take 'a positive MRI' as proof that a patient has axSpA, especially in the absence of clear clinical symptoms indicative of the disease.
- These data suggest that the current definition of MRI changes in the SIJ used for the classification of axSpA need an update.

INTRODUCTION

Axial spondyloarthritis (axSpA) is a chronic inflammatory disease of the axial skeleton with a prevalence between 0.1% and 1.4%.[1] Classification to axSpA is possible by using the Assessment of SpondyloArthritis international Society (ASAS) classification criteria.[2] According to the *imaging arm* of these criteria, patients aged <45 years with chronic back pain and a symptom onset can be classified as axSpA if either definite periarticular structural sacroiliac joint (SIJ) changes are detected on conventional radiographs (CR), or if SpA-related bone marrow oedema (BME) are found by MRI examinations on the SIJs.[3] Within this context, a SIJ MRI with either ≥2 inflammatory lesions in form of BME in one slice or with only one BME lesion in ≥2 consecutive slices has been defined as 'positive'.[4] For the evaluation of the spine, a spinal MRI that is 'highly suggestive' of axSpA consists of ≥3 anterior or posterior BME lesions ('spondylitis') or several vertebral corners (VC) with postinflammatory fatty lesions (FL). Also, these lesions must be visible in ≥2 consecutive slices in the sagittal view of the spinal MRI. Overall, BME and FL have confirmed their value as predictors of structural damage, as seen on spinal CR during the course of the disease.[5]

The sensitivity and specificity of the above-mentioned cut-offs for identifying axSpA have recently been tested in different cohorts with

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limited diagnostic value.[6] In consequence, the value of the proposed definitions for a 'positive' MRI especially in the spine have been challenged, since a combination of both, BME and structural changes in the SIJ to classify axSpA, was found to perform superior compared with BME alone.[3]

Overall, due to a recently reported low specificity,[7] the described limitations may lead to overinterpretation of positive MRI findings in daily practice with the consequence of overdiagnosis and, consequently, overtreatment.

Therefore, we investigated the frequency of BME and FL, suggestive of axSpA, on MRI examinations of the spine and the SIJ in a general-population sample.

METHODS

Study sample

Our study sample was selected from the population-based cohort study 'Study of Health in Pomerania' (SHIP) in Germany. SHIP is part of the Community Medicine Research net of the University of Greifswald, Germany, and comprises of the separate cohorts SHIP and SHIP-TREND. Volunteers of both cohorts were sampled and selected from the two counties of North Pomerania and East Pomerania and the two cities of Greifswald and Stralsund. SHIP baseline examinations started in 1997. SHIP-TREND was initiated in 2008 with a similar sampling scheme to allow for examinations of population trends. A core set of examinations (physical examinations, interviews, self-reported questionnaires and biomaterials) has constant financing. However, further examinations, such as MRI, require additional funding. Whole-body MRI (with orientations dedicated to different areas of examination) was funded as of 2008 and implemented in the second follow-up of SHIP and the baseline examinations of SHIP-TREND which were conducted in parallel. The entire SHIP project and the sampling process has been described in more detail elsewhere.[8 9] For the present study, we planned to start double readings as soon as MRIs of n=800 volunteers were available. From these, n=793 volunteers being aged <45 years of the MRI examination had complete MRI sets (both spine and SIJ) and were included in the analyses (figure 1).

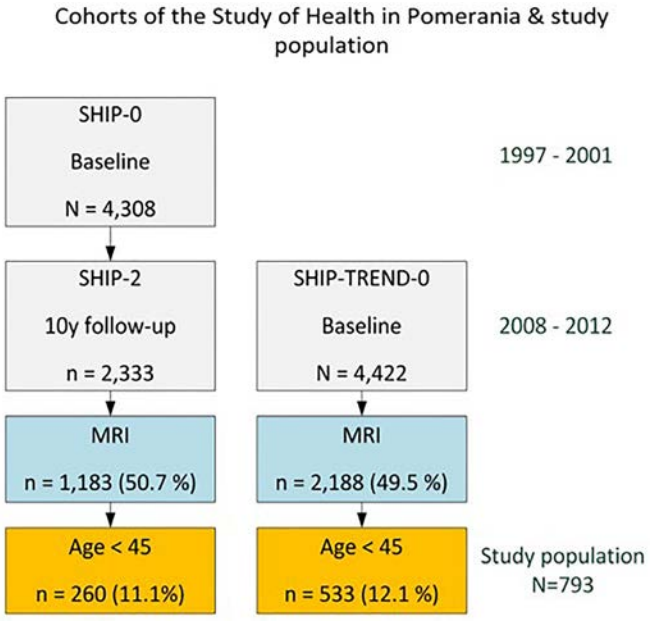

Figure 1 Cohort design of the Study of Health in Pomerania (SHIP). Percentages of MRI participation and the sample of this study refer to either SHIP-2 or SHIP-TREND. The sampling process is described in detail in Ittermann *et al*.[9]

Patient and public involvement

The SHIP study was initiated in 1997 and is conducted in the general population. Participation is based on voluntary commitment. An invitation letter to participate in the study was sent to a random selection of the population of Mecklenburg-Western Pomerania based on the data from population registries. The design of the population-based cohort study with deep phenotyping was conducted before the design of the research question targeted in the paper. This is a common approach in population-based epidemiology. Given the large set of measurements and variables (>40 000 in total), not all uses can be anticipated a priori. However, the design of the reading of MRI images was specifically designed for this study, given the available MRI sequences. The study is not conducted in patients but in the general population. Regarding the design, participants were not involved. The recruitment was designed to ensure representativity. Participants were not involved in the invitation approach. Pilot studies with feedback from participants influenced study conduct. The SHIP study is observational and has no intended intervention. The required time for study participation is communicated in the invitation letter, newsletter and in personal phone calls arranging appointments. After participation, participants were asked whether they liked participation or had any complaints. They were also asked about their satisfaction of having participated in an MRI examination. In both cases, the vast majority was very content. Participants were involved by giving their consent to various potential uses of the results.

MRI and reading of images

All MRIs were performed at one site with the same MRI device (Magnetom Avanto, Siemens Medical Systems, Erlangen, Germany) under the same standardised protocol.[10] T1 and T2 MRI sequences in a sagittal orientation for the whole spine (figure 2A) and short tau inversion recovery (STIR) sequences in a semicoronal orientation for the SIJ (figure 2B) were available. All images were blinded for age, sex and clinical findings. Two trained readers evaluated the MR images independently in a paired fashion[3] to assess BME (defined as hyperintense signal on T2-weighted and hypointense signal on T1-weighted images in VC of the spine or as hyperintense signal on STIR sequences in the SIJs) and chronic MRI lesions (FL defined as hyperintense signal on both T1-weighted and T2-weighted images of the VC).

Prior to MRI evaluation, a training session with 30 reference sets of images, including axSpA patients, guided by an experienced reader was performed. A lesion was judged as being present if fulfilling the ASAS definitions for a positive MRI for inflammatory (SIJ and VC) or FL (VC).[4 11 12] For all definitions, a lesion had to be present in at least two consecutive slices to be counted positive. Clearly, degenerative spinal lesions defined as pathologic changes involving the vertebral endplate or being accompanied by abnormalities of the intervertebral disc (obvious dehydration, protrusion or prolapse), lesions of oedema in the lateral or posterior elements, lesions that looked like spondylodiscitis or any lesions that were suspicious to not comply to the ASAS definitions were not counted (figure 2A).

In case of disagreement between readers (lesion present or not present in SIJ or VC), discrepant cases were discussed by both readers in front of the blinded MRI, to reach an agreement.

MRI lesions were also scored based on the Berlin MRI score for BME for the SIJ and the spine.[13] Once the lesions were

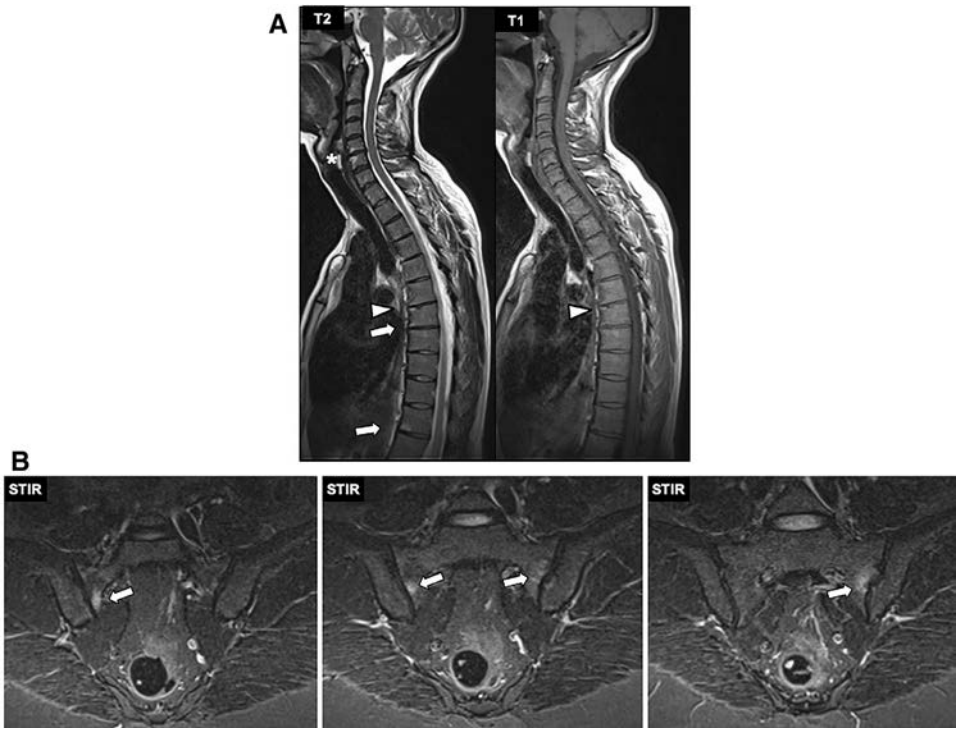

Figure 2 (A) Example of hyperintense signals in the T2-weighted and T1-weighted spinal MRI sequences of a male patient, age 39 years. *Arrowhead*: Fatty lesion at the edge of a thoracic vertebra, seen as hyperintense signal in both T2-weighted and T1-weighted MRI sequences. *Arrows:* Bone marrow oedema at the edge of a thoracic vertebra, seen as hyperintense signal only in the T2-weighted MRI sequence. '*': Example of a lesion that was not counted as positive despite hyperintense signal on the T2-weighted sequence, due to the concomitant dehydration of the adjacent intervertebral disk and the small protrusion in this segment. (B) Example of hyperintense signals in the STIR MRI sequences of the SIJ of a female patient, age 41 years. *Arrows*: Bone marrow oedema seen as periarticularly located hyperintense signal on STIR MRI. SIJ, sacroiliac joints; STIR, short tau inversion recovery.

identified as described aforementioned and judged positive, BME score was calculated based on the mean score of both readers. VC FLs were captured in a binary manner (presence or absence of lesion).

Collection of clinical information

The collected clinical information was age (in years), sex, smoking (ever vs never smoked), mean spinal back pain level in the last 3 months prior to MRI examination (on a numerical rating scale (NRS-rated) 0–10), physical activity (none, <1 hour, 1–2 hours and ≥2 hours on average per week), high-sensitivity C reactive protein (hsCRP, mg/dL), human leucocyte antigen (HLA)-B27 status (positive/negative) and body mass index category according to the WHO definition (underweight, normal, overweight and obese).

Handling of missing values

Missing values, such as not evaluable segments due to artefacts or missing clinical information, were not imputed. Results are always presented based on the available data for each parameter.

Statistical analysis

Characteristics of volunteers are summarised using descriptive statistics. Depending on the distribution of each clinical feature, the mean±SD or the median and IQR are shown. Percentages of frequencies presented were calculated with respect to the number of available sites. Univariate logistic regression was applied to examine associations between clinical characteristics and the presence of MRI lesions.

RESULTS

Clinical characteristics

Overall, data of 793 individuals were evaluated based on the inclusion criteria aforementioned. The mean age was 37.3 (SD 6.3 years) years, 392 (49.4%) were male and 401 (50.6%) were female. The median NRS of back pain in the last 3 months prior to MRI examination was 2 (0.0–4.0).

Available and missing values

The total amount of variables to be scored in the 793 volunteers was 54 (BME in 8 SIJ quadrants, and VC BME and FL each in 23 spinal segments, respectively), resulting in 6344 SIJ quadrants and 18 293 VC for the analysis for BME and FL. For SIJ BME, no missings were reported. For VC BME, a total of 6/18 293 (0.03%) segments were not available (n=2 in segments C2/3, C3/4 and Th12/L1, respectively). For VC FL, a total of 743/18 293 (4.1%) segments were not available for scoring (n=487 in C2/3, n=205 in C3/4, n=25 in C4/5, n=9 in Th2/3, n=5 in Th1/2, n=4 in Th3/4, n=2 in C7/Th1 and in L1/2, and n=1 in C5/6, C6/7, Th1/2, Th2/3 and Th8/9, respectively).

Clinical information was available from all 793 patients with exception of HLA-B27, hsCRP and smoking, where information from n=756, n=761 and n=792 patients was available, respectively (table 1).

Agreement in the evaluation of images between readers

Discrepancies between readers were seen in 1071 (2.5%) of the scored variables. Most discrepant cases were found in variables related to VC FL (n=943), while less were found in variables

Table 1 Occurrence rate of MRI lesions of the sacroiliac joints and in the spine according to subcategories from the number of participants ('n') with available information

Parameter (n patients with data available) and subcategory		n (%) patients in each subcategory	Sacroiliac joints	Spine	
			n (%) quadrants with BME on MRI	n (%) segments with BME on MRI	n (%) segments with FL on MRI
Age, years (n=793)	<30	114 (14.4%)	24 (2.63%)	19 (0.72%)	208 (7.93%)
	30–35	120 (15.1%)	20 (2.08%)	57 (2.07%)	288 (10.43%)
	35–40	198 (25.0%)	51 (3.22%)	104 (2.28%)	499 (10.96%)
	40–45	361 (45.5%)	92 (3.19%)	182 (2.19%)	1309 (15.77%)
Sex (n=793)	Male	392 (49.4%)	100 (3.19%)	158 (1.75%)	1284 (14.24%)
	Female	401 (50.6%)	87 (2.71%)	204 (2.21%)	1020 (11.06%)
hsCRP, mg/dL (n=761)	Normal	708 (93%)	169 (2.98%)	337 (2.07%)	2054 (12.61%)
	Increased	53 (7%)	15 (3.54%)	18 (1.48%)	165 (13.54%)
HLA-B27 (n=756)	Negative	689 (91.1%)	157 (2.85%)	307 (1.94%)	1995 (12.59%)
	Positive	67 (8.9%)	26 (4.85%)	38 (2.47%)	195 (12.65%)
BMI category, kg/m^2 (n=793)	<25 (under–normal weight)	357 (45%)	60 (2.1%)	159 (1.94%)	803 (9.78%)
	25–30 (overweight)	287 (36.2%)	83 (3.61%)	146 (2.21%)	918 (13.91%)
	>30 (obese)	149 (18.8%)	44 (3.69%)	57 (1.66%)	583 (17.01%)
Ever smoked (n=792)	Yes	497 (62.8%)	126 (3.17%)	240 (2.1%)	1499 (13.11%)
	No	295 (37.2%)	61 (2.58%)	122 (1.8%)	800 (11.79%)
Back pain (n=793)	NRS=0	342 (43.1%)	63 (2.30%)	170 (2.16%)	985 (12.52%)
	NRS=1–3	223 (28.1%)	69 (3.87%)	99 (1.93%)	648 (12.63%)
	NRS≥4	228 (28.8%)	55 (3.02%)	93 (1.77%)	671 (12.8%)

Percentages were calculated on the basis of the numbers of available sites.
BME, bone marrow oedema; BMI, body mass index; FL, fatty lesions; HLA-B27, human leucocyte antigen-B27; hsCRP, high-sensitivity C reactive protein; NRS, numerical rating scale.

related to VC BME (n=88) or SIJ BME (n=40) lesions. In all discrepant cases, a consensus was reached.

Frequency and quantification of BME lesions in the SIJs

BME in the SIJ according to the ASAS definition for positive MRI was found in 136 (17.2%) volunteers with little differences between males (n=71, 18.1%) and females (n=65, 16.2%), p=0.47. In total, 187 of 6344 (2.9%) evaluated SIJ quadrants showed BME. SIJ BME in ≥1 quadrant was found in 136 (17.2%) volunteers, while 7 volunteers (0.9%) had ≥3, and 1 (0.1%) had ≥5 SIJ quadrants showing BME lesions (table 2).

In the whole study sample, the mean number of SIJ quadrants with BME lesions was 0.24 (SD 0.62), while in the group with BME in SIJ, the mean number of SIJ quadrants with BME lesions was 1.38 (SD 0.83).

The distribution of SIJ quadrants with BME lesions according to subcategories based on patient's characteristics is presented in table 1.

The mean Berlin SIJ-BME score in all 793 volunteers was 0.78 (SD 1.56), while in the subgroup of the 136 with ≥1 SIJ BME lesion, the median Berlin score was 2.36 (SD 2.63).

Table 2 Frequency of patients with 'positive' lesions based on different lesion cut-offs (≥1 to ≥5 lesions) for bone marrow oedema (BME) and fatty lesions (FL) in the sacroiliac joints (SIJ) and the spine

Site and lesion		Cut-off numbers of lesions				
		≥1	≥2	≥3	≥4	≥5
SIJ	BME	136	37	7	3	1
Spine	BME	218	86	38	13	6
	FL	645	500	351	270	185

Frequency and quantification of BME lesions in the spine

Similar to the SIJ, the frequency of VC BME according to the ASAS definition for positive MRI was almost equally distributed between males (n=102, 26.0%) and females (n=116, 28.9%) (p=0.36). Out of the total of 18 239 spinal segments, 362 (2%) showed signs of VC BME: ≥1 lesion was found in 218 (27.5%) volunteers, while 38 (4.8%) volunteers had ≥3 and 6 volunteers (0.8%) had ≥5 VC BME in any spinal segment (table 2). In the whole study sample, the mean number of VC BME was 0.45 (SD 0.91), while in those with ≥1 VC BME, it was 1.66 (SD 1.01).

The distribution VC BME in single spinal segments (cervical, thoracic or lumbar) is shown in figure 3A. The most frequently affected area by VC BME was found in the lower part of the thoracic spine. The distribution of VC BME to subcategories based on the clinical patient's characteristics is presented in table 1.

The mean Berlin spine score in all 793 volunteers was 0.49 (SD 1.04), while in the subgroup of the 218 patients with ≥1 VC BME, the median Berlin spine score was 1.81 (SD 1.25).

Frequency and quantification of FL in the spine

There was no statistically significant difference in the frequency of VC FL between males (n=326, 83.2%) and females (n=319, 79.6%) (p=0.19).

Out of the total of 18 239 spinal segments, 2407 (13.3%) showed signs of VC FL. Based on the individual volunteers, VC FL (≥1 lesion) were found in 645 (81.3%) volunteers, while 351 (44.3%) volunteers had ≥3 and 185 volunteers (23.3%) had ≥5 VC FL lesions in any of the spinal segments (table 2). The mean number of VC FL in all volunteers was 2.91 (SD 2.69), while the mean number of VC FL in the group of 645 volunteers with ≥1 VC FL was 3.57 (SD 2.55).

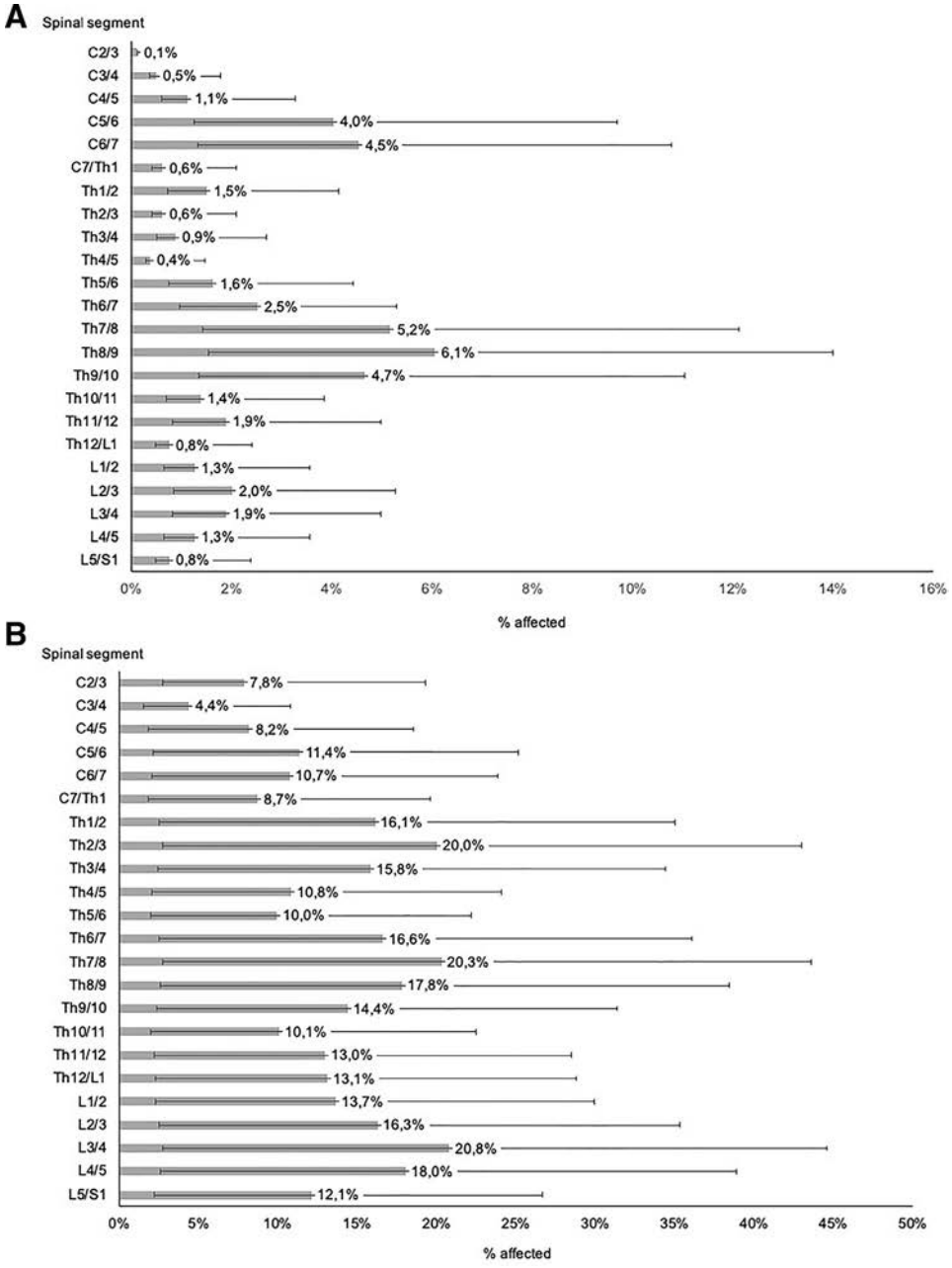

Figure 3 Distribution of spondylitis lesions (A, scale x-axis 0%–16%) and FL (B, scale x-axis 0%–50%) in the 23 different vertebral segments. The numbers/percentages at each bar represent the mean proportion of lesions in each spinal segment for all participating volunteers where the respective spinal segment was available for evaluation. The lines represent the upper (to the right) and lower (to the left) confidence limit per spinal segment. C, cervical spine; FL, fatty lesions; L, lumbar spine; Th, thoracic spine.

The distribution of VC FL lesions in single spinal segments (cervical, thoracic or lumbar) is shown in figure 3B. The most frequently affected areas by VC FL were the upper and lower part of the thoracic spine and the lower part of the lumbar spine.

Dependency of MRI findings to clinical and laboratory characteristics

Logistic regression analysis for spine showed that VC BME and VC FL were significantly related to increasing age, per decade increase: OR 1.33, 95% CI 1.02 to 1.72, and OR 1.73, 95% CI 1.32 to 2.27, respectively. No further clinical characteristic had a significant association with the frequency of VC BME or VC FL (table 3).

DISCUSSION

To the best of our knowledge, this study is the first to assess the frequency of both, inflammatory and fatty MRI changes of the spine and inflammatory changes of the SIJ, in a general population sample. The volunteers participating in SHIP,[8] a large population-based cohort study, showed a high frequency of sacroiliac and spinal MRI changes suggestive of axSpA according to the ASAS definitions.[2 11] There was a difference between inflammatory and fatty MRI changes: while the frequency of BME in SIJ or the spine (VC BME) was 17%–28%, VC FL were even more frequent with >80% individuals showing such changes. However, when the cut-offs for positive scores were raised from ≥1 to ≥3 or ≥5 lesions, this frequency decreased

Table 3 Univariate ORs (CIs) for the association of clinical predictors and the frequency of bone marrow oedema (BME) (in sacroiliac joints (SIJ) and spine) and fatty lesions (FL) (in spine)

Parameter	BME SIJ	BME spine	FL spine
Age (per 10 years increase), years	1.19 (0.88 to 1.62)	1.33 (1.02 to 1.72)	1.73 (1.32 to 2.27)
Sex (male vs female)	1.14 (0.79 to 1.66)	0.86 (0.63 to 1.18)	1.27 (0.89 to 1.82)
BMI<25 (reference), kg/m^2	N/A	N/A	N/A
BMI (25–30 vs reference)	1.46 (0.96 to 2.21)	1.31 (0.93 to 1.86)	1.48 (1.00 to 2.19)
BMI (>30 vs reference)	1.61 (0.98 to 2.65)	1.09 (0.71 to 1.68)	3.27 (1.76 to 6.07)
Smoking (ever vs never)	1.25 (0.84 to 1.84)	1.06 (0.77 to 1.47)	1.00 (0.69 to 1.44)
Back pain last 3 months (yes vs no)	1.32 (0.90 to 1.93)	1.00 (0.73 to 1.37)	0.91 (0.63 to 1.31)
HLA-B27 (pos. vs neg.)	1.27 (0.68 to 2.36)	1.15 (0.66 to 1.99)	1.14 (0.58 to 2.24)
hsCRP (>0.5 vs <0.5), mg/dL	0.96 (0.46 to 2.03)	0.83 (0.43 to 1.58)	1.13 (0.54 to 2.37)
Physical activity (none=reference)	N/A	N/A	N/A
>2 hours vs none	0.79 (0.47 to 1.35)	0.88 (0.55 to 1.39)	1.30 (0.75 to 2.26)
1–2 hours vs none	0.73 (0.45 to 1.20)	0.89 (0.58 to 1.36)	1.01 (0.62 to 1.63)
<1 hour vs none	0.64 (0.38 to 1.08)	0.85 (0.55 to 1.32)	0.97 (0.59 to 1.59)

BMI, body mass index; HLA-B27, human leucocyte antigen-B27; hsCRP, high-sensitivity C reactive protein; N/A, not applicable; neg., negative; pos., positive.

substantially. This suggests a good performance of the existing definitions of a 'positive spinal MRI' (cut-off≥5 lesions[11]) related to specificity. Furthermore, these results are also in line with previously published data on SIJ MRIs only or in groups with smaller patient numbers[7] from different samples, such as athletes[14] and military recruits.[15] There, the relative frequency of BME lesions ranged largely between 6% and 60%. The only study that also considered a low number of healthy volunteers[7] reported a proportion of SIJ BME of 23.4%, which is comparable to our study. On the other hand, the rates of volunteers presenting with VC BME or FL are different from data reported in another study, also with low numbers of volunteers with spinal MRIs,[6] with rates of 43.8% of subjects showing ≥1 VC BME and 65% of subjects showing ≥1 VC FL. Overall, our data suggest that the sensitivity of MRI to detect minor changes is high and that caution is needed to take 'a positive MRI' as proof for a patient having axSpA. This statement holds for both, diagnosis and classification of axSpA. Importantly, the quantification of lesions based on the Berlin MRI score for the SIJ and the spine indicated a rather low extent of the detected BME lesions, with median scores around zero for BME at both examined sites of the axial skeleton. This finding is consistent with recent data showing that 'deep' lesions (defined as signal ≥1 cm from the articular surface) were almost exclusively found in axSpA patients but not in other conditions.[7] Our large population-based sample confirms this result for the SIJ and extends it to BME in the spine. Taken together, these findings are important for the diagnosis and classification of axSpA.

One other important finding of our study is the dependency of spinal MRI lesions to age. Both, VC BME and FL, were more frequent in older individuals—even below the age of 45 years. Physical activity had no impact on the frequency of BME or FL. Thus, minor inflammatory MRI changes may occur in individuals with no evidence of an inflammatory condition. This has already guided us to challenge the specificity of small MRI signals at the SIJ, the spine and the entheses for axSpA.[16]

Whether the occurrence of the described MRI signals can be attributed to mechanical stress needs further study. In that regard, one recent study has clearly shown that degenerative changes in the axial skeleton are associated with pain, also in patients with axSpA.[17] Small changes may just disappear once the mechanical load is gone. If mechanical stress continues to be present, this will probably be different. It seems very likely that the cause of the detected MRI changes is of mechanical nature and that the immune system has no or a minor role in that process. To our opinion, these MRI signals are early degenerative changes which potentially lead to osteoarthritis later in life. According to two recent publications, there is no doubt that degenerative changes, potentially in addition to BME, are already present in young patients with and without axSpA.[18 19]

Another result of this study worth mentioning is the distribution of VC BME and FL in the spine. The majority of lesions were observed in the lower part of the thoracic spine. This pattern of involvement confirms earlier data spinal MRIs of patients with radiographic axSpA from our group[20] and also recent CT data[21] and is also in line with the well-known higher mechanical load of this spinal area in other conditions, as compared with the cervical or the lumbar spine alone.[22]

Since all MRIs were performed in the same centre, under the same standardised protocol and with the same device, there is no reason to consider methodologic variability as a limitation of our study.

A limitation of our study may be that the participants were volunteers from the general population, and potential selection bias might have occurred. However, another population-based study gives evidence of a rather small impact of initial non-response and attrition on back pain-related point estimates.[23] In line with this, the reported back pain in the past 7 days in our sample was 41%. This is not much different from epidemiologic data provided in recent reviews.[19 24] Furthermore, HLA-B27 frequency in our cohort is in line with other population-based results.[25]

Since many axSpA patients are not diagnosed or diagnosed late,[26] we cannot exclude that there were axSpA patients in our cohort. With an estimated prevalence of about 1%,[1] it is possible that about 6–10 individuals in this study could indeed have (yet undiagnosed) axSpA. It is, for example, possible that those subjects with a lot of MRI changes or more HLA-B27 positives with chronic back pain did have the disease. Furthermore, the fact that we are currently unable to provide any follow-up information of the study volunteers may be considered as a limitation. However, this was currently not the target of the analyses presented here. Such data would be interesting for the understanding of the natural course of the described lesions and the possible development of defined inflammatory rheumatic conditions. Finally, an important limitation of the study is also the fact that the MRI sequences available in the cohort were not the ones ideally recommended by ASAS. ASAS recommends that sequences designed to identify inflammation or depict structural damage are simultaneously reviewed—this was, for technical reasons, not done in this study. For the SIJ, we had only STIR but no T1 images available. Nevertheless, since we were looking for clear lesions of BME, we do not think that missing the information on T1 images has a significant impact on the results, but it may even have led to an underestimation of 'positive' images. On the other hand, for spinal MRIs, we only had T1 and T2 but no STIR images available. Although it is not stated explicitly

in the ASAS paper on descriptions of spinal MRI lesions,[11] the vast majority of the studies have been made by using the STIR sequence for the detection of inflammatory lesions, due to the high sensitivity of this sequence.[27] In the present study, we evaluated inflammatory lesions based on the occurrence of high-intensity signal on the available T2-weighted sequences and only if the corresponding area on T1-weighted sequences was showing a hypointense signal. Similar to the SIJ, this might have influenced our data towards a rather lower prevalence of lesions due to the missing, sensitive, STIR lesion, but on the other hand, may have also led to a more specific evaluation of the pathologic findings.

In conclusion, in this first large population-based study, a high frequency of inflammatory and fatty vertebral corner lesions and inflammatory SIJ MRI lesions suggestive of axSpA has been found. These data suggest that the current definition of MRI changes used for the classification of axSpA requires an update. A small size and a small number of MRI signals detected in the axial skeleton of patients under suspicion of axSpA is inconclusive for diagnosis or classification of axSpA, while higher cut-offs may be more adequate for assessment of positive axSpA-related MRI findings. Finally, such MRI changes tend to occur more frequently in individuals of higher age groups, suggesting the influence of a mechanical factor and potential development of osteoarthritis.

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REFERENCES

1 Braun J, Sieper J. Ankylosing spondylitis. *Lancet* 2007;369:1379–90.
2 Rudwaleit M, van der Heijde D, Landewé R, *et al*. The development of assessment of spondyloarthritis International Society classification criteria for axial spondyloarthritis (Part II): validation and final selection. *Ann Rheum Dis* 2009;68:777–83.
3 Lambert RGW, Bakker PAC, van der Heijde D, *et al*. Defining active sacroiliitis on MRI for classification of axial spondyloarthritis: update by the ASAS MRI Working group. *Ann Rheum Dis* 2016;75:1958–63.
4 Rudwaleit M, Jurik AG, Hermann K-GA, *et al*. Defining active sacroiliitis on magnetic resonance imaging (MRI) for classification of axial spondyloarthritis: a consensual approach by the ASAS/OMERACT MRI group. *Ann Rheum Dis* 2009;68:1520–7.
5 Baraliakos X, Heldmann F, Callhoff J, *et al*. Which spinal lesions are associated with new bone formation in patients with ankylosing spondylitis treated with anti-TNF agents? a long-term observational study using MRI and conventional radiography. *Ann Rheum Dis* 2014;73:1819–25.
6 Weber U, Zhao Z, Rufibach K, *et al*. Diagnostic utility of candidate definitions for demonstrating axial spondyloarthritis on magnetic resonance imaging of the spine. *Arthritis Rheumatol* 2015;67:924–33.
7 de Winter J, de Hooge M, van de Sande M, *et al*. Magnetic resonance imaging of the Sacroiliac joints indicating sacroiliitis according to the assessment of spondyloarthritis International Society definition in healthy individuals, runners, and women with postpartum back pain. *Arthritis Rheumatol* 2018;70:1042–8.
8 Völzke H, Alte D, Schmidt CO, *et al*. Cohort profile: the study of health in Pomerania. *Int J Epidemiol* 2011;40:294–307.
9 Ittermann T, Wittfeld K, Nauck M, *et al*. High thyrotropin is associated with reduced hippocampal volume in a population-based study from Germany. *Thyroid* 2018;28:1434–42.
10 Hegenscheid K, Kühn JP, Völzke H, *et al*. Whole-Body magnetic resonance imaging of healthy volunteers: pilot study results from the population-based SHIP study. *Rofo* 2009;181:748–59.
11 Hermann K-GA, Baraliakos X, van der Heijde DMFM, *et al*. Descriptions of spinal MRI lesions and definition of a positive MRI of the spine in axial spondyloarthritis: a consensual approach by the ASAS/OMERACT MRI study Group. *Ann Rheum Dis* 2012;71:1278–88.
12 Sieper J, Rudwaleit M, Baraliakos X, *et al*. The assessment of spondyloarthritis International Society (ASAS) Handbook: a guide to assess spondyloarthritis. *Ann Rheum Dis* 2009;68 Suppl 2:ii1–44.
13 Haibel H, Rudwaleit M, Brandt HC, *et al*. Adalimumab reduces spinal symptoms in active ankylosing spondylitis: clinical and magnetic resonance imaging results of a fifty-two-week open-label trial. *Arthritis Rheum* 2006;54:678–81.
14 Weber U, Jurik AG, Zejden A, *et al*. Frequency and Anatomic Distribution of Magnetic Resonance Imaging Features in the Sacroiliac Joints of Young Athletes: Exploring "Background Noise" Toward a Data-Driven Definition of Sacroiliitis in Early Spondyloarthritis. *Arthritis Rheumatol* 2018;70:736–45.
15 Varkas G, de Hooge M, Renson T, *et al*. Effect of mechanical stress on magnetic resonance imaging of the sacroiliac joints: assessment of military recruits by magnetic resonance imaging study. *Rheumatology* 2018;57:508–13.
16 Braun J, Baraliakos X. Active and chronic sacroiliitis spondylitis and enthesitis, how specific are imaging findings for axial spondyloarthritis? *Rheumatology* 2018.
17 de Hooge M, de Bruin F, de Beer L, *et al*. Is the site of back pain related to the location of magnetic resonance imaging lesions in patients with chronic back pain? results from the spondyloarthritis caught early cohort. *Arthritis Care Res* 2017;69:717–23.
18 de Bruin F, ter Horst S, Bloem HL, *et al*. Prevalence of degenerative changes of the spine on magnetic resonance images and radiographs in patients aged 16-45 years with chronic back pain of short duration in the spondyloarthritis caught early (space) cohort. *Rheumatology* 2016;55:56–65.
19 de Bruin F, Treyvaud MO, Feydy A, *et al*. Prevalence of degenerative changes and overlap with spondyloarthritis-associated lesions in the spine of patients from the DESIR cohort. *RMD Open* 2018;4:e000657.
20 Baraliakos X, Landewé R, Hermann K-G, *et al*. Inflammation in ankylosing spondylitis: a systematic description of the extent and frequency of acute spinal changes using magnetic resonance imaging. *Ann Rheum Dis* 2005;64:730–4.
21 de Koning A, de Bruin F, van den Berg R, *et al*. Low-Dose CT detects more progression of bone formation in comparison to conventional radiography in patients with ankylosing spondylitis: results from the SIAS cohort. *Ann Rheum Dis* 2018;77:293–9.
22 Qiu T-X, Teo E-C, Lee K-K, *et al*. Validation of T10-T11 finite element model and determination of instantaneous axes of rotations in three anatomical planes. *Spine* 2003;28:2694–9.
23 Schmidt CO, Raspe H, Pfingsten M, *et al*. Does attrition bias longitudinal population-based studies on back pain? *Eur J Pain* 2011;15:84–91.
24 Hoy D, Brooks P, Blyth F, *et al*. The epidemiology of low back pain. *Best Pract Res Clin Rheumatol* 2010;24:769–81.
25 Braun J, Bollow M, Remlinger G, *et al*. Prevalence of spondylarthropathies in HLA-B27 positive and negative blood donors. *Arthritis Rheum* 1998;41:58–67.
26 Regierer AC, Weiß A, Baraliakos X, *et al*. [RABBIT-SpA: a new disease register for axial spondyloarthritis and psoriatic arthritis]. *Z Rheumatol* 2019. doi:10.1007/s00393-019-0613-z
27 Baraliakos X, Hermann K-GA, Landewé R, *et al*. Assessment of acute spinal inflammation in patients with ankylosing spondylitis by magnetic resonance imaging: a comparison between contrast enhanced T1 and short tau inversion recovery (stir) sequences. *Ann Rheum Dis* 2005;64:1141–4.

CLINICAL SCIENCE

Development of ASAS quality standards to improve the quality of health and care services for patients with axial spondyloarthritis

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ABSTRACT:

Objectives The Assessment of SpondyloArthritis International Society (ASAS) aimed to develop a set of quality standards (QS) to help improve the quality of healthcare provided to adult patients affected by axial spondyloarthritis (axSpA) worldwide.

Methods An ASAS task force developed a set of QS using a stepwise approach. First, key areas for quality improvement were identified, discussed, rated and agreed on. Thereafter, areas were prioritised and statements for the most important key areas were phrased on consensus. Appropriate quality measures were defined to allow quantification of the QS at the community level.

Results The ASAS task force, consisting of 20 rheumatologists, two physiotherapists and two patients, selected and proposed 34 potential key areas for quality improvement which were then commented by 140 ASAS members and patients. Within that process three new key areas came up, which led to a re-evaluation of all 37 key areas by 120 ASAS members and patients. Five key areas were identified as most important to determine quality of care: referral including rapid access, rheumatology assessment, treatment, education/self-management and comorbidities. Finally, nine QS were agreed on and endorsed by the whole ASAS membership.

Conclusions ASAS successfully developed the first set of QS to help improving healthcare for adult patients with axSpA. Even though it may currently not be realistic to achieve the QS in all healthcare systems, they provide high-quality of care framework for patients with axSpA that should be aimed for.

Key messages

What is already known about this subject?

- Wide variation in the delivery and quality of healthcare for patients with axial spondyloarthritis (axSpA) exists.
- Provision of measurable constructs can help to identify key gaps in the current provision care at the community level
- Quality assessment tools have been published for several rheumatological conditions; none relate specifically to patients with axSpA.

What does this study add?

- Assessment of SpondyloArthritis International Society (ASAS) developed the first set of quality standards to help improving health care for adult patients with axSpA.
- Quality standards have been formulated for key area of referral, rheumatology assessment, treatment, education/self-management and comorbidities.

How might this impact on clinical practice or future developments?

- ASAS quality standards provide high-quality of care framework for patients with axSpA intending to help organisations improve quality of care and to monitor service improvements.
- ASAS quality standards are achievable in daily care in an optimised situation and intend to minimise variation in quality of care.

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INTRODUCTION

Axial spondyloarthritis (axSpA) is a chronic inflammatory disease of the axial skeleton with inflammatory back pain as the major symptom, and spinal/sacroiliac joint inflammation and new bone formation as the most pathognomonic features.[1] There is wide variation in the delivery and quality of healthcare for patients with axSpA. The mission of the Assessment of SpondyloArthritis International Society (ASAS) as an international group of experts in the field of spondyloarthritis (SpA) is to support and promote the study of axial and peripheral SpA, to increase awareness and early diagnosis of the disease, to develop and validate assessment tools, and to evaluate treatment modalities in order to promote clinical research with the ultimate goal to improve outcome of the disease (ASAS website: www.asas-group.org). Several unmet needs such as delayed diagnosis and restricted access to treatment have been described in many countries

worldwide.[2 3] These and other gaps in current care provision prompted ASAS in 2016 to start developing a set of quality standards (ASAS-QS) to help optimise access, treatment and patient outcomes in axSpA. Although the diagnostic delay has somewhat decreased in recent years, this is still significant when compared with rheumatoid or psoriatic arthritis.[4 5] Furthermore, the availability and quality of health and care provision across rheumatological diseases varies worldwide due to different economic and political realities and healthcare systems.[3] Thus, assessing the quality of care provided to patients with axSpA is important not only to patients and physicians, but also to providers and purchasers of healthcare.[6]

There is no agreed methodology to quantify quality of care. According to the US Institute of Medicine quality measures assess "the degree to which health services for individuals and populations increase the likelihood of desired health outcomes and are consistent with current professional knowledge".[7] Different validated measurement sets such as quality indicators, performance measures or quality standards (QS) have been suggested to establish measurable constructs.[8 9] To date, quality assessment tools have been published for several rheumatological conditions like inflammatory arthritis, rheumatoid arthritis and gout,[10–12] but none of these relate specifically to patients with axSpA.

The UK based National Institute of Clinical Excellence (NICE) has a long trajectory of developing QS defined as a set of statements to help improve quality of health and care services and very recently published a quality assessment tool for patients with axSpA.[13] The different measurement sets relate to measurable aspects of healthcare, and in contrast to guidelines or recommendations, the measurement sets do not reflect all stages and levels of disease status.

ASAS decided to choose QS as quality assessment tool, based on the methodology used by NICE. NICE specifies QS should be developed to cover areas where there is variation of care and identify resources and processes which need to be optimised in order to achieve quality improvement.[14] QS offer the opportunity to define measurable constructs which can be operationalised in structural or process related characteristics of healthcare as defined by Donabedian.[15] Donabedian's classical theoretical framework for measuring healthcare quality includes three related categories of which the first two, that is, structures (innate characteristics of providers and the system), and processes (what healthcare providers do in delivering care), influence the third category: outcomes (what happens to patients, particularly with respect to their health).

Improvement in quality of care should focus on those areas in which there is evidence of variation in the delivery of care. Those key areas for quality improvement should identify key requirements for high-quality care or service provision that are expected to contribute to improving the experience of care or services as well as their safety and effectiveness. However, topics have to be balanced between ideal settings and actual clinical practice settings and QS should therefore describe enhanced practice, which is aspirational but achievable in daily care.

Thus, our objective was to develop international QS to improve the quality of care for adult patients with axSpA by identification of key gaps in the current provision care at the community level.

METHOD

First, in order to operate its QS programme, ASAS nominated two groups: a Steering Committee (JB, UK, RL, MR, DvdH, MW) and a QS group tasked with the development of an ASAS-QS set. This QS group was formed by ASAS members proposed by the ASAS Executive Committee, based on their clinical and research experience in outcome and management of SpA. The ASAS-QS group decided to (1) invite additional patients and healthcare professionals in order to meet priorities and to guarantee representativeness of the group and (2) to utilise the pre-existing ASAS recommendations for early referral for patients with a suspicion of axSpA and ASAS recommendations for the management of axSpA as scientific key source guidance.[16 17] All ASAS members are actively involved in scientific projects and ASAS members are referred here as 'ASAS community'.

The ASAS-QS group used a stepwise approach to develop a disease-specific set of QS for adult patients with axSpA (figure 1). ASAS decided to base any subsequent methodological approach on NICE quality standard process guide excluding any reference or involvement to payers of the health system as this differs widely across countries.[14] In order to meet stakeholder priorities, ASAS members were asked twice to identify key areas for quality improvement in an attempt to understand all relevant medical needs and gaps at a national level.

Each quality standard consists of two components: a quality statement and a quality measure. A quality standard set addresses key areas for quality of care improvement by providing specific, clear, concise, and measurable statements that are derived from evidence-based guidance. Each quality statement is accompanied by a quality measure which is meant to quantify the quality of care or service provision specified in the statement by providing a numerator and a denominator. Further, each quality standard is accompanied by a rationale providing the scientific evidence and the guidance and definitions of the terms used for each specific quality statement.

The following steps were undertaken to develop the ASAS-QS for adult patients with axSpA:

1. *Provisional list of key areas for quality improvement*: ASAS-QS group members convened at a face-to-face 1-day meeting to set up the remit of the work in Berlin in January 2016. After an extensive open discussion, it was decided to use a stepwise approach by starting with the identification of possible key areas for quality improvement. This should be areas in which variation in care exists but which can be improved and which are measurable and achievable. Key areas for quality improvement were grouped into the categories structure, process and outcomes to help guiding the subsequent steps.
2. *Evaluation of the provisional list of key areas for quality improvement*: During summer 2016, ASAS members and patient representatives were invited via a web-based survey to comment on this provisional list and to identify additional key areas for quality improvement not mentioned before in the provisional list. Patient representatives were invited via the national patient organisation. Participants were asked to agree or disagree to each single QS item. ASAS-QS group agreed that a key area for quality improvement would be considered as important when ≥25% of the participants—independent of being patient or professional—agreed to the key areas for quality improvement. Participants received background information on the definition and process development of QS prior to starting the survey to reduce probability of misinterpretation or misunderstanding. ASAS-QS group and two patient representatives met to discuss the findings of the web-based survey in a face-to-face meeting (September 2016).
3. *Prioritisation of key areas for quality improvement:* All participants who completed step 2 were then invited to actively contribute to the development process and to comment on

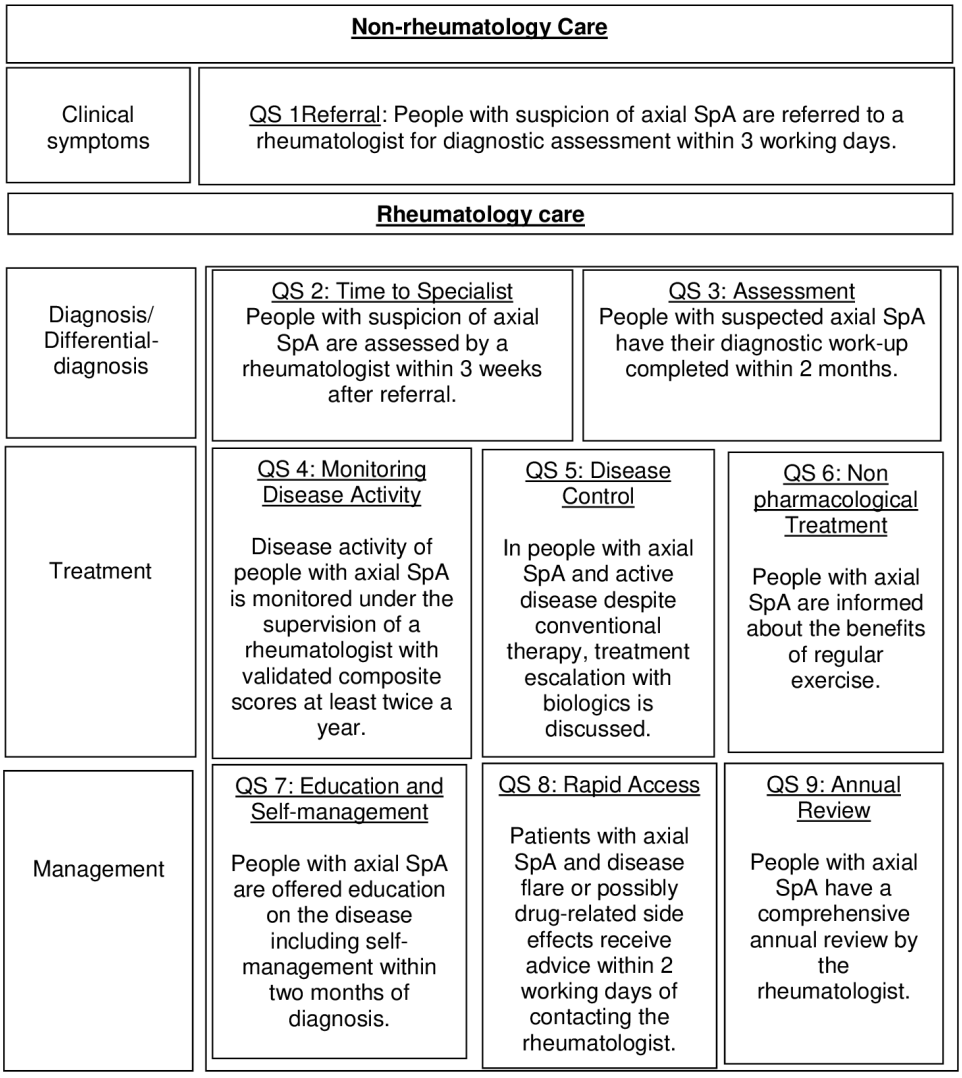

Figure 1 Summary of the nine Assessment of SpondyloArthritis International Society quality statements. SpA, spondyloarthritis.

the complete list of key areas for quality improvement via a web-based survey. They received background information on QS prior to starting the survey. Participants were then asked to prioritise key areas for quality improvement by indicating level of importance on a numerical rating scale (NRS) 0 (not important at all) to 10 (very important). A threshold of 75% of participants reaching ≥8 on NRS—independent of being patient or professional—was considered as reflecting an important key area for quality improvement. As there was no guidance for a cut-off in the literature, the steering group decided to use a strict cut-off for agreement to ensure a select decisions made based on wide agreement.

4. *Identification of final key areas for quality improvement:* ASAS-QS members discussed in a 1-day meeting (January 2017) the further methodology. As part of this process, the most important key areas for quality improvement from step 3 were regrouped into domains for which quality statements and measures should be developed. The ASAS-QS group was then asked to propose phrasing of important QS.
5. *Phrasing of QS:* ASAS-QS members drafted a statement, rationale and measure for selected key areas in a 1-day meeting (January 2018). If necessary, aspects were phrased in more than one statement. Phrasing of the QS was influenced by proposals of the ASAS community.
6. *Voting on ASAS-QS:* ASAS-QS group presented the ASAS-QS set to the ASAS community in January 2018 and discussed the content, applicability and implementation of the final ASAS-QS set. Subsequent to the meeting the ASAS members were again asked to give the level of agreement on an NRS 0 (I do not agree at all) to 10 (I fully agree) in a web-based survey. A threshold of 75% of participants reaching ≥7 on NRS was needed to be agreed by the ASAS community.
   a. *Re-evaluation on QS 1 and 2:* QS 1 and 2 provoked disagreement and required further discussions. Therefore, the ASAS-QS group provided background information and education sessions on meaning and intention of QS and discussed rephrasing of final ASAS-QS set. ASAS members were invited in November 2018 to comment on QS 1 and 2 in a web-based survey by answering the following questions: "I agree to the phrasing of QS 1 (2, respectively) and do not wish a change of this quality statement". Participants who affirmed the question were asked to rate the level of agreement on an NRS 0 (I do not agree at all) to 10 (I fully agree). Participants who denied the question were asked to share thoughts about QS 1 (2, respectively) and to specify disagreement.
7. *Endorsement by the ASAS membership:* 'Pro' and 'con' arguments to QS 1 and 2 were exchanged during the annual

ASAS meeting in January 2019 in Amsterdam. After extensive discussion, a simple vote for agreement/disagreement of QS 1 and 2 was taken from ASAS members.

RESULTS

The ASAS-QS group consists of 20 rheumatologists, two physiotherapists and two patient research partners.

1. *Provisional list with key areas for quality improvement:* The ASAS-QS group developed in January 2016 (Berlin) a list with 34 potential key areas for quality improvement (online supplementary file 1). Key areas were grouped into the categories structure (n=7), process (n=23) and outcome (n=4).
2. *Evaluation of the provisional list of key areas for quality improvement:* 140 participants (86 ASAS members and 42 axSpA patients from 10 countries (Belgium, France, Germany, Israel, the Netherlands, Portugal, Spain, Turkey, UK, USA), 12 participants did not provide demographics, see online supplementary file 2) evaluated the provisional list of key areas for quality improvement in summer 2016 via a web-based survey. Five new key areas for quality improvement were proposed (one item for the category structure (structural support for physiotherapist-led exercise), two items for the category process (extra-articular manifestation and assessment of current treatment), and two items for the category outcome (percentage of patients who improved in mobility as measured by physiotherapists tests and percentage of hospital admissions for complicated disease)). The rate of misunderstanding was low, in most cases <5%, except for comorbidities (8.6%), morphometric assessment (26.4%), corrective osteotomy (15.0%), and total arthroplasty (13.6%). All key areas for quality improvement initially proposed by the group reached the threshold of 25% agreement (mean agreement to the categories structure 83.8%, process 79.5% and outcome 56.8%. Agreement with each single key area is shown in online supplementary file 1. The results of the survey were presented to the ASAS-QS group and two patient representatives at a meeting in Ghent in September 2016 where future steps regarding the prioritisation of key areas for quality improvement and content validity of possible QS were extensively discussed.
3. Prioritisation of key areas for quality improvement: In autumn 2016, 120 participants (86 physicians, 29 patients, five participants did not provide demographics, see online supplementary file 2) prioritised in a web-based survey 39 key areas for quality improvement, across three categories structure, process and outcome (see online supplementary file 2). Key areas were prioritised between 4.5 and 8.6 (see online supplementary file 1). Five key areas were rated as most important: timely diagnosis, documentation of diagnosis, patient information, assessment of disease activity, and assessment of infection risks when starting biologicals.
4. *Identification of final key areas for quality improvement:* ASAS-QS group met in January 2017 in Leeds and decided to omit the strict segmentation in structure, process and outcome and rather phrase QS which may cover more than one of the categories structure, process and outcome. ASAS QS grouped selected key areas into superordinate domains: referral including rapid access, rheumatology assessment, treatment, education including self-management and comorbidities. The ASAS community provided detailed proposals about phrasing of most important domains.
5. *Phrasing of QS:* the ASAS-QS group drafted nine QS for axSpA encompassing a statement and a rationale, in January 2018 in Lisbon (tables 1–3). Since the identified key areas represent domains which can be applied in a wide context, it was necessary to think about their application in different clinical settings leading to more than one QS for one key area. Phrasing of QS was influenced by proposals of the ASAS community from step 4. Statements were provided for aspects of referral and rapid access (n=4), rheumatology assessment (n=1), treatment (n=2), education (n=1) and comorbidities (n=1). Whenever timelines were mentioned, timelines were developed by consensus within the ASAS-QS group. The timelines were not data driven and represent the 'aspirational but achievable' aspect of the ASAS-QS which are presumably related to different national perception.
6. *Voting on ASAS-QS:* the proposed ASAS-QS were presented to the membership at the annual ASAS meeting in Lisbon in January 2018. At this meeting, the ASAS community intensively discussed the content, applicability and implementation of the final ASAS-QS set. One item in particular, the timeframes given in QS 1 and 2 was a matter of debate. Subsequent, voting in August 2018 among 115 ASAS members showed (too) low agreement for QS 1 and 2, whereas agreement was substantially higher for QS 3–9 (tables 1–3).
    a. *Re-evaluation of the final set of ASAS-QS:* During August and November 2018, the ASAS-QS group provided background information and education sessions on meaning and intention of QS in general to ASAS community. In November 2018, 73 ASAS members provided feedback to QS 1 and 2. Disagreement to QS 1 was raised by 24 members of the ASAS community (32.9%) whereas agreement to QS 1 was stated by 49 members (67.1%). Agreement was documented by a high value of mean level of agreement (NRS 8.1 (1.9), ≥7:92.1%). Disagreement to QS 2 was raised by 18 members (25%) whereas agreement to QS 2 was stated by 54 members (75%) (one missing response). Agreement was documented by a high value of mean level of agreement (NRS 7.9 (1.9), ≥7:87.1%). Arguments for disagreement of QS 1 were based on defined timeframe of three working days ('unrealistic setting'), exertion of rheumatologists on decisions made in general practitioner (GP) area, and unclear definition of the term 'suspicion of axSpA'. Arguments for disagreement of QS 2 were based on the defined timeframe of 3 weeks and lack of possibility of rheumatologist to intervene with a centralised appointment system.
7. *Endorsement:* QS 1 and 2 were discussed at the ASAS annual meeting in Amsterdam in January 2019. After extensive discussion, it was decided not to change the initially proposed phrasing of QS 1 and 2 and 98 ASAS members voted to retain QS 1 and 2 unchanged (QS 1: 77 approval, 10 decline, 0 abstention; QS 2: 72 approval, 11 decline, four abstention) (table 1).

Quality measures were drafted after the wording of the quality statements was agreed by the ASAS-QS group. All quality measures related to processes are expressed as a numerator and a denominator to define a proportion (numerator/denominator) (tables 1–3).

DISCUSSION

ASAS successfully developed the first QS set for the improvement of the quality of health and care services provided to adults with axSpA. These QS include a clear description of high priority areas for quality improvement and monitoring. Significant differences in the availability and quality of healthcare may

Table 1 Quality standards (QS) for axial spondyloarthritis, clinical symptoms and diagnosis

No	Domain	Statement	Rationale	Quality measure, category structure	Quality measure, category process, numerator	Quality measure, category process, denominator	Level of agreement, NRS 0–10	Agreement (NRS ≥7 by 75% of ASAS members)
QS1	Referral	Patients with suspicion of axSpA are referred to a rheumatologist for diagnostic assessment within three working days	When axSpA is suspected, ASAS recommendations for the early referral of patients with a clinical suspicion of axSpA provide criteria for deciding whether the patient should be referred to rheumatology for special diagnostic assessment. AxSpA is often missed in non-specialist settings, resulting in substantial delays in diagnosis and treatment. No single test has been shown to have sufficient sensitivity or specificity to diagnose axSpA. Timeframe of three working days is expert-driven intending to trigger immediate referrals.	Evidence of local arrangements (including local arrangements to raise awareness of signs and symptoms of axSpA) and written protocols to ensure that patients with suspicion for axSpA are referred to rheumatology within three working days.	The number of patients with a suspicion of axSpA that is referred to rheumatology within three working days.	The number of patients with a suspicion of axSpA.	6.0±3.1 second vote: 88.5%.	47.8
QS2	Time to specialist	Patients with suspicion of axSpA are assessed by a rheumatologist within 3 weeks after referral	Rapid referral of patients with suspicion of axSpA is important to avoid delay in diagnosis and increase the likelihood of early treatment initiation. A rheumatologist (which implies the rheumatology team including physicians, nurses, and other health professionals) is able to identify axial and peripheral manifestations as well as extra-articular manifestations and comorbidities. Given the potentially detrimental effects of delayed diagnosis, patients with these symptoms and signs are in need of a first appointment within 3 weeks. Timeframe is expert-driven intending to trigger timely appointments. Timeframe of 3 weeks refers to a first appointment. Additional examinations required for decision-making process can follow after the first appointment.	Evidence of local arrangements including sufficient number of rheumatologists to ensure that patients with suspicion of axSpA can be seen by a rheumatology specialist within 3 weeks after referral.	The number of patients with a suspicion of axSpA that is assessed by a rheumatologist within 3 weeks after referral.	The number of patients with suspicion of axSpA referred to a rheumatologist.	7.2±2.5 second vote: 86.7%.	69.6
QS3	Assessment	Patients with suspicion of axSpA have their diagnostic work-up completed within 2 months.	Timely diagnostic work-up by a rheumatologist is needed to ensure correct diagnosis and to achieve better long-term outcomes and improve their quality of life. Diagnostic work-up includes identification of SpA variables, laboratory and imaging results. Diagnostic work-up should be completed within 2 months after first appointment.	Evidence of local arrangements including sufficient number of rheumatologists and facilities and access to facilities in the given timeframe to ensure that patients with suspicion of axSpA have a diagnostic work-up within 2 months after first appointment by a rheumatologist.	The number of patients with a suspicion of axSpA, in whom a diagnostic work up was completed within 2 months after first appointment.	The number of patients with suspicion of axSpA seen for the first time by the rheumatologist more than 2 months ago.	8.5±2.0	89.6

ASAS, Assessment of SpondyloArthritis International Society; axSpA, axial spondyloarthritis; NRS, numerical rating scale.

exist within each country and between different countries, and the ASAS-QS may serve as a tool for assessing, delivering and demanding optimal care for patients with axSpA in any country. QS are intended to help organisations improve quality of care and to monitor service improvements by supporting comparison of current performance. All ASAS-QS are achievable in daily care in an optimised situation and intend to minimise variation in quality of care. It is emphasised that ASAS is well aware that all QS are ideal visions of an optimal care provision which may currently not be realistic in many countries. ASAS-QS are aspirational but they may guide a wide range of purposes both locally and nationally. For example, people using services, care-providers and the public can use the QS to identify components of a high-quality service that is achievable.

Assessing quality of care provided to patients with rheumatic diseases is challenging because various areas need to be improved

Table 2 Quality standards (QS) for axial spondyloarthritis, treatment

No	Domain	Statement	Rationale	Quality measure, category structure	Quality measure, category process, numerator	Quality measure, category process, denominator	Level of agreement, NRS 0–10	Agreement (NRS ≥7 by 75% of ASAS members)
QS4	Monitoring	Disease activity of patients with aSpA is monitored under the supervision of a rheumatologist with validated composite scores at least every 6 months.	Assessment of disease activity is of importance because of the correlation between clinical disease activity and syndesmophyte formation and between disease activity, function and health-related quality of life. Monitoring of disease activity by a rheumatologist (which implies the rheumatology team including physicians, nurses, other health professionals) is required because of multifaceted and ambiguous clinical symptoms of disease activity such as pain and disability. Assessment of disease activity using ASDAS is recommended. Repeating the assessment at regular intervals will ensure that the treatment of patients with axSpA is adapted when they need it.	Evidence of local arrangements to ensure that patients with aSpA have an assessment with validated composite scores at least every 6 months.	The number of patients diagnosed with axSpA more than 6 months ago in whom disease activity was monitored with validated composite scores at least every 6 months.	The number of patients diagnosed with axSpA more than 6 months ago.	8.0±2.2	81.7
QS5	Disease control	In patients with axSpA and active disease despite conventional therapy, treatment escalation with biological drugs is discussed.	Treatment escalation is important to achieving disease control, which ideally results in remission or a low disease activity state, and therefore lower disease impact on functioning and everyday living. Patients who have high disease activity despite conventional therapy should discuss the use of biological drugs with their rheumatologist, taking patient profile, cost and access to biologicals into account. The 2016 update of the ASAS-EULAR management recommendations for axSpA provides criteria for recommending use of biologicals in patients with axial disease and high disease activity. The choice of intervention should be a joint decision between patient and rheumatologist.	Evidence of local arrangements to ensure that patients with axSpA and active disease despite conventional therapy are offered biologicals according to the ASAS recommendations to improve the chance of remission or low disease activity in the future.	The number of patients with axSpA and active disease despite conventional therapy in whom treatment with biologicals has been discussed.	The number of patients with axSpA and active disease despite conventional therapy.	9.2±1.5	94.8
QS6	Treatment, non-pharma	Patients with axSpA are informed about the benefits of regular exercise.	Physical activity should be an integral part of standard care throughout the course of disease in patients with axSpA. It is important that patients with axSpA are given information about benefits of regular exercise to reduce pain and stiffness and improve cardiorespiratory fitness and by doing so, also reducing the risk for cardiovascular disease. Actively raising the usefulness of exercising regularly will support patients in improving functioning and maintaining quality of life.	Evidence of local arrangements to encourage patients with axSpA to exercise on a regular basis.	The number of patients diagnosed with axSpA who are informed about the benefits of regular exercise.	The number of patients diagnosed with axSpA.	9.5±0.9	98.3

ASAS, Assessment of SpondyloArthritis International Society; ASDAS, Ankylosing Spondylitis Disease Activity Score; axSpA, axial spondyloarthritis; NRS, numerical rating scale.

worldwide. For example, the number of rheumatologists for a certain population, healthcare utilisation for groups of patients with rheumatological disorders and appropriate outcome measures are some of the many issues which are also relevant for patients with axSpA. Moreover, scientific evidence might be scarce for the identified key areas for quality improvement. This was the case for example, when phrasing the ASAS-QS topic 'referral'. Whereas the delay in diagnosis is clearly an important gap in daily care experienced by many patients with axSpA, the challenge faced by the ASAS-QS group was twofold: (1) systems of referral to specialists vary worldwide and (2) evidence for the optimal time period is lacking. However, the ASAS-QS group was convinced that given a specific timeframe instead of phrasing like 'timely or immediately' is needed to force substantial decrease in diagnostic delay. Moreover, the concept of QS requires the QS to be measurable (quantifiable).[15] Hence, the

Table 3 Quality standards (Qs) for axial spondyloarthritis, management

No	Domain	Statement	Rationale	Quality measure, category structure	Quality measure, category process, numerator	Quality measure, category process, denominator	Level of agreement, NRS 0–10	Agreement (NRS ≥7 by 75% of ASAS members)
QS7	Education and self-management	Patients with axSpA are offered education on the disease including self-management within 2 months of diagnosis.	Education is essential in enabling understanding and self-management of axSpA and reducing the risk of complications. It should start at diagnosis and continue throughout a patient's life. It is important that the patients learn how to manage their symptoms, reduce their pain and distress and improve their functioning and quality of life. Educational tasks should cover information about the disease, diagnostic utilities, treatment options including side effects, and a healthy lifestyle (physical activity and smoking cessation). Healthcare professionals can support the patient's ability to self-manage their condition by giving reassuring advice about the inflammatory cause and the risk of progressive disability of the condition, and the importance of an active lifestyle.	Evidence of local arrangements to ensure that health professionals have access to information and the knowledge needed to fully address educational needs of patients.	The number of patients diagnosed with axSpA who will have educational and self-management activities within 2 months of diagnosis.	The number of patients diagnosed with axSpA.	8.6±2.0	87.0
QS8	Rapid access	Patients with axSpA and disease flare or possibly drug-related side effects receive advice within two working days of contacting the rheumatologist.	Patients with axSpA may experience disease flares, pain intensification due to other causes or drug related side effects and may therefore have complex needs. Providing rapid access to a rheumatology service without delay maximises the impact on the person's quality of life, allowing them to continue with their usual activities and reduce the likelihood of harm from adverse events. Rapid access can be provided by all possible ways of contacting the rheumatologist (personal, by telephone or internet) (word rheumatologist implies the rheumatology team including physicians, nurses, and other health professionals).	Evidence of local arrangements to ensure that patients with axSpA receive advice within two working days of contacting the rheumatologist	The number of patients with axSpA, experiencing flares or potential side effects who contact the rheumatologist that received advice within two working day of contacting the rheumatologist	The number of patients diagnosed with axSpA.	7.8±2.4	90.0
QS9	Annual review	Patients with axSpA have a comprehensive annual review by the rheumatologist.	Annual review is important to ensure that all aspects of the disease are under control. It provides a regular opportunity to assess the patient in terms of current disease management, and any further support they may need in the future, in order to enable them to maximise their health, participation in society and life satisfaction. Focus should not only be on clinical symptoms and severity of disease but also on comorbidities like CV risk management or osteoporosis, employment, psychological factors, and life-style including physical activity. Applicability of areas covered should be individualised. A rheumatologist (which implies the rheumatology team including physicians, nurses, and other health professionals) is able to identify those aspects during an assessment and can refer to other specialty for investigations.	Evidence of local arrangements for patients with axSpA to have a comprehensive annual review that is coordinated by the rheumatology service.	The number of patients with axSpA diagnosed more than 1 year ago whose most recent comprehensive review was within 12 months of diagnosis or the previous review	The number of patients with axSpA diagnosed more than 1 year ago.	8.8±1.7	89.6

ASAS, Assessment of SpondyloArthritis International Society; axSpA, axial spondyloarthritis; CV, cardiovascular; NRS, numerical rating scale.

intention of the referral QS (QS1) is to reach high quality of care and therefore, enhance the practice of referrals to rheumatologists in patients with 'suspicion of axSpA' taking into account that rheumatologists are the medical specialists primarily responsible for diagnosing and treating patients with axSpA. There is ample evidence that recognition of axSpA can be optimised by an adequate preselection of patients to be referred to the rheumatologist based on combination of different parameters such as inflammatory back pain, HLA-B27 or sacroiliitis.[17] An obvious consideration is whether the ASAS-QS could be applied to other rheumatic diseases since many of the concepts utilised in these QS are relevant to other rheumatic conditions. However, three arguments may be against operationalisation of ASAS-QS into a different context: (1) axSpA is still less recognised than other inflammatory rheumatic diseases, has less recognisable features and takes longer time to diagnose, (2) ASAS is a group of experts in the field of spondyloarthritis and each step of the methodology relates explicitly to patients with SpA, and (3) the ASAS-QS group might not be representative for other rheumatic diseases. ASAS group apprehend that utilisation of disease-specific QS in a wider context might reduce the impact of such QS. Therefore, we suggest that ASAS-QS cannot be extrapolated to other rheumatic diseases directly, but may form a template for other diseases. QS are different from recommendations or guidelines. Recommendations imply evidence-based actions that should be done in order to optimally diagnose and treat the disease. Usually, every important aspect of the disease is covered. QS are measurable constructs relating to specific aspects of the disease where there is unwarranted performance variation at the community level. Moreover, quality standards are going beyond the intention of recommendations because they intend to measure improvement in quality of care. Yazdany *et al* showed in a community-based cohort that following systemic lupus erythematosus (SLE) quality measures was significantly protective against increased disease damage (Brief Index of Lupus Damage adjusted OR 0.4, 95% CI 0.4 to 0.7).[18]

Our experience in developing disease-specific QS within an international group of SpA experts showed that the endorsement of QS sets into national public health domains can be ambitious. After the approval of QS 1 (referral) and QS 2 (time to specialist) failed in the initial voting round by the ASAS community, intensive discussion, explanation and evaluation followed, resulting in an approval of the initial wording of QS 1 and 2. We learnt from that experience that implementation strategies must be accompanied at national levels by education about the meaning of QS in order to specify the intention of the QS: optimise quality of care at a community level instead of describing current practice of daily care.

In fact, implementation is a crucial aspect in the process of using the ASAS-QS at a national level. When implementing QS at a national level, several components such as data source, target population and reporting period have to be defined nationally prior to analysing QS in an individual country.[19] A separate project is usually necessary for a successful implementation. ASAS did not decide on specific implementation strategies but leave it up to the national ASAS members. A major strength of this ASAS initiative is the participation of SpA experts from all over the world including other health professionals (physiotherapists), and patients with axSpA. Thus, we think that inclusion of a variety of stakeholders adds to the representativeness of the ASAS-QS set. Another strength is the restriction to the most important areas in which variation in quality of care has been identified by the panel. Focusing on five key areas (referral, rheumatology assessment, treatment, education/self-management and comorbidities) increases the probability to induce a substantial improvement in quality of care. A limitation of our work is that we were not able to test the feasibility of the ASAS-QS in clinical practice worldwide. This is an important issue since previous research in the field of QS operationalised as indicators for rheumatoid arthritis showed that less than 50% of information was available for measuring quality indicators in registries.[20]

With the help of the ASAS-QS one can focus on which resources and processes are needed to deliver high quality of care at the community level, thereby reducing significant healthcare disparities among populations and across regions. As indicated earlier, NICE published recently a quality assessment tool for patients with axSpA which was developed in parallel to the ASAS-QS set.[13] None of the ASAS members participated in NICE guidance and no ideas were exchanged between both groups. Interestingly, areas addressed are quite similar and topics covered in both sets are the domains of referral and assessment as well as the importance of exercise and education about the disease.

The ASAS-QS set is not intended to replace other methods to improve quality of care. Furthermore, other methods, such as medical education, effective use of information technologies, and the development of evidence-based guidelines and practice recommendations, should complement the implementation of the ASAS-QS set. The proposed ASAS-QS for axSpA do not provide a comprehensive service specification. They rather define priority areas for quality of care improvement. The ASAS-QS should now be implemented at a national level for local quality improvement.

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REFERENCES

1 Sieper J, Poddubnyy D. Axial spondyloarthritis. *Lancet* 2017;390:73–84.
2 Putrik P, Ramiro S, Kvien TK, *et al*. Inequities in access to biologic and synthetic DMARDs across 46 European countries. *Ann Rheum Dis* 2014;73:198–206.
3 Nikiphorou E, van der Heijde D, Norton S, *et al*. Inequity in biological DMARD prescription for spondyloarthritis across the globe: results from the ASAS-COMOSPA study. *Ann Rheum Dis* 2018;77:405–11.
4 Sørensen J, Hetland ML, All Departments of Rheumatology in Denmark. Diagnostic delay in patients with rheumatoid arthritis, psoriatic arthritis and ankylosing spondylitis: results from the Danish nationwide DANBIO registry. *Ann Rheum Dis* 2015;74:e12.
5 Feldtkeller E, Khan MA, van der Heijde D, *et al*. Age at disease onset and diagnosis delay in HLA-B27 negative vs. positive patients with ankylosing spondylitis. *Rheumatol Int* 2003;23:61–6.
6 Mennini FS, Viti R, Marcellusi A, *et al*. Economic evaluation of spondyloarthritis: economic impact of diagnostic delay in Italy. *Clinicoecon Outcomes Res* 2018;10:45–51.
7 Initiative IHCQ. *Crossing the quality chasm*. The IOM Health Care Quality Initiative, 2006.
8 Solomon DH, Gabriel SE. Quality measures 101: what every rheumatologist should know. *Clin Exp Rheumatol* 200;25:18–21.
9 Wilson BA, Cooper M, Barber CEH. Standards of care for inflammatory arthritis: a literature review. *Semin Arthritis Rheum* 2017;47:22–8.
10 Barber CEH, Marshall DA, Mosher DP, *et al*. Development of system-level performance measures for evaluation of models of care for inflammatory arthritis in Canada. *J Rheumatol* 2016;43:530–40.
11 Petersson IF, Strömbeck B, Andersen L, *et al*. Development of healthcare quality indicators for rheumatoid arthritis in Europe: the eumusc.net project. *Ann Rheum Dis* 2014;73:906–8.
12 Mikuls TR, MacLean CH, Olivieri J, *et al*. Quality of care indicators for gout management. *Arthritis Rheum* 2004;50:937–43.
13 McAllister K, Goodson N, Warburton L, *et al*. Spondyloarthritis: diagnosis and management: summary of NICE guidance. *BMJ* 2017;356.
14 NIfHaCE. *Quality Standards process guide*, 2016.
15 Donabedian A. Methods for deriving criteria for assessing the quality of medical care. *Med Care Rev* 1980;37:653–98.
16 van der Heijde D, Ramiro S, Landewé R, *et al*. 2016 update of the ASAS-EULAR management recommendations for axial spondyloarthritis. *Ann Rheum Dis* 2017;76:978–91.
17 Poddubnyy D, van Tubergen A, Landewe R, *et al*. Assessment of spondyloarthritis international S. development of an ASAS-endorsed recommendation for the early referral of patients with a suspicion of axial spondyloarthritis. *Ann Rheum Dis* 2015;74:1483–7.
18 Yazdany J, Trupin L, Schmajuk G, *et al*. Quality of care in systemic lupus erythematosus: the association between process and outcome measures in the lupus outcomes study. *BMJ Qual Saf* 2014;23:659–66.
19 Suter LG, Barber CE, Herrin J, *et al*. American College of rheumatology white paper on performance outcome measures in rheumatology. *Arthritis Care Res* 2016;68:1390–401.
20 Navarro-Compán V, Smolen JS, Huizinga TWJ, *et al*. Quality indicators in rheumatoid arthritis: results from the Meteor database. *Rheumatology* 2015;54:1630–9.

EPIDEMIOLOGICAL SCIENCE

Determining factors related to poor quality of life in patients with axial spondyloarthritis: results from the British Society for Rheumatology Biologics Register (BSRBR-AS)

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ABSTRACT

Objective To determine modifiable factors associated with poor quality of life (QoL) in patients with axial spondyloarthritis (axSpA).

Methods Analysis of data from the British Society for Rheumatology Biologics Register in Ankylosing Spondylitis (BSRBR-AS) and validation of a previous model using data from 1810 patients with axSpA recruited during 2012–2017. Data collected included clinical and patient-reported measures. QoL was assessed using the Ankylosing Spondylitis Quality of Life (ASQoL) measure. Linear regression models predicting ASQoL scores were used first to validate a previous model from a national study, to extend this with additional information available in BSRBR-AS and finally to identify a 'de novo' model from BSRBR-AS of which factors impact on poor QoL.

Results Four out of five factors included in a previous model of poor QoL in patients with axSpA were confirmed: Bath Ankylosing Spondylitis Disease Activity Index and Bath Ankylosing Spondylitis Functional Index, fatigue and widespread pain, although the performance of the model was improved by the addition of measures of mood and sleep disturbance. In a de novo model in BSRBR-AS, there were six factors (other than disease activity and function) that predicted ASQoL: depression (β=0.16), sleep disturbance (β=0.08), activity impairment (β=0.04), fibromyalgia (Symptom Severity Scale (β=0.24) and Widespread Pain Index (β=0.10)) and tobacco smoking (β=0.66).

Conclusion This study confirms that poor QoL in patients with axSpA, in addition to high disease activity and poor function, is independently influenced by sleep disturbance, mood and widespread pain. These additional factors are not considered targets for treatment in current European League Against Rheumatism (EULAR) guidelines for managing the condition.

Key messages

What is already known about this subject?

- Maximising quality of life is the stated aim of EULAR management recommendations in axSpA, and the focus is on reducing disease activity and improving function.

What does this study add?

- Poor mental health, sleep problems, and widespread pain all additionally, independently, contribute to poor quality of life in patients with axSpA.

How might this impact on clinical practice or future developments?

- Management of axSpA needs to focus on a wider set of targets than disease activity and function.

INTRODUCTION

When treating people with axial spondyloarthritis (axSpA) and other inflammatory conditions, rheumatologists are focused on reducing disease activity and, in so doing, aim to reduce the impact which the disease has on their lives. The ultimate aim, however, is to improve patients' quality of life (QoL). Reducing disease activity is one way to do that, but there may be other factors not directly captured by disease activity measures, which impact on QoL. axSpA has an impact on people's working lives; mental health and physical health symptoms, such as pain and fatigue, have been shown to have an important influence on QoL.[1 2] Further, we (and others) have shown that pharmacological therapy targeted at reducing disease activity in inflammatory arthritis may have modest effects on aspects such as mental health,[3] fatigue[4] and work productivity.[5]

Previously, in an analysis of 959 patients from a national disease register (Scotland Registry for Ankylosing Spondylitis (SIRAS)), we have shown that five potentially modifiable factors predict poor QoL (using the Ankylosing Spondylitis Quality of Life Scale (ASQoL)[6]): high disease activity, poor physical function, fatigue, chronic widespread pain (CWP) and poor spinal mobility.[7] Of these factors, disease activity had the lowest (20%) population attributable risk for poor QoL. In addition, there were a number of nonmodifiable factors or at least not easily modifiable in the clinic, which were also related to poor QoL: female sex, fewer years of education, not in full-time employment, living in areas with higher deprivation, not being able to drive and history of peripheral joint involvement. We concluded that 'these findings provide evidence that in addition to traditional clinical targets…, focus on nonspecific symptoms (CWP and fatigue), perhaps with nonpharmacological therapies, may

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yield important improvements in QoL'. The positive predictive value for poor QoL varied from around 0% and 15% in those with one or two modifiable factors to around 60% and 80% in those with four or five such factors, respectively.

The aims of the current study, using a Great Britain-wide registry of axSpA (British Society for Rheumatology Biologics Register in Ankylosing Spondylitis (BSRBR-AS)), were (1) to validate the previous model of modifiable factors linked to poor QoL; (2) given that the BSRBR-AS has collected a wider set of variables, to determine whether these additional variables (related to mood and sleep disturbance) are independent in predicting poor QoL; and (3) using the BSRBR-AS to develop a model predicting poor QoL 'de novo', to determine how consistent are the factors that predict poor QoL across both populations.

METHODS

The BSRBR-AS is a prospective cohort study of people with axSpA. Patients were naïve to biological therapy on recruitment, but some were about to start such therapy (the biological cohort), while others continued on other therapy (non-biological cohort). The study protocol has been published previously.[8] Briefly, recruitment took place across 83 secondary care centres between December 2012 and December 2017, initially for those patients, aged at least 16 years, meeting the Assessment of Spondyloarthritis International Society (ASAS) imaging criteria for axSpA[9] or the modified New York (mNY) definition of ankylosing spondylitis.[10] From November 2014, those meeting the ASAS clinical criteria were also eligible. Clinical data were collected from medical notes, and patients completed questionnaires that were handed out in the clinic and could be completed there, or at home and posted back to the recruitment centre. For the current study, the data used were from the time of recruitment (for non-biological cohort) and just prior to commencing biological therapy (biological cohort), which was also mainly at the time of recruitment. QoL was assessed by ASQoL,[6] an 18-item questionnaire which gives a score between 0 (best QoL) and 18 (worst QoL).

Clinical information included extraspinal manifestations (uveitis, psoriasis, inflammatory bowel disease, enthesitis and dactylitis), inflammatory markers (C reactive protein), peripheral joint involvement, symptom duration, body mass index (BMI) and information on 14 comorbidities (related to cardiovascular, respiratory, gastrointestinal, renal and neurological conditions and cancer). Patient-reported measures included age, gender, level of education, employment status and lifestyle factors (tobacco smoking and alcohol intake), as well as Bath Ankylosing Spondylitis Disease Activity Index (BASDAI),[11] Bath Ankylosing Spondylitis Functional Index (BASFI)[12] and Bath Ankylosing Spondylitis Metrology Index (BASMI).[13] These Bath indices all produce scores ranging from 0 to 10 (least to most severe). Participant postcodes were used to determine a deprivation quintile, with reference to either the population of Scotland or England and Wales.[14–16] This ranged from 1 (most deprived) to 5 (least deprived). Mental health was assessed by the Hospital Anxiety and Depression Scale (HADS) scored from 0 (best) to 21 (worst).[17] Overall work impairment (including absenteeism, presenteeism) and other activity (non-work) impairment by the Work Productivity and Activity Impairment (WPAI) Specific Health Problem, all scored from 0% to 100%.[18 19] The Widespread Pain Index (WPI) (0–19) and Symptom Severity Scale (SSS) scores were assessed through the 2011 fibromyalgia 'research' criteria.[20] This was collected only among persons recruited from August 2015. Fatigue was collected through the Chalder Fatigue Scale (0–33)[21] and sleep disturbance by the Jenkins Sleep Evaluation Questionnaire (0–20),[22] with higher scores on each indicating worse state.

Patients attended meetings to identify priority areas for analysis.

Analysis

In validating the model linking modifiable factors to QoL previously reported by Dean *et al*,[7] the five modifiable factors reported (fatigue, BASFI, CWP, BASDAI and BASMI) were included in a multivariable linear regression model. The model was adjusted for non-modifiable factors associated with QoL on univariable analysis at $p \leq 0.2$. The current study did not have information available to determine the presence of CWP according to the American College of Rheumatology 1990 criteria for fibromyalgia[23] and instead used the WPI subscale of the 2011 'research' criteria for fibromyalgia.[20] Building on this five-factor model, the second analysis offered three additional modifiable factors (anxiety, depression and sleep disturbance), which were available in BSRBR-AS but had not been available within SIRAS. The stepwise selection therefore had eight candidate variables. Variables were entered into the model at $p \leq 0.1$ and excluded at $p \geq 0.15$. Adjustment for nonmodifiable factors was applied as previously. The third analysis was a multivariable linear regression model, de novo, with forward stepwise selection, using modifiable factors from BSRBR-AS with $p \leq 0.2$ from the univariable analysis. The model examined which factors, in addition to disease activity and function, influenced poor QoL but omitted the work productivity factors (absenteeism, presenteeism and work impairment), which were only available for employed participants. Adjustment for nonmodifiable factors was applied as previously. For each model, once the variables to be included were determined, the model was re-run using all the participants with data for the included variables (rather than only participants with data available for each of the candidate variables).

All statistical analysis was undertaken using STATA V.15 and on the August 2017 version of the BSRBR-AS dataset.

RESULTS

A total of 1810 participants were eligible for the current analyses. Approximately two-thirds were male (67%), their median age was 49 years (IQR 38–61), with a median time since symptom onset of 17 years (IQR 8–31) (table 1). Of those who had been tested, 80% were HLA-B27 positive. Most participants (67%) met the mNY criteria for ankylosing spondylitis (AS), an additional 29% fulfilled ASAS imaging criteria but not mNY, and 4% fulfilled only ASAS clinical criteria for axSpA. The median BASDAI and ASQoL scores were 4.8 (IQR 2.5–6.8) and 9 (IQR 3–13), respectively.

Factors associated with poor QoL

Among clinical factors, all extraspinal manifestations, with the exception of uveitis, were associated with poorer QoL (table 2). Higher BMI and a greater number of comorbidities were also associated with poorer QoL. Longer symptom duration, however, was associated with better QoL (β per year=−0.05, 95% CI −0.07 to −0.04). Most patient-reported factors demonstrated a relationship. Worse disease activity, function and metrology were significantly associated with poorer QoL (BASDAI β per unit increase=1.82, BASFI β=1.56, BASMI β=0.97). Females reported slightly poorer QoL (β=1.58, 95% CI 1.02 to 2.13), and there were important associations with low socioeconomic

Table 1 Characteristics of the British Society for Rheumatology Biologics Register in Ankylosing Spondylitis patients

Variables		n	% or median (IQR)*
Quality of life (ASQoL)	Continuous	1810	9 (3–13)
Clinical factors			
Symptom duration (years)	Continuous	1809	17.3 (7.6–30.8)
Uveitis	Not present	1364	76.0
	Present	431	24.0
Psoriasis	Not present	1598	89.0
	Present	197	11.0
Inflammatory bowel disease	Not present	1617	90.1
	Present	178	9.9
Enthesitis	Not present	1612	89.8
	Present	183	10.2
Peripheral joint disease	Not present	1477	82.3
	Present	318	17.7
Dactylitis	Not present	1726	96.2
	Present	69	3.8
Spinal mobility (BASMI)	Continuous	1340	3.8 (2.4–5.4)
Inflammation (C reactive protein) (mg/dL)	Continuous	1404	0.5 (0.2–2.0)
Body mass index (kg/m^2)	Continuous	1810	26.9 (23.9–30.8)
Number of comorbidities	Continuous	1788	0 (0, 1)
Patient-reported factors			
Disease activity (BASDAI)	Continuous	1785	4.8 (2.5–6.8)
Physical function (BASFI)	Continuous	1801	4.5 (2.0–7.0)
Age (years)	Continuous	1810	49.1 (37.6–60.8)
Gender	Male	1208	66.8
	Female	602	33.2
Education	Secondary school	583	32.5
	Apprenticeship	173	9.7
	Further education college	539	30.0
	University degree	354	19.7
	Further degree	146	8.1
Employment	Working full-time	870	48.2
	Working part-time	258	14.3
	Retired	318	17.6
	Retired early (ill health)	103	5.7
	Unemployed (ill health), not seeking work	164	9.1
	Other†	93	5.1
Deprivation, quintiles of the general population	1, most deprived	278	15.4
	2	313	17.3
	3	382	21.1
	4	430	23.8
	5, least deprived	407	22.5
Smoking status	Never	787	44.1
	Ex	664	37.2
	Current	334	18.7
Alcohol consumption	Never	122	6.8
	Ex	311	17.5
	Current	1350	75.7
Chalder fatigue	Continuous	1806	14 (11–19)
Symptom Severity Scale	Continuous	675	6 (3–8)
Widespread Pain Index	Continuous	863	4 (2–7)
Sleep disturbance (Jenkins)	Continuous	1796	10 (5–16)
Anxiety (HADS)	Continuous	1788	7 (4–11)
Depression (HADS)	Continuous	1787	5 (2–9)
Absenteeism (WPAI) (%)	Continuous	1011	0 (0–0)
Presenteeism (WPAI) (%)	Continuous	1015	20 (10–50)
Work impairment (WPAI) (%)	Continuous	987	30 (10–50)
Activity (non-work) impairment (%)	Continuous	1774	40 (20–70)

Jenkins indicates the Jenkins Scale for Sleep Disturbance.
*% given for discrete variables, median (IQR) for continuous variables.
†Because of small numbers in certain categories, we collapsed employment status (unpaid work; unemployed, but seeking work; or student) into other.
ASQoL, Ankylosing Spondylitis Quality of Life; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; BASMI, Bath Ankylosing Spondylitis Metrology Index; CRP, C reactive protein; HADS, Hospital Anxiety and Depression Scale; WPAI, Work Productivity and Activity Impairment.

status (eg, those who had attended only secondary school/those living in the highest levels of deprivation had on average around four points higher ASQoL score than those with a further degree/living in an area with lowest levels of deprivation, respectively). Current smoking was associated with significantly worse QoL (β=4.45, 95% CI 3.74 to 5.16). Higher level of fatigue (β per one unit increase in score=0.63, 95% CI 0.60 to 0.67), fibromyalgia (SSS) (β=1.34 per unit increase, 95% CI 1.24 to 1.44), fibromyalgia (WPI) (β=0.69, 95% CI 0.61 to 0.77), sleep disturbance (β=0.56, 95% CI 0.52 to 0.59), anxiety (β=0.72, 95% CI 0.67 to 0.76) and depression (β=0.97, 95% CI 0.92 to 1.01) were all related to poorer QoL. Non-work activity impairment due to AS (β=0.16 per % impairment, 95% CI 0.15 to 0.16) was also associated with poor QoL. Among those working, a higher percentage of time absent (β=0.11 per % time absent, 95% CI 0.09 to 0.13) or working with reduced productivity (presenteeism) (β=0.14 per % time work impaired, 95% CI 0.13 to 0.15) were related to poor QoL.

Validation of model predicting poor QoL

There were 555 participants in the current study who provided the necessary information to be part of the validation of the QoL model reported in the SIRAS study (the lower number was due mainly to the fact that WPI was only collected partway through the study, and there were missing data for BASMI). Four of the previously reported five factors (BASDAI (β=0.69, 95% CI 0.51 to 0.87), BASFI (β=0.85, 95% CI 0.69 to 1.01), fatigue (β=0.14, 95% CI 0.08 to 0.19) and widespread pain (WPI) (β=0.07, 95% CI 0.00 to 0.14)) were related to poor QoL, but there was no independent relationship with BASMI (β=0.01, 95% CI −0.16 to 0.18) (table 3). A stepwise model was then run with these five factors and additional psychosocial factors available in the current study. All three extra added factors (sleep disturbance (β=0.10, 95% CI 0.07 to 0.12), anxiety (β=0.12, 95% CI 0.09 to 0.16) and depression (β=0.19, 95% CI 0.14 to 0.24)) were included in the new best-fitting model, together with

Table 2 Predictors of Ankylosing Spondylitis Quality of Life from univariable linear regression analysis

Variable		Regression coefficient, β (95% CI)*
Clinical factors		
Symptom duration (years)	Continuous	−0.05 (−0.07 to −0.04)
Uveitis	Not present	–
	Present	−1.32 (−1.94 to −0.70)
Psoriasis	Not present	–
	Present	1.46 (0.62 to 2.31)
Inflammatory bowel disease	Not present	–
	Present	1.25 (0.36 to 2.13)
Enthesitis	Not present	–
	Present	1.72 (0.85 to 2.60)
Peripheral joint disease	Not present	–
	Present	1.82 (1.13 to 2.51)
Dactylitis	Not present	–
	Present	0.99 (−0.39 to 2.37)
Spinal mobility (BASMI)	Continuous	0.97 (0.82 to 1.11)
Inflammation (CRP) (mg/dL)	Continuous	0.02 (0.00 to 0.04)
Body mass index (kg/m^2)	Continuous	0.16 (0.10 to 0.21)
Number of comorbidities	Continuous	0.75 (0.43 to 1.07)
Patient-reported factors		
Disease activity (BASDAI)	Continuous	1.82 (1.75 to 1.88)
Physical function (BASFI)	Continuous	1.56 (1.51 to 1.62)
Age (years)	Continuous	−0.05 (−0.06 to −0.03)
Gender	Male	–
	Female	1.58 (1.02 to 2.13)
Education	Secondary school	–
	Apprenticeship	−1.25 (−2.20 to −0.30)
	Further education college	−0.97 (−1.62 to −0.31)
	University degree	−2.78 (−3.52 to −2.04)
	Further degree	−3.95 (−4.96 to −2.93)
Employment	Working full-time	–
	Working part-time	1.90 (1.89 to 2.61)
	Retired	0.18 (−0.48 to 0.83)
	Retired early (ill health)	5.07 (4.02 to 6.11)
	Unemployed (ill health), not seeking work	8.54 (7.69 to 9.40)
	Other†	2.71 (1.61 to 3.80)
Deprivation, quintiles of the general population	1, most deprived	–
	2	−2.20 (−3.09 to −1.30)
	3	−3.00 (−3.86 to −2.14)
	4	−3.64 (−4.48 to −2.80)
	5, least deprived	−4.37 (−5.22 to −3.52)
Smoking status	Never	–
	Ex	1.37 (0.80 to 1.94)
	Current	4.45 (3.74 to 5.16)
Alcohol consumption	Never	–
	Ex	−0.07(−1.23 to 1.09)
	Current	−3.60 (−4.63 to −2.58)
Chalder fatigue	Continuous	0.63 (0.60 to 0.67)
Symptom Severity Scale	Continuous	1.34 (1.24 to 1.44)
Widespread Pain Index	Continuous	0.69 (0.61 to 0.77)
Sleep disturbance (Jenkins)	Continuous	0.56 (0.52 to 0.59)
Anxiety (HADS)	Continuous	0.72 (0.67 to 0.76)
Depression (HADS)	Continuous	0.97 (0.92 to 1.01)
Absenteeism (WPAI) (%)	Continuous	0.11 (0.09 to 0.13)
Presenteeism (WPAI) (%)	Continuous	0.14 (0.13 to 0.15)
Work impairment (WPAI) (%)	Continuous	0.14 (0.13 to 0.15)
Activity (non-work) impairment (%)	Continuous	0.16 (0.15 to 0.16)

Jenkins indicates the Jenkins Scale for Sleep Disturbance.
*A positive regression coefficient means a poorer quality of life compared with a reference category or per unit increase in the risk factor, for continuous variables.
†Because of small numbers in certain categories, we collapsed employment status (unpaid work; unemployed, but seeking work; or student) into other.
BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; BASMI, Bath Ankylosing Spondylitis Metrology Index; CRP, C reactive protein; HADS, Hospital Anxiety and Depression Scale; WPAI, Work Productivity and Activity Impairment.

disease activity (BASDAI) (β=0.55, 95% CI 0.45 to 0.64) and function (BASFI) (β=0.85, 95% CI 0.77 to 0.93) (table 4).

Developing a new model predicting poor QoL

Finally, a de novo stepwise model within BSRBR-AS used data from 642 participants. The patient-reported factors included in the final model were disease activity (BASDAI (β=0.31, 95% CI 0.14 to 0.47)), function (BASFI (β=0.59, 95% CI 0.45 to 0.73)), depression (β=0.16, 95% CI 0.09 to 0.24) and sleep disturbance (β=0.08, 95% CI 0.04 to 0.13), in agreement with results of previous models (table 5). In addition, activity impairment (β=0.04, 95% CI 0.02 to 0.05), fibromyalgia (SSS) (β=0.24, 95% CI 0.13 to 0.35) and fibromyalgia (WPI) (β=0.10, 95% CI 0.03 to 0.17) entered the model as did current tobacco smoking (β=0.66, 95% CI 0.10 to 1.21).

DISCUSSION

The results of this study provide consistent evidence of which modifiable factors are associated with poor QoL in patients with axSpA. A model previously proposed in a Scotland-wide study was partly validated, confirming that worse disease activity, poor function and high levels of fatigue and widespread nature of pain symptoms were strongly related to poor QoL. The results were then extended by showing that in addition, mood (anxiety and depression), sleep disturbance, and both widespread pain and somatic symptom components related to fibromyalgia were importantly related to poor QoL.

The participants in BSRBR-AS are broadly representative of the patients with axSpA in clinics across Great Britain, with the exception that none of them were currently prescribed biologics (although around one-third of the participants were about to commence biologics). We have used BASDAI as the measure of disease activity as it is the measure most commonly used in the UK and is part of national guidelines by the National Institute of Health and Clinical Excellence and the British Society for Rheumatology. A second reason is that the necessity to have a measure of inflammation (such as for the Ankylosing Spondylitis Disease Activity Score)[24] would have resulted in a much higher level of missing data, where this had not been measured in the required timeframe.

Some models have many fewer subjects included than others. This was partly a result of variables related to fibromyalgia (WPI

Table 3 Validation of a model predicting poor quality of life

Variable	Model derived from SIRAS study		BSRBR-AS model*
		Relative risk (95% CI)	Regression coefficient, β (95% CI)†
Disease activity (BASDAI)	BASDAI<4	1.00	0.69 (0.51 to 0.87)
	BASDAI≥4	1.52 (1.09 to 2.12)	
Physical function (BASFI)	BASFI<4	1.00	0.85 (0.69 to 1.01)
	BASFI≥4	3.46 (1.76 to 6.82)	
Spinal mobility (BASMI)	BASMI<4	1.00	0.01 (–0.16 to 0.18)
	BASMI≥4	1.52 (0.93 to 2.50)	
Fatigue	None/mild	1.00	0.14 (0.08 to 0.19)
	Moderate/severe	1.60 (1.13 to 2.28)	
Widespread Pain Index‡	No	1.00	0.07 (0.00 to 0.14)
	Yes	1.92 (1.33 to 2.75)	

N=555.
*Model adjusted for gender, age, education, symptom duration, employment, deprivation, alcohol consumption and history of peripheral joint involvement.
†All variables in the model are continuous (in contrast to dichotomous in SIRAS).
‡Chronic widespread pain was available in SIRAS, and WPI was available in BSRBR-AS.
BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; BASMI, Bath Ankylosing Spondylitis Metrology Index; BSRBR-AS, British Society for Rheumatology Biologics Register in Ankylosing Spondylitis; SIRAS, Scotland Registry for Ankylosing Spondylitis.

and SSS), which were only collected for part of the duration of the study and in relation to BASMI, which was only measured on some participants. Once the variables included in a specific model were determined, the analysis was, however, re-run, including all participants with the required data available. In validating the model predicting poor QoL, the information collected was not in exactly the same format between studies (for pain, WPI was collected instead of CWP). However, the information collected in BSRBR-AS was more detailed and was analysed in a statistically more efficient manner (ie, using continuous variables where available). Overall the results, however, were consistent across both studies. There can be a certain amount of circularity, since factors that are potentially associated with poor QoL can themselves be used in assessing QoL. Within ASQoL, for example, there are items on function, mental health, sleep and pain. These are all aspects (assessed by specific questionnaires) that the current study has found were associated with overall QoL. Such circularity is unavoidable, but in the current study, the regressions model the association per unit change in score, so there is limited influence of one aspect of QoL on the overall score and second in our analysis as part of the SIRAS study, items were removed (in turn) from ASQoL on pain and tiredness, and associations with CWP and fatigue (respectively) were still observed.[7] Finally, one may debate whether function is modifiable independent of disease activity. In this dataset, there is a clear correlation between them (correlation=0.76). We have considered both as EULAR recommendations include each as targets for management, and the results suggest they make an independent contribution to QoL. The correlations between SSS and BASDAI (correlation=0.68) and between BASFI and non-work physical impairment as measured by WPAI (correlation=0.78) also suggest important relationships. However, as part of the model assessment, we calculated the variance inflation factor as a method of assessing potential multicollinearity, and this confirmed that there were no concerns.

The current study confirms the important role of disease activity and function in terms of QoL but adds to the literature by emphasising the important independent role of additional features associated with axSpA: mental health, fatigue and sleep problems, and widespread pain. Fatigue has been recognised to

Table 4 Validation of a model predicting poor QoL: the additional role of depression, anxiety and sleep disturbance

Variable	Regression coefficient, β (95% CI)*†
Disease activity (BASDAI)	0.55 (0.45 to 0.64)
Physical function (BASFI)	0.85 (0.77 to 0.93)
Sleep disturbance (Jenkins)	0.10 (0.07 to 0.12)
Anxiety (HADS)	0.12 (0.09 to 0.16)
Depression (HADS)	0.19 (0.14 to 0.24)

N=1692.
Jenkins indicates the Jenkins Scale for Sleep Disturbance.
*Model adjusted for gender, age, education, symptom duration, employment, deprivation, alcohol consumption, history of peripheral joint involvement and number of comorbidities.
†Regression coefficients represent change in QoL per unit increase in predictor.
BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; HADS, Hospital Anxiety and Depression Scale; QoL, quality of life.

Table 5 Variables associated with poor quality of life: the British Society for Rheumatology Biologics Register in Ankylosing Spondylitis study

Variable*		Regression coefficient, β (95% CI)†
Disease activity (BASDAI)	Per unit increase	0.31 (0.14 to 0.47)
Physical function (BASFI)	Per unit increase	0.59 (0.45 to 0.73)
Symptom Severity Scale	Per unit increase	0.24 (0.13 to 0.35)
Widespread Pain Index	Per unit increase	0.10 (0.03 to 0.17)
Sleep disturbance (Jenkins)	Per unit increase	0.08 (0.04 to 0.13)
Depression (HADS)	Per unit increase	0.16 (0.09 to 0.24)
Activity (non-work) impairment (%)	Per % increase	0.04 (0.02 to 0.05)
Smoking—current smoker	Yes/no	0.66 (0.10 to 1.21)

N=642.
Jenkins indicates the Jenkins Scale for Sleep Disturbance.
*Work variables were not offered to the model as these were only relevant to persons in employment. Inflammation not offered to the model because of the level of missing data.
†Model adjusted for gender, age, education, symptom duration, current employment, deprivation, history of peripheral joint involvement, uveitis, psoriasis, inflammation bowel disease, enthesitis, dactylitis and number of comorbidities.
BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; HADS, Hospital Anxiety and Depression Scale.

have an important influence on health-related QoL in rheumatoid arthritis (RA)[25] and indeed it was a priority for RA patients who participated in a focus group study in Sweden, in terms of being a key component to measure in evaluating treatment success.[26] Zhao *et al*,[27] in a meta-analysis of 16 studies, estimated the prevalence of at least moderate depression in patients with axSpA as 15%, based on a HADS score of ≥11. Garrido-Cumbrera *et al*,[28] in a sample of 680 patients as part of the Atlas of Axial Spondyloarthritis in Spain, reported that high disease activity was a risk factor for poor mental health, but the current study emphasises that poor mental health independently predicts worse QoL. A meta-analysis of the co-occurrence of fibromyalgia in axSpA estimated a prevalence of 13%,[29] compared with a prevalence in the general population of around 2%–5%,[30] and we have previously shown within BSRBR-AS that patients who have comorbid fibromyalgia have the same absolute improvement in QoL when treated with anti-TNFα therapy, but their QoL prior to and on treatment remains worse. Further, a high score on the SSS (rather than the WPI) is predicative of lack of improvement.[31] The fact that these additional features are common has been recognised, but not that they contribute independently to poor QoL, and there has been a lack of studies on how they can be effectively treated (including alongside inflammatory arthritis). Nevertheless, we acknowledge that there are some who propose that it is sufficient to focus on inflammation and that, in so doing, other aspects that impinge on quality of life will also improve.[32]

The most recent EULAR/ASAS guidelines for the management of axSpA state, in Recommendation 2, that 'the primary goal of treating the patient with axSpA is to maximise long-term health-related quality of life through control of symptoms and inflammation, prevention of progressive structural damage, preservation/normalisation of function and social participation'.[33] The results of the current study confirm that disease activity and function, as a focus, are appropriate. However, it is not sufficient. In the guidelines, there is no mention of sleep problems, widespread pain or mental health and specifically how these aspects should be managed. The results from this study suggest that their role is important, and independent of disease activity and functional limitation. Results from others studies, as noted previously, suggest that patients will continue to experience fatigue, poor mental health and fibromyalgia-like symptoms if management is focused on inflammation alone.[3 4] Effecting improvement in such additional disease features is challenging; studies are under way to test behavioural approaches to management and/or physical activity for fatigue and fibromyalgia symptoms in patients with a range of inflammatory arthritides.[34 35] Currently, evidence suggests, for example, that community-based exercise programmes exert a positive (although modest) effect on anxiety[36 37] and depression[38] among patients with arthritis and other rheumatic conditions. A recent trial demonstrated that group-based cognitive behavioural therapy delivered within rheumatology teams reduced the impact of fatigue in patients with RA.[39] Such therapy aims to reduce the impact of, for example, fatigue and widespread pain rather than improving symptoms per se, and not all patients are willing to engage with them. Further, the expertise and resources to deliver the interventions to target these additional factors are not easily available to many rheumatology teams.

In summary, the current study has shown that, through analysis of factors related to poor QoL and validation of a previously published model, improving the QoL of patients with axSpA means that, in addition to improving disease activity and function in patients, there must be attention to the comorbid features of fatigue, poor sleep and mental health and other common symptoms.

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Contributors All authors discussed and contributed to designing this study. GJM, OR and LED designed the analysis plan, which was undertaken by OR and overseen by LED and GJM. Results were reviewed by all authors. OR and GJM both contributed to drafting the first version of the manuscript, which was critically reviewed by all authors.

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REFERENCES

1 López-Medina C, Schiotis RE, Font-Ugalde P, *et al*. Assessment of fatigue in spondyloarthritis and its association with disease activity. *J Rheumatol* 2016;43:751–7.10.3899/jrheum.150832
2 Bedaiwi M, Sari I, Thavaneswaran A, *et al*. Fatigue in ankylosing spondylitis and nonradiographic axial spondyloarthritis: analysis from a longitudinal observation cohort. *J Rheumatol* 2015;42:2354–60.
3 Matcham F, Galloway J, Hotopf M, *et al*. The impact of targeted rheumatoid arthritis pharmacologic treatment on mental health: a systematic review and network meta-analysis. *Arthritis Rheumatol* 2018;70:1377–91.
4 Druce KL, Bhattacharya Y, Jones GT, *et al*. Most patients who reach disease remission following anti-TNF therapy continue to report fatigue: results from the British Society for rheumatology biologics register for rheumatoid arthritis. *Rheumatology* 2016;55:1786–90.
5 Shim J, Jones GT, Pathan EMI, *et al*. Impact of biological therapy on work outcomes in patients with axial spondyloarthritis: results from the British Society for rheumatology biologics register (BSRBR-AS) and meta-analysis. *Ann Rheum Dis* 2018;77:1578–84.
6 Doward LC, Spoorenberg A, Cook SA, *et al*. Development of the ASQoL: a quality of life instrument specific to ankylosing spondylitis. *Ann Rheum Dis* 2003;62:20–6.
7 Dean LE, Macfarlane GJ, Jones GT. Five potentially modifiable factors predict poor quality of life in ankylosing spondylitis: results from the Scotland Registry for ankylosing spondylitis. *J Rheumatol* 2018;45:62–9.
8 Macfarlane GJ, Barnish MS, Jones EA, *et al*. The British Society for rheumatology biologics registers in ankylosing spondylitis (BSRBR-AS) study: protocol for a prospective cohort study of the long-term safety and quality of life outcomes of biologic treatment. *BMC Musculoskelet Disord* 2015;16:347.
9 Sieper J, Rudwaleit M, Baraliakos X, *et al*. The assessment of spondyloarthritis International Society (ASAS) Handbook: a guide to assess spondyloarthritis. *Ann Rheum Dis* 2009;68:ii1–44.
10 van der Linden S, Valkenburg HA, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the new York criteria. *Arthritis Rheum* 1984;27:361–8.
11 Garrett S, Jenkinson T, Kennedy LG, *et al*. A new approach to defining disease status in ankylosing spondylitis: the Bath ankylosing spondylitis disease activity index. *J Rheumatol* 1994;21:2286–91.
12 Calin A, Garrett S, Whitelock H, *et al*. A new approach to defining functional ability in ankylosing spondylitis: the development of the Bath ankylosing spondylitis functional index. *J Rheumatol* 1994;21:2281–5.
13 Jenkinson TR, Mallorie PA, Whitelock HC, *et al*. Defining spinal mobility in ankylosing spondylitis (as). The Bath as Metrology index. *J Rheumatol* 1994;21:1694–8.

14 The Scottish Government. The Scottish index of multiple deprivation, 2016. Available: www.gov.scot/Topics/Statistics/SIMD/ [Accessed 1 Aug 2019].
15 GOV.UK. English indices of deprivation, 2015. Available: https://www.gov.uk/government/statistics/english-indices-of-deprivation-2015 [Accessed 1 Aug 2019].
16 StatsWales. The Welsh index of multiple deprivation, 2014. Available: https://statswales.gov.wales/Catalogue/Community-Safety-and-Social-Inclusion/Welsh-Index-of-Multiple-Deprivation/WIMD-2014 [Accessed 1 Aug 2019].
17 Zigmond AS, Snaith RP. The hospital anxiety and depression scale. *Acta Psychiatr Scand* 1983;67:361–70.
18 Reilly MC, Zbrozek AS, Dukes EM. The validity and reproducibility of a work productivity and activity impairment instrument. *Pharmacoeconomics* 1993;4:353–65.
19 Reilly MC, Gooch KL, Wong RL, *et al*. Validity, reliability and responsiveness of the work productivity and activity impairment questionnaire in ankylosing spondylitis. *Rheumatology* 2010;49:812–9.
20 Wolfe F, Clauw DJ, Fitzcharles MA, *et al*. Fibromyalgia criteria and severity scales for clinical and epidemiological studies: a modification of the ACR preliminary diagnostic criteria for fibromyalgia. *J Rheumatol* 2011;38:1113–22.
21 Chalder T, Berelowitz G, Pawlikowska T, *et al*. Development of a fatigue scale. *J Psychosom Res* 1993;37:147–53.
22 Jenkins CD, Stanton BA, Niemcryk SJ, *et al*. A scale for the estimation of sleep problems in clinical research. *J Clin Epidemiol* 1988;41:313–21.
23 Wolfe F, Smythe HA, Yunus MB, *et al*. The American College of rheumatology 1990 criteria for the classification of fibromyalgia. Report of the multicenter criteria committee.. *Arthritis & Rheumatism* 1990;33:160–72.
24 Lukas C, Landewé R, Sieper J, *et al*. Assessment of spondyloarthritis international Society.Development of an ASAS-endorsed disease activity score (ASDAS) in patients with ankylosing spondylitis. *Ann Rheum Dis* 2009;68:18–24.
25 Rupp I, Boshuizen HC, Jacobi CE, *et al*. Impact of fatigue on health-related quality of life in rheumatoid arthritis. *Arthritis Care Res* 2004;51:578–85.
26 Ahlmén M, Nordenskiöld U, Archenholtz B*et al*. Rheumatology outcomes: the patient's perspective. A multicentre focus group interview study of Swedish rheumatoid arthritis patients. *Rheumatology* 2005;44:105–10.
27 Zhao S, Thong D, Miller N, *et al*. The prevalence of depression in axial spondyloarthritis and its association with disease activity: a systematic review and meta-analysis. *Arthritis Res Ther* 2018;20:140.
28 Garrido-Cumbrera M, Delgado-Domínguez CJ, Gálvez-Ruiz D, *et al*. The effect of axial spondyloarthritis on mental health: results from the atlas. *J Rheumatol* 2019;46:1284–9.
29 Duffield SJ, Miller N, Zhao S, *et al*. Concomitant fibromyalgia complicating chronic inflammatory arthritis: a systematic review and meta-analysis. *Rheumatology* 2018;57:1453–60.
30 Jones GT, Atzeni F, Beasley M, *et al*. The prevalence of fibromyalgia in the general population: a comparison of the American College of rheumatology 1990, 2010, and modified 2010 classification criteria. *Arthritis & Rheumatology* 2015;67:568–75.
31 Macfarlane GJ, MacDonald RIR, Pathan E, *et al*. Influence of co-morbid fibromyalgia on disease activity measures and response to tumour necrosis factor inhibitors in axial spondyloarthritis: results from a UK national register. *Rheumatology* 2018;57:1982–90.
32 Figueiredo-Braga M, Cornaby C, Cortez A, *et al*. Influence of biological therapeutics, cytokines, and disease activity on depression in rheumatoid arthritis. *J Immunol Res* 2018;2018:1–9.
33 van der Heijde D, Ramiro S, Landewé R, *et al*. 2016 update of the ASAS-EULAR management recommendations for axial spondyloarthritis. *Ann Rheum Dis* 2017;76:978–91.
34 Martin KR, Bachmair E-M, Aucott L, *et al*. Protocol for a multicentre randomised controlled parallel-group trial to compare the effectiveness of remotely delivered cognitive-behavioural and graded exercise interventions with usual care alone to lessen the impact of fatigue in inflammatory rheumatic diseases (lift). *BMJ Open* 2019;9:e026793.
35 Arthritis V. 1360 results for 'how can we effectively manage patients who have axial spondyloarthritis and fibromyalgia', 2019. Available: https://www.versusarthritis.org/search?query=how+can+we+effectively+manage+patients+who+have+axial+spondyloarthritis+and+fibromyalgia [Accessed 1 Aug 2019].
36 Kelley GA, Kelley KS. Exercise reduces depressive symptoms in adults with arthritis: Evidential value. *WJR* 2016;6:23–9.
37 Kelley GA, Kelley KS, Callahan LF. Community-deliverable exercise and anxiety in adults with arthritis and other rheumatic diseases: a systematic review with meta-analysis of randomised controlled trials. *BMJ Open* 2018;8:e019138.
38 Kelley GA, Kelley KS, Hootman JM. Effects of exercise on depression in adults with arthritis: a systematic review with meta-analysis of randomized controlled trials. *Arthritis Res Ther* 2015;17:21.
39 Hewlett S, Almeida C, Ambler N, *et al*. Reducing arthritis fatigue impact: two-year randomised controlled trial of cognitive behavioural approaches by rheumatology teams (raft). *Ann Rheum Dis* 2019;78:465–72.

EPIDEMIOLOGICAL SCIENCE

Clinical manifestations, disease activity and disease burden of radiographic versus non-radiographic axial spondyloarthritis over 5 years of follow-up in the DESIR cohort

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ABSTRACT

Objectives To compare the clinical manifestations, disease activity and disease burden between patients with radiographic (r-axSpA) and non-radiographic axial spondyloarthritis (nr-axSpA) over a 5-year follow-up period in the Devenir des Spondylarthropathies Indifférenciées Récentes (DESIR) cohort.

Methods Patients from the DESIR cohort who had X-ray images of the sacroiliac joints available at baseline and did not leave the study during the 5-year follow-up period because of a diagnosis other than axSpA were included. A unilateral rating of 'obvious sacroiliitis' by the local reader was considered sufficient for classification as r-axSpA. The incidence of first episodes of peripheral and extra-rheumatic manifestations was compared between the two groups using the incidence rate ratio and Cox regressions adjusted for sex, age and tumour necrosis factor blocker (TNFb) intake. Mean values of patient-reported outcomes (PROs) and days of sick leave over 5 years of follow-up were compared using mixed models adjusted for sex, age, TNFb intake and baseline values.

Results In total, 669 patients were included, of whom 185 (27.7%) and 484 (72.3%) were classified as r-axSpA and nr-axSpA, respectively. At baseline, the r-axSpA patients showed a significantly higher prevalence of males. After adjusting for age, sex and TNFb intake, Cox regressions for peripheral and extra-rheumatic manifestations did not show any significant differences between groups. Mixed models also showed similar mean levels in PROs and days of sick leave between groups over time.

Conclusion The incidence of peripheral and extra-rheumatic manifestations as well as the disease burden over time remained similar between r-axSpA and nr-axSpA groups after adjusting for intermediate variables.

Trial registration number NCT01648907

Key messages

What is already known about this subject?

► A debate exists concerning the concept of non-radiographic axial spondyloarthritis (nr-axSpA): some people suggest that patients with nr-axSpA might actually be suffering from a disease different from radiographic axSpA (r-axSpA), while others consider that nr-axSpA could be a self-limited form of axSpA with a rapidly favourable course or an early stage of the same spectrum.

What does this study add?

► This study suggests that both r-axSpA and nr-axSpA behave similarly over time since the incidence of peripheral and extra-rheumatic manifestations as well as the disease burden are not different after 5 years of follow-up.

How might this impact on clinical practice or future developments?

► These results confirm the concept of axSpA as one single disease, which implies that both r-axSpA and nr-axSpA patients should be treated with equal priority. For this reason, the distinction between r-axSpA and nr-axSpA should only have implications for clinical research and not for clinical practice.

INTRODUCTION

Axial spondyloarthritis (axSpA) is a chronic inflammatory rheumatic disease that encompasses patients with radiographic axSpA (r-axSpA; also known as ankylosing spondylitis—AS, with advanced structural damage on X-ray) and non-radiographic axSpA (nr-axSpA; no definitive signs of structural damage on X-ray).[1] The fact that only 5.1% of patients with recent axSpA shifts from nr-axSpA to r-axSpA over 5 years[2] prompts a debate concerning the concept of nr-axSpA: some people suggest that patients classified as nr-axSpA might actually be suffering from a disease different from r-axSpA, while others suggest that nr-axSpA could be a self-limited form of axSpA with a rapidly favourable course. This debate is particularly important in North America, where the Food and Drug Administration expressed several concerns about the incompletely characterisation of the natural history of axSpA, which led to the non-approval of several biological disease-modifying antirheumatic drugs (bDMARDs) to treat patients with nr-axSpA.[3] These questions resulted in the publication of some studies that compare these two groups of patients, showing a similar disease burden but a higher prevalence of males and smokers, a larger mean disease duration and a higher level of acute phase reactants in r-axSpA patients.[4–8] However, most of these studies have a cross-sectional design which does not allow

us to understand the natural history of these two subgroups. Only a few studies have evaluated the course of the disease with a longitudinal and prospective approach, but most of them focused on radiographic progression and/or the effectiveness of bDMARDs as the main outcome.[9–12] In 2015, two independent studies that compared the clinical course (ie, patient-reported outcomes (PROs) and acute phase reactants) of r-axSpA and nr-axSpA were published: the first study reported no between-group differences in pain and quality of life over 3 years, while mean C reactive protein (CRP) levels remained higher in the r-axSpA group[13]; the second study reported a similar disease activity (measured with the Bath AS Disease Activity Index—BASDAI) and functional status (measured with the Bath AS Function Index—BASFI) in both groups, but higher mean CRP levels in r-axSpA patients after a 2-year follow-up.[14] However, none of these studies compared the incidence of peripheral and extra-rheumatic manifestations between groups.

DESIR (Devenir des Spondylarthropathies Indifférénciées Récentes) is a prospective cohort of patients with recent onset axSpA. We conducted this study with the aim of comparing the clinical manifestations and disease burden between r-axSpA and nr-axSpA patients over 5 years of follow-up. By estimating the risk of extra-spinal manifestations, we planned to determine whether nr-axSpA can be considered as the same disease as r-axSpA. By estimating the level of activity/severity over time, we intended to explore whether nr-axSpA can be considered as a self-limited disease.

METHODS

Patients

For this analysis, 5-year follow-up data from the DESIR cohort were used. Patients who had X-ray images of the sacroiliac joints (SIJ) available at baseline were included. The DESIR cohort has been previously described.[15] Briefly, consecutive patients aged 18–50 years from 25 centres in France who had inflammatory back pain (evaluated by either the Calin or the Berlin criteria)[16 17] that lasted ≥3 months but <3 years were included if the treating rheumatologist considered the symptoms suggestive of axSpA (a score ≥5 on a scale from 0 to 10). Moreover, we excluded patients who had a different diagnosis than that of axSpA after at least 2 years of follow-up according to the treating rheumatologist. Visits were scheduled every 6 months during the first 2 years and yearly thereafter.

The study was conducted according to good clinical practice guidelines and was approved by the appropriate local medical ethical committees.

Patient and public involvement

Patients were not involved in the design of the study, conduct of the study, development or dissemination of study results.

Definitions of r-axSpA and nr-axSpA

Pelvic radiographs collected at baseline were used to define r-axSpA and nr-axSpA. Local radiologists or rheumatologists read all available baseline radiographs of the SIJ in their own centre, hereafter called 'local reading'. Local readers were asked to rate each SIJ as either 'normal', 'doubtful sacroiliitis', 'obvious sacroiliitis' or 'SIJ fusion'.[18] According to this scoring method, a unilateral rating of 'obvious sacroiliitis' was considered sufficient for the classification as r-axSpA in this study, while the remaining patients were classified as nr-axSpA. We used this scoring system because it more closely resembles common clinical practice than does the modified New York (mNY) criteria and because it has been used in previous studies.[19]

Moreover, a sensitivity analysis was performed using the results from the central readings. Baseline radiographs of the SIJ were read independently by three trained readers. Each reader evaluated each SIJ according to the mNY grading method (ie, at least a unilateral grade 3 sacroiliitis or at least a bilateral grade 2 sacroiliitis). A radiograph of the SIJ was considered positive for sacroiliitis if two of the three central readers agreed on fulfilment of the mNY criteria, and hereafter referred to as 'central reading'.

Collected data

Baseline information about sociodemographics, smoking status, alcohol, HLA-B27, axial symptom duration, good non-steroidal anti-inflammatory drugs (NSAIDs) response, Assessment of Spondyloarthritis International Society (ASAS) axial, European Spondylarthropathy Study Group (ESSG) and AMOR criteria fulfilment were used.[20 21]

At baseline and during the follow-up (at 0, 6, 12, 18, 24, 36, 48 and 60 months), the following data were analysed: peripheral arthritis (either detected via physical examination or considering patients who reported having received intra-articular corticosteroids between visits), dactylitis, enthesitis at any location, uveitis, psoriasis, inflammatory bowel disease (IBD), CRP, Ankylosing Spondylitis Disease Activity Score (ASDAS-CRP), SpondyloArthritis Research Consortium of Canada (SPARCC) score on the SIJ,[22] BASDAI, BASFI, SF-36 questionnaire[23] and days of sick leave. Treatment intake, including NSAIDs by the ASAS-NSAID score,[24] conventional synthetic disease modifying antirheumatic drugs (csDMARDs) and TNFb were also analysed.

Statistical analysis

Baseline characteristics

Baseline clinical characteristics of patients with and without at least unilateral 'obvious sacroiliitis' according to the local reading were compared using χ^2 and t-test (or Fisher and Mann-Whitney U test for non-parametric data). In order to confirm that differences across r-axSpA and nr-axSpA were similar regardless the use of local and central reading, the same analysis was conducted using the central reading definition (ie, fulfilment of mNY criteria according to two of the three central readers).

Peripheral and extra-rheumatic manifestations over 5 years of follow-up

Three types of statistical models were conducted to compare peripheral and extra-rheumatic manifestations between r-axSpA and nr-axSpA: (1) a cross-sectional model at baseline; (2) a pseudo-longitudinal model, in which the prevalence of these manifestations at 5-year time-point as well as the incidence rate ratio (IRR) between the r-axSpA and nr-axSpA groups were compared; (3) a longitudinal model (Cox regressions) in which data from intermediate visits was used to compare the time-to-event of these manifestations (firstly as a crude analysis and thereafter adjusted by age, gender and TNFb intake over follow-up) between both groups.

csDMARDs and TNFb initiation over 5 years of follow-up

The same three analysis as that used for peripheral and extra-rheumatic manifestations were conducted for csDMARDs and TNFb initiation.

Disease activity, PROs and days of sick leave over 5 years of follow-up

Disease activity (CRP and SPARCC-SIJ), PROs and days of sick leave over the 5 years of follow-up were compared between the

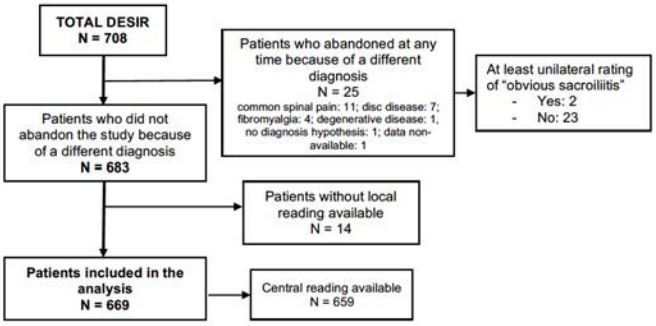

Figure 1 Flow-chart with regard to the patients included in the analysis. DESIR, Devenir des Spondylarthropathies Indifférenciées Récentes.

r-axSpA and nr-axSpA using a crude mixed model with random effects (here, the subject was considered a random effect and the absence of 'obvious sacroiliitis' based on the local reading a fixed effect) and also adjusting for baseline values, age, sex and time-changing variables (TNFb use).

Exploratory analysis of the variables influencing disease activity over time

In order to determine which variables had an impact on the disease activity over the 5 years of follow-up, a multivariate linear regression was performed to determine the different factors that may explain the change in mean BASDAI and ASDAS-CRP over time.

Handling of missing data

For the cross-sectional analysis, baseline missing data were not imputed. For the IRR analysis, the last observation carried forward (LOCF) method was used (ie, a patient with at least one episode in the follow-up was considered positive at the end of the study). For the longitudinal analysis, LOCF was used for Cox models, while continuous variables were imputed via the use of mixed models.

RESULTS

Of the 708 patients included in the DESIR cohort, a total of 25 patients were excluded for this analysis because they had a different diagnosis other than axSpA after at least 2 years of follow-up (figure 1). Among the remaining 683 patients, 669 and 659 had local and central X-ray reading data available, respectively.

Among the 669 patients evaluated by local readers, 185 (27.7%) had at least a unilateral rating of 'obvious sacroiliitis', and 484 (72.3%) were classified as either 'normal' or 'doubtful sacroiliitis'. Of the 659 patients with central reading data available, 92 (14.0%) fulfilled the mNY criteria according to two of the three central readers, and 567 (86.0%) did not fulfil the mNY classification criteria.

Baseline characteristics

Baseline characteristics and differences between r-axSpA and nr-axSpA patients according to the local reading definition are shown in table 1. A sensitivity analysis was performed using the mNY classification from the central reading (online supplementary table S1) showing similar results.

Peripheral and extra-rheumatic manifestations over 5 years of follow-up

Using r-axSpA and nr-axSpA definition from the local reading, we compared the prevalence of peripheral and extra-rheumatic manifestations at baseline and after 5 years follow-up between the two groups, as well as the IRR by excluding patients who had a positive event at baseline (table 2). At baseline, r-axSpA patients showed a significantly lower prevalence of 'ever' peripheral enthesitis than nr-axSpA patients, while the prevalence of 'ever' peripheral arthritis, dactylitis and extra-rheumatic manifestations were similar between the two groups. Overall, all peripheral and extra-rheumatic manifestations showed a higher prevalence in the whole population after 5 years (figure 2).

The incidence of first episodes of peripheral arthritis was higher in r-axSpA than in nr-in axSpA patients, with a significant IRR of 1.75 (95% CI 1.14 to 2.67) for r-axSpA versus nr-axSpA. Uveitis, IBD and psoriasis also showed a higher incidence of first episodes in r-axSpA, while peripheral enthesitis and dactylitis were more incident among nr-axSpA patients, although these differences were not significant.

Cox regressions comparing the incidence of peripheral and extra-rheumatic manifestations over time are shown in table 3. R-axSpA patients showed a significantly higher risk for the development of peripheral arthritis than nr-axSpA (crude HR 1.73, 95% CI 11.13 to 2.64), but these differences were no longer significant after adjusting for age, sex and TNFb intake. Similarly, peripheral enthesitis, dactylitis and extra-rheumatic manifestations did not show any differences after both adjusting and not adjusting for intermediate variables, reflecting a similar risk of appearance of these manifestations between r-axSpA and nr-axSpA patients.

csDMARDs and TNFb initiation over 5 years of follow-up

Regarding TNFb (table 2), at baseline per protocol, not a single patient had been exposed to or was receiving TNFb, while 252 initiated TNFb after 5 years of follow-up, with significant differences between r-axSpA and nr-axSpA groups. The incidence of TNFb initiation was also different between r-axSpA and nr-axSpA (IRR 1.59, 95% CI 1.23 to 2.07).

The prevalence of patients under csDMARDs at baseline and after 5 years was similar between the r-axSpA and nr-axSpA groups.

Cox regressions (online supplementary table S2) showed a significantly higher risk of TNFb initiation among r-axSpA patients after adjusting for sex, age and CRP mean levels.

Disease activity, PROs and days of sick leave over 5 years of follow-up

Table 4 shows the results of the mixed model with random effects for disease activity variables, PROs and days of sick leave. Mean CRP over time was significantly higher among r-axSpA patients, even after adjusting for intermediate variables. MRI-SIJ inflammation evaluated with SPARCC was higher among the r-axSpA than among nr-axSpA group, but these differences disappeared after adjusting for intermediate variables.

Compared with r-axSpA, nr-axSpA patients showed significantly higher levels of BASDAI and BASFI, poorer scores in the SF-36 questionnaire and a larger number of days of sick leave over time. However, these differences disappeared after adjusting for intermediate variables.

Table 1 Baseline characteristics of the 669 patients with local X-ray reading available

		At least unilateral rating of 'obvious sacroiliitis' (local reader)		
	Total patients n=669	Yes (r-axSpA) n=185 (%)	No (nr-axSpA) n=484 (%)	P value
Sex (male)	312/669 (46.6%)	110/185 (59.5%)	202/484 (41.7%)	**<0.001**
Age, mean (SD)	33.6 (8.6)	31.3 (8.9)	34.5 (8.4)	**<0.001**
Ethnicity (Caucasian)	600/642 (89.7%)	162/185 (87.6%)	438/484 (90.5%)	0.263
High level of education	394/666 (59.2%)	106/185 (57.3%)	288/481 (59.9%)	0.544
Smoking (ever)	245/664 (36.9%)	81/184 (44.0%)	164/480 (34.17%)	**0.018**
Alcohol (ever)	98/667 (14.7%)	39/185 (21.1%)	59/482 (12.2%)	**0.004**
Symptoms duration, mean (SD)	1.5 (0.9)	1.6 (0.9)	1.5 (0.9)	0.178
HLA-B27 positive	397/668 (59.4%)	137/185 (74.0%)	260/483 (53.8%)	**<0.001**
Family history of SpA	280/631 (44.4%)	81/177 (43.8%)	199/454 (45.8%)	0.661
Good NSAIDs response	573/663 (86.4%)	169/185 (91.4%)	404/478 (84.5%)	**0.021**
Positive MRI-SIJ according to ASAS definition	233/657 (35.5%)	132/181 (72.9%)	101/476 (21.2%)	**<0.001**
ASAS criteria (according to local reading)	422/669 (63.8%)	161/185 (87.03%)	261/484 (53.93%)	**<0.001**
ASAS or ESSG or AMOR criteria	623/669 (93.1%)	185/185 (100.0%)	438/484 (94.9%)	**<0.001**
Peripheral arthritis (ever)	158/664 (23.8%)	48/185 (25.9%)	110/479 (23.0 %)	0.419
Any peripheral enthesitis (ever)	379/669 (56.7%)	88/185 (47.6%)	291/484 (60.1%)	**0.003**
Heel enthesitis (ever)	282/618 (45.6%)	63/170 (37.1%)	219/448 (48.9%)	**0.008**
Dactylitis (ever)	95/666 (14.3%)	25/185 (13.5%)	70/481 (14.6%)	0.731
Uveitis (ever)	62/669 (9.3%)	22/185 (12.0 %)	40/484 (8.3%)	0.148
Inflammatory bowel disease (ever)*	34/669 (5.1%)	14/185 (7.6%)	20/484 (4.1%)	0.070
Psoriasis	115/669 (17.2%)	29/185 (15.7%)	86/484 (17.8%)	0.521
Abnormal CRP (>5 mg/dL)	189/648 (29.2%)	84/178 (47.2%)	105/470 (22.3%)	**<0.001**
ASDAS-CRP ≥2.1	446/640 (69.7%)	121/177 (68.4%)	325/463 (70.2%)	0.651
SPARCC, mean (SD)	13.0 (19.1)	25.4 (22.3)	8.2 (15.3)	**<0.001**
NSAID-ASAS score (6 months), mean (SD)	45.6 (40.7)	53.4 (44.7)	42.6 (38.6)	**0.002**
csDMARDs intake during the last 6 months	90/668 (13.5%)	67/184 (12.4%)	23/484 (13.9%)	0.626
BASDAI, mean (SD)	44.5 (20.2)	40.0 (20.6)	46.2 (19.7)	**<0.001**
BASDAI Q1 (Fatigue)	56.4 (23.5)	49.5 (24.3)	59.1 (22.7)	**<0.001**
BASDAI Q2 (Spinal pain)	53.0 (24.9)	49.2 (26.7)	54.4 (24.1)	**0.015**
BASDAI Q3 (Joint pain)	26.9 (27.3)	22.4 (26.8)	28.6 (27.4)	**0.009**
BASDAI Q4 (Enthesis pain)	40.2 (29.5)	36.2 (28.9)	41.8 (28.9)	**0.031**
BASDAI (Q5+Q6)/2 (Stiffness)	45.5 (23.7)	42.4 (24.6)	46.7 (23.3)	**0.040**
BASFI, mean (SD)	30.3 (22.8)	28.3 (22.0)	31.1 (23.1)	0.156
SF-36 MCS, mean (SD)	40.0 (9.1)	41.5 (8.6)	39.4 (9.2)	0.051
SF-36 PCS, mean (SD)	40.3 (11.2)	41.6 (11.6)	39.8 (10.9)	**0.010**
HAQ-AS, mean (SD)	0.7 (0.5)	0.6 (0.5)	0.7 (0.5)	0.068
Level of confidence in SpA diagnosis (0–10)	7.0 (2.6)	8.3 (1.7)	6.5 (2.7)	**<0.001**

*Fisher test or Mann-Whitney U test.

ASAS, Assessment of SpondyloArthritis international Society; ASDAS, Ankylosing Spondylitis Disease Activity Score; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; CRP, C reactive protein; ESSG, European Spondylarthropathy Study Group; HAQ-AS, Health Assessment Questionnaire; SF-36 MCS, Mental Component Score from the SF-36 questionnaire; MRI-SIJ, MRI from the sacroiliac joints; nr-axSpA, non-radiographic axial Spondyloarthritis; NSAID, non-steroidal anti-inflammatory drug; SF-36 PCS, Physical Component Score from the SF-36 questionnaire; Q, Question; r-axSpA, radiographic axial Spondyloarthrits; SpA, Spondyloarthritis; SpondyloArthritis Research Consortium of Canada.

Exploratory analysis of the variables influencing disease activity over time

Finally, multivariate linear regressions (online supplementary tables S3 and S4) showed that the variability of BASDAI over the 5 years was explained by age, sex, level of education, radiographic sacroiliitis, TNFb and mean CRP, while ASDAS-CRP was explained by sex, level of education and TNFb.

DISCUSSION

This study permitted to evaluate the natural history of patients with recent IBP classified as r-axSpA or nr-axSpA in daily clinical practice.

In the DESIR cohort, the probability of observing structural damage on the SIJ (at least unilateral rating of 'obvious sacroiliitis') in patients with recent IBP suspicious of axSpA was 27.6%, while the probability of observing patients fulfilling the mNY criteria was 14.0%. We decided to use the 'local reading' scoring system because it closely reflects the procedure in clinical practice; that is, even if a patient does not fulfil the mNY criteria, a rheumatologist or radiologist usually classifies the patient as r-axSpA if he/she has obvious unilateral structural damage.

At baseline, the results showed similarities between patients with r-axSpA and nr-axSpA (especially in extra-rheumatic manifestations and disease burden) with some differences mainly in relation to the clinical presentation and inflammation. A larger number of r-axSpA patients were smoking males and had higher CRP and SPARCC levels compared with nr-axSpA patients. These characteristics have been classically described as risk

Table 2 Prevalence and incidence of peripheral and extra-rheumatic manifestations, as well as treatment initiation

		Prevalence at baseline		Prevalence after 5 years of follow-up		Incidence/100 person-years		
Handling missing data		**Without imputation**		**LOCF and imputed '0' at baseline**		**LOCF and excluding patients with positive event at baseline**		
		Per group	**Whole population**	**Per group**	**Whole population**	**Per group**	**Whole population**	**IRR (95% CI)**
Peripheral arthritis (ever)	r-axSpA*	48/185 (25.9%)	158/664 (23.8%)	82/185 (44.3%)†	249/669 (37.2%)	5.85	3.66	1.75 (1.14 to 2.67)†
	nr-axSpA	110/479 (23.0%)		167/484 (34.5%)		3.34		
Any peripheral enthesitis (ever)	r-axSpA	88/185 (47.6%)†	379/669 (56.7%)	124/185 (67.9%)†	493/669 (73.7%)	9.60	10.54	0.87 (0.58 to 1.29)
	nr-axSpA	291/484 (60.1%)		369/484 (76.2%)		11.05		
Dactylitis (ever)	r-axSpA	25/185 (13.5%)	95/666 (14.3%)	36/185 (19.5%)	146/669 (21.8%)	1.44	1.87	0.70 (0.36 to 1.37)
	nr-axSpA	70/481 (14.6%)		110/484 (22.7%)		2.03		
Uveitis (ever)	r-axSpA	22/185 (12.0%)	62/669 (9.3%)	35/185 (18.9%)†	96/669 (14.3%)	1.65	1.15	1.70 (0.85 to 3.39)
	nr-axSpA	40/484 (8.3%		61/484 (12.6%)		0.97		
Inflammatory bowel disease (ever)	r-axSpA	14/185 (7.6%)	34/669 (5.1%)	23/185 (12.4%)	60/669 (9.0%)	1.08	0.84	1.45 (0.64 to 3.25)
	nr-axSpA	20/484 (4.1%)		37/484 (7.6%)		0.75		
Psoriasis (ever)	r-axSpA	29/185 (15.7%)	115/669 (17.2%)	46/185 (24.9%)	164/669 (24.5%)	2.30	1.85	1.37 (0.76 to 2.47)
	nr-axSpA	86/484 (17.8%)		118/484 (24.4%)		1.68		
TNFb intake (ever)	r-axSpA	0 (0%)	0 (0%)	87/185 (47.0%)†	252/669 (37.7%)	14.77	10.63	1.59 (1.23 to 2.07)†
	nr-axSpA	0 (0%)		165/484 (34.1%)		9.26		
csDMARDs intake (ever)	r-axSpA	23/185 (12.4%)	90/668 (13.47%)	37/185 (20.0%)	152/669 (22.7%)	1.86	2.34	0.73 (0.40 to 1.33)
	nr-axSpA	67/483 (13.9%)		115/185 (23.8%)		2.54		

*Baseline classification according to local reading definition: at least unilateral 'obvious sacroiliitis'.
†P value <0.05 between r-axSpA and nr-axSpA.
csDMARDs, conventional synthetic disease-modifying anti-rheumatic drugs; IRR, incidence rate ratio for r-axSpA versus nr-axSpA; LOCF, last observation carried forward; nr-axSpA, non-radiographic axial Spondyloarthritis; r-axSpA, radiographic axial Spondyloarthritis; TNFb, tumour necrosis factor blockers.

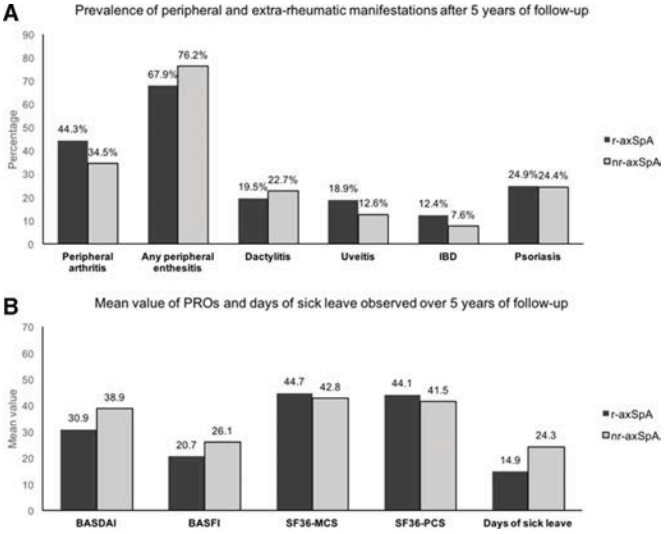

Figure 2 Main outcomes after 5 years of follow-up. BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Function Index; IBD, inflammatory bowel disease; PRO, patient-reported outcome; SF36-MCS, Mental Component Score from the SF-36 questionnaire; SF36-PCS, Physical Component Score from the SF-36 questionnaire; nr-axSpA, non-radiographic axial spondyloarthritis; r-axSpA, radiographic axial spondyloarthritis.

factors for structural damage in the SIJ and the spine,[25] which lead to a higher probability of TNF initiation. In fact, in this study we demonstrated that inflammation measured with CRP was permanently higher among r-axSpA despite the greater use of TNFb in this group; however, this can be partly explained because 31% of r-axSpA patients in the DESIR cohort did not received a TNFb treatment despite a high disease activity over 5 years of follow-up.[26] HLA-B27 positivity and alcohol intake were also more frequent among nr-axSpA, being these results consistent with previous studies in the DESIR cohort.[2 19] We also found a higher prevalence of heel enthesitis and other peripheral enthesitis among the nr-axSpA group at baseline. However, these peripheral manifestations might be artificially overrepresented among nr-axSpA patients because they help to diagnose nr-axSpA in the absence of radiographic sacroiliitis.

In this study we aimed to evaluate the incidence of first episodes of peripheral and extra-rheumatic manifestations between the two groups after diagnosis. For this reason, in the longitudinal analysis (ie, IRR and Cox regressions), we decided to remove patients with a positive event at baseline to avoid the bias caused by the prevalence at the inclusion visit. Although the nr-axSpA group showed a higher prevalence of peripheral enthesitis at baseline, the incidence was similar between groups after the exclusion of patients with a positive event at the inclusion visit, confirming the theory that peripheral manifestations might be overestimated among nr-axSpA patients at the time of diagnosis. The incidence of dactylitis and extra-rheumatic manifestations were also similar, demonstrating a clinical pattern that is comparable between these two groups with regard to these manifestations. These data suggest that axSpA should be considered a single disease entity.

Because r-axSpA patients showed higher CRP levels over time, we expected to find a greater disease burden in this group. Curiously, and contrary to our expectations, nr-axSpA patients presented higher scores in the BASDAI and BASFI, a poorer quality of life and a larger number of days of sick leave at the follow-up. We have two theories that can explain these results. One theory is that a percentage of nr-axSpA patients may have concomitant fibromyalgia, as this phenomenon has been described in a study published by Moltó *et al*: patients without radiographic sacroiliitis more frequently had concomitant fibromyalgia according to the American College of Rheumatology (ACR) 1990 criteria than did r-axSpA patients.[27] The second hypothesis is that other factors and patient characteristics influence disease activity and disease burden. Thus, we adjusted the mixed models using variables that might lead to the higher scores observed among nr-axSpA group (ie, age, sex and TNFb use). The rationale to adjust for these variables was based on the fact that they were, a priori, clinically relevant to differentiate both groups. These variables could be considered as 'intermediate' variables since they could influence the pathological pathway between the 'exposure' (ie, axSpA subgroup) and the outcomes; on the other hand, TNFb can be associated with both the outcome and the 'exposure' (eg, prescription rates were more important in the r-axSpA group), and as such it could also be considered as a 'confounder' variable. In any case, differences between r-axSpA and nr-axSpA were no longer evident after adjusting for these variables. To test the hypothesis that the answering to these questionnaires depend not only on the presence of sacroiliitis, and in an exploratory approach, we decided to evaluate factors that explain the mean level of BASDAI over time in these patients through the use of a multivariate linear regression. Interestingly, the mean BASDAI over the 5 years was explained by age, sex, education, radiographic sacroiliitis, TNFb and mean CRP. These results suggest that several factors influence the association between axSpA subgroups and the PROs.

This study has some weaknesses and strengths. One weakness is that we did not use central reading to classify r-axSpA and nr-axSpA patients. However, in the protocol, we planned to not use central reading in order to mimic clinical practice, in which only one rheumatologist or radiologist evaluates X-ray images. It should be noted that we did not separately analyse patients who switched from nr-axSpA to r-axSpA during the follow-up for two reasons: first, because as described by Dougados *et al* in this same cohort, only 5.1% of patients shifts from nr-axSpA to

Table 3 Cox regressions to compare the incidence of peripheral and extra-rheumatic manifestations over 5 years of follow-up between r-axSpA and nr-axSpA

	Crude HR (95% CI)	P value	HR adjusted for sex, age and TNFb intake (95% CI)	P value
Peripheral arthritis	1.73 (1.13 to 2.64)	**0.011**	1.30 (0.83 to 2.05)	0.256
Any peripheral enthesitis	0.88 (0.59 to 1.30)	0.517	0.88 (0.58 to 1.33)	0.555
Dactylitis	0.71 (0.36 to 1.38)	0.310	0.82 (0.42 to 1.63)	0.578
Uveitis	1.69 (0.85 to 3.38)	0.135	1.76 (0.86 to 3.61)	0.124
Inflammatory bowel disease	1.45 (0.64 to 3.24)	0.371	1.29 (0.56 to 2.99)	0.552
Psoriasis	1.37 (0.76 to 2.47)	0.296	1.18 (0.64 to 2.17)	0.590

HR, Hazard Ratio; TNFb, tumour necrosis factor blockers.

Table 4 Mixed models with random effects to compare disease activity, PROs and days of sick leave over 5 years of follow-up between r-axSpA and nr-axSpA

	All patients n=669 mean (SD)	Obvious sacroiliitis (r-axSpA) n=185 mean (SD)	No obvious sacroiliitis (nr-axSpA) n=484 mean (SD)	MM crude p-value	MM adjusted for baseline value p-value	MM adjusted for baseline value, age, sex and TNFb intake p-value
CRP	5.6 (10.2)	7.7 (13.4)	4.8 (8.5)	**<0.001**	**0.001**	**<0.001**
SPARCC	7.3 (15.5)	13.5 (21.0)	4.9 (11.9)	**<0.001**	0.479	0.333
BASDAI	36.6 (21.7)	30.9 (20.9)	38.9 (21.5)	**<0.001**	**<0.001**	0.130
BASFI	24.6 (22.0)	20.7 (20.3)	26.1 (22.5)	**0.002**	**0.004**	0.236
SF-36 MCS	43.3 (11.3)	44.7 (11.2)	42.8 (11.3)	**0.016**	0.148	0.662
SF-36 PCS	42.3 (9.4)	44.1 (8.59)	41.5 (9.6)	**<0.001**	**0.003**	0.214
Days of sick leave	21.7 (61.1)	14.9 (45.5)	24.3 (65.9)	**0.009**	0.082	0.424

BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Function Index; CRP, C reactive protein; MM, mixed model; SF36-MCS, Mental Component Score from the SF-36 questionnaire; SF36-PCS, Physical Component Score from the SF-36 questionnaire; SPARCC, SpondyloArthritis Research Consortium of Canada; TNFb, tumour necrosis factor blockers.

r-axSpA over 5 years[2]; and second, because we wanted to understand the behaviour of patients classified either as nr-axSpA or r-axSpA at baseline, regardless of the development of structural damage over time. Another limitation is the difficulty of precisely evaluating peripheral and extra-rheumatic manifestations that occur between two study visits. Thus, we used variables based on physical exploration and clinical interviews and accumulated information from previous study visits. Moreover, we considered the occurrence of the first episode of each manifestation as a primary outcome because we did not have information about flares between visits. One strength of this study is that we removed from the analysis patients who left the DESIR cohort because of a diagnosis other than axSpA according to the rheumatologist's opinion; thus, all patients included in this study were diagnosed as patients with axSpA.

In summary, in this study we observed that both r-axSpA and nr-axSpA seem to behave similarly over time since the incidence of peripheral and extra-rheumatic manifestations are not different after 5 years of follow-up. Although the nr-axSpA group showed a greater disease burden, these differences disappeared after adjusting for intermediate variables, suggesting the influence of multiple factors on questionnaires scores. These highlighted results confirm the concept of axSpA as a single disease, which implies that both r-axSpA and nr-axSpA patients should be treated with equal priority.

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REFERENCES

1 Rudwaleit M, van der Heijde D, Landewé R, *et al*. The development of assessment of SpondyloArthritis international Society classification criteria for axial spondyloarthritis (part II): validation and final selection. *Ann Rheum Dis* 2009;68:777–83.
2 Dougados M, Sepriano A, Molto A, *et al*. Sacroiliac radiographic progression in recent onset axial spondyloarthritis: the 5-year data of the DESIR cohort. *Ann Rheum Dis* 2017;76:1823–8.
3 Deodhar A, Reveille JD, van den Bosch F, *et al*. The concept of axial spondyloarthritis: joint statement of the spondyloarthritis research and treatment network and the assessment of SpondyloArthritis international Society in response to the US Food and Drug Administration's comments and concerns. *Arthritis Rheumatol* 2014;66:2649–56.
4 Kiltz U, Baraliakos X, Karakostas P, *et al*. Do patients with non-radiographic axial spondyloarthritis differ from patients with ankylosing spondylitis? *Arthritis Care Res* 2012;64:1415–22.
5 Wallis D, Haroon N, Ayearst R, *et al*. Ankylosing spondylitis and nonradiographic axial spondyloarthritis: part of a common spectrum or distinct diseases? *J Rheumatol* 2013;40:2038–41.
6 Poddubnyy D, Haibel H, Listing J, *et al*. Baseline radiographic damage, elevated acute-phase reactant levels, and cigarette smoking status predict spinal radiographic progression in early axial spondyloarthritis. *Arthritis Rheum* 2012;64:1388–98.
7 Mease PJ, Heijde DVD, Karki C, *et al*. Characterization of patients with ankylosing spondylitis and nonradiographic axial spondyloarthritis in the US-Based Corrona Registry. *Arthritis Care Res* 2018;70:1661–70.
8 Burgos-Varga R, Wei JC-C, Rahman MU, *et al*. The prevalence and clinical characteristics of nonradiographic axial spondyloarthritis among patients with inflammatory back pain in rheumatology practices: a multinational, multicenter study. *Arthritis Res Ther* 2016;18.
9 Glintborg B, Sørensen IJ, Østergaard M, *et al*. Ankylosing spondylitis versus nonradiographic axial spondyloarthritis: comparison of tumor necrosis factor inhibitor effectiveness and effect of HLA-B27 status. An observational cohort study from the Nationwide DANBIO registry. *J Rheumatol* 2017;44:59–69.
10 Poddubnyy D, Rudwaleit M, Haibel H, *et al*. Effect of non-steroidal anti-inflammatory drugs on radiographic spinal progression in patients with axial spondyloarthritis:

results from the German Spondyloarthritis Inception Cohort. *Ann Rheum Dis* 2012;71:1616–22.
11 Dougados M, Demattei C, van den Berg R, *et al*. Rate and predisposing factors for sacroiliac joint radiographic progression after a two-year follow-up period in recent-onset spondyloarthritis. *Arthritis Rheumatol* 2016;68:1904–13.
12 Ciurea A, Scherer A, Exer P, *et al*. Tumor necrosis factor α inhibition in radiographic and nonradiographic axial spondyloarthritis: results from a large observational chort. *Arthritis Rheum* 2013;65:3096–106.
13 Wallman JK, Kapetanovic MC, Petersson IF, *et al*. Comparison of non-radiographic axial spondyloarthritis and ankylosing spondylitis patients--baseline characteristics, treatment adherence, and development of clinical variables during three years of anti-TNF therapy in clinical practice. *Arthritis Res Ther* 2015;17.
14 Poddubnyy D, Haibel H, Braun J, *et al*. Brief report: clinical course over two years in patients with early nonradiographic axial spondyloarthritis and patients with ankylosing spondylitis not treated with tumor necrosis factor blockers: results from the German Spondyloarthritis Inception Cohort. *Arthritis Rheumatol* 2015;67:2369–75.
15 Dougados M, Etcheto A, Molto A, *et al*. Clinical presentation of patients suffering from recent onset chronic inflammatory back pain suggestive of spondyloarthritis: the DESIR cohort. *Joint Bone Spine* 2015;82:345–51.
16 Calin A, Porta J, Fries JF, *et al*. Clinical history as a screening test for ankylosing spondylitis. *JAMA* 1977;237:2613–4.
17 Rudwaleit M, Metter A, Listing J, *et al*. Inflammatory back pain in ankylosing spondylitis: a reassessment of the clinical history for application as classification and diagnostic criteria. *Arthritis Rheum* 2006;54:569–78.
18 Blachier M, Coutanceau B, Dougados M, *et al*. Does the site of magnetic resonance imaging abnormalities match the site of recent-onset inflammatory back pain? the DESIR cohort. *Ann Rheum Dis* 2013;72:979–85.
19 Chung HY, Machado P, van der Heijde D, *et al*. Smokers in early axial spondyloarthritis have earlier disease onset, more disease activity, inflammation and damage, and poorer function and health-related quality of life: results from the DESIR cohort. *Ann Rheum Dis* 2012;71:809–16.
20 Dougados M, van der Linden S, Juhlin R, *et al*. The European Spondylarthropathy Study Group preliminary criteria for the classification of spondylarthropathy. *Arthritis Rheum* 1991;34:1218–27.
21 Amor B, Dougados M, Listrat V, *et al*. [Evaluation of the Amor criteria for spondylarthropathies and European Spondylarthropathy Study Group (ESSG). A cross-sectional analysis of 2,228 patients]. *Ann Med Interne* 1991;142:85–9.
22 Maksymowych WP, Inman RD, Salonen D, *et al*. Spondyloarthritis research Consortium of Canada magnetic resonance imaging index for assessment of sacroiliac joint inflammation in ankylosing spondylitis. *Arthritis Rheum* 2005;53:703–9.
23 Perneger TV, Leplège A, Etter JF, *et al*. Validation of a French-language version of the MOS 36-item Short Form Health Survey (SF-36) in young healthy adults. *J Clin Epidemiol* 1995;48:1051–60.
24 Dougados M, Simon P, Braun J, *et al*. ASAS recommendations for collecting, analysing and reporting NSAID intake in clinical trials/epidemiological studies in axial spondyloarthritis. *Ann Rheum Dis* 2011;70:249–51.
25 Taurog JD, Chhabra A, Colbert RA. Ankylosing spondylitis and axial spondyloarthritis. *N Engl J Med* 2016;374:2563–74.
26 López-Medina C, Dougados M, Collantes-Estévez E, *et al*. Adherence to recommendations for the use of anti-tumour necrosis factor and its impact over 5 years of follow-up in axial spondyloarthritis. *Rheumatology* 2018;57:880–90.
27 Moltó A, Etcheto A, Gossec L, *et al*. Evaluation of the impact of concomitant fibromyalgia on TNF alpha blockers' effectiveness in axial spondyloarthritis: results of a prospective, multicentre study. *Ann Rheum Dis* 2018;77:533–40.

CLINICAL SCIENCE

Factors associated with long-term cardiac dysfunction in neonatal lupus

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ABSTRACT

Objectives Cardiac manifestations of neonatal lupus (NL) have been associated with significant morbidity and mortality; however, there is minimal information on long-term outcomes of affected individuals. This study was initiated to evaluate the presence of and the risk factors associated with cardiac dysfunction in NL after birth in multiple age groups to improve counselling, to further understand pathogenesis and to provide potential preventative strategies.

Methods Echocardiogram reports were evaluated in 239 individuals with cardiac NL: 143 from age 0–1 year, 176 from age >1–17 years and 64 from age >17 years. Logistic regression analyses evaluated associations of cardiac dysfunction at each age group with demographic, fetal and postnatal factors, using imputation to address missing data.

Results Cardiac dysfunction was identified in 22.4% at age 0–1 year, 14.8% at age >1–17 years and 28.1% at age >17 years. Dysfunction in various age groups was significantly associated with male sex, black race, lower fetal heart rates, fetal extranodal cardiac disease and length of time paced. In 106 children with echocardiograms at ages 0–1 year and >1–17 years, 43.8% with dysfunction at age 0–1 year were also affected at age >1–17 years, while the others reverted to normal. Of children without dysfunction at age 0–1 year, 8.9% developed new dysfunction between ages >1 and 17 years. Among 34 with echocardiograms at ages >1–17 years and >17 years, 6.5% with normal function at age >1–17 years developed dysfunction in adulthood.

Conclusions Risk factors in fetal life can influence cardiac morbidity into adulthood.
Although limited by a small number of cases, cardiac dysfunction in the first year often normalises by later childhood. New-onset dysfunction, although rare, can occur de novo after the first year.

Key messages

What is already known about this subject?

- Cardiac dysfunction can persist and occur de novo after birth in individuals born with cardiac neonatal lupus (NL).

What does this study add?

- Postnatal cardiac dysfunction in individuals with cardiac NL is more prevalent in the first year of life compared with later childhood, and highest in adults older than age 17 years.
- Factors associated with disease severity as early as the fetal life are associated with cardiac dysfunction into adulthood. Underlying early subclinical damage from the fetal or neonatal life may increase susceptibility to future cardiac insults or the effects of prolonged pacing.

How might this impact on clinical practice or future developments?

- Close monitoring and aggressive treatment of early extranodal disease and incomplete block may have long-term benefit in preventing subsequent morbidity in cardiac NL. In utero, the use of fluorinated steroids to halt progression or to reverse these non-immutable manifestations of cardiac injury, as well as early pacemaker placement or treatment of heart failure, could potentially forestall subsequent cardiac dysfunction.

INTRODUCTION

Neonatal lupus (NL) results from placental transport of maternal anti-Ro with or without anti-La antibodies during gestation.[1] The most serious features are cardiac manifestations (cardiac NL), including congenital heart block (CHB) and/or extranodal disease, such as dilated cardiomyopathy (DCM), endocardial fibroelastosis (EFE) and hydrops fetalis.[2–4] While some mothers have systemic lupus erythematosus or Sjogren's syndrome, the majority are asymptomatic, and these pathogenic antibodies are sought only based on fetal disease. The prevalence of anti-Ro has been approximated between 0.12% and 2.0% in blood donors or pregnant women.[5] Thus, a significant number of women are faced with the risk of cardiac NL in their offspring. Discussion of this outcome is integral to pregnancy counselling in these women, since 1%–3% will face the possibility that their child will have a permanent cardiac abnormality that can be fatal (17%) and/or require lifelong pacing (>70%).[3 6–9]

Until recently, there has been minimal substantive data on long-term outcomes associated with cardiac NL. Postnatal development of cardiomyopathy in neonates with CHB despite normal in utero heart function has been reported, and has been considered related to continued inflammation induced by anti-Ro antibodies or the effects of right ventricular (RV) pacing, which is associated with dyssynchrony-induced cardiomyopathy.[2 10 11] In several studies, the incidence of postnatal cardiomyopathy has been estimated at 19%–29%, with cardiac dysfunction documented after the first months of life in 8%–15%.[12–16]

The study reported herein leverages the largest extant cohort of cardiac NL to evaluate factors associated with cardiac dysfunction and aortic dilation (online supplementary information) in three age groups: postnatal, 0–1 year, where maternal autoantibodies may be present in circulation; childhood, >1–17 years; and adults, >17 years (the age individuals have generally completed puberty).[17] A subset of patients assessed serially was evaluated for regression or de novo development of dysfunction over time. It is anticipated that these data will improve counselling of anti-Ro-positive mothers and identify risk factors to further understand the pathogenesis of anti-Ro-mediated injury and provide potential preventative strategies.

METHODS

Study population

Cardiac NL cases were identified from the Research Registry for Neonatal Lupus (RRNL), initially established in 1994.[18] Families are enrolled in the RRNL if a mother has anti-Ro and/or anti-La antibodies and at least one child has either cardiac or cutaneous NL manifestations. Cardiac NL is defined in this study as anti-Ro exposure and high grade CHB (second or third degree), and/or presence of extranodal disease, which includes echocardiographic evidence of EFE, DCM and/or hydrops fetalis. Patients with isolated first-degree block or sinus bradycardia were excluded. Cases included were those born between January 1963 and January 2016. A total of 239 individuals had at least one postnatal echocardiogram available to assess cardiac dysfunction and aortic dilation (online supplementary information). Four individuals died subsequent to the last available postnatal echocardiogram, three after echocardiogram at the age >1–17 years and one after echocardiogram at the age >17 years. Risk factors for mortality in these individuals have been previously described.[3]

Study design and data collection

Serial postnatal echocardiogram reports were separated into age groups 0–1, >1–17 and >17 years. When multiple echos were available in each group, the report at the oldest age was used in the analyses done for this study. The composite outcome for cardiac dysfunction was defined as at least one of the following: (1) qualitative description of left ventricular (LV) dysfunction on echocardiogram report; (2) concurrent cardiac medication use (beta blockers, ACE inhibitors and/or digoxin, excluding use solely for hypertension or other causes per record review); and (3) heart transplant.

Several demographic factors were evaluated for association with cardiac dysfunction, including sex of the affected individual, age at the time of postnatal echocardiogram and maternal race/ethnicity. Fetal factors evaluated included gestational age at detection of cardiac NL and exposure to maternal use of hydroxychloroquine, fluorinated steroids and beta agonists (such as terbutaline, used to treat low fetal heart rates). Fetal echocardiographic factors included ventricular heart rate at the time of cardiac NL detection, nadir ventricular rate detected during pregnancy, presence of EFE, DCM or hydrops fetalis, and a composite of fetal extranodal disease inclusive of at least one of EFE, DCM or hydrops. A previously described severity score representing overall fetal disease, weighted by features associated with cardiac NL mortality (bradycardia and extranodal disease) and created to account for the low rate of these comorbidities, was also associated with postnatal cardiac dysfunction.[19] Factors evaluated after birth included the presence of a pacemaker, the length of time with a pacemaker, the number of pacemakers placed and the length of time paced via RV, dual-chamber and/or biventricular pacemaker (see online supplementary information for additional information and aortic dilation definitions).

Statistical analysis

Demographic, fetal and postnatal potential risk factors were summarised by the presence of cardiac dysfunction at ages 0–1, >1–17 and >17 years. Bivariate associations of each factor with cardiac dysfunction at each age period were cross-sectionally assessed with two-sample t-test or Wilcoxon rank-sum test for continuous variables, and χ^2 or Fisher's exact test for categorical variables as appropriate. Multivariable logistic regression analysis was performed to identify independent risk factors for cardiac dysfunction at each age period. Models for the first two age groups included demographic factors that were significant at $p \leq 0.10$ in at least one age group in bivariate analysis. Of the fetal factors associated with in utero bradycardia (beta agonist use, ventricular rate at detection and nadir), the factor with the highest level of statistical significance in each age group was selected for inclusion to avoid collinearity. The presence of any in utero extranodal disease and the length of time with a pacemaker at the time of echocardiogram were also chosen as predictor variables because event rates were higher and missing data rates were lower compared with other fetal echocardiographic and postnatal factors. The model for cardiac dysfunction at age >17 years included only four predictors due to small sample size and low outcome event rates. As a sensitivity analysis, an alternative logistic regression model for each age period included severity score as a predictor instead of in utero ventricular nadir/rate at detection and in utero extranodal disease (see online supplementary information for information on multiple imputation to address missing data and aortic dilation analyses). All analyses were conducted using SAS V.9.4.

RESULTS

Patient demographics

A total of 239 individuals with cardiac NL from 226 mothers were evaluated by echocardiogram at least once after birth. Demographic factors of the overall cohort are described in table 1.

Factors associated with cardiac dysfunction at age 0–1 year

Echocardiogram reports were available for 143 children between ages 0 and 1 year, with cardiac dysfunction identified in 32 (22.4%, 95% CI 15.8% to 30.1%) (figure 1). In bivariate analyses, demographic, fetal and postnatal risk factors were compared between individuals with and without cardiac dysfunction (table 2).

Those with cardiac dysfunction were less likely to be female, had an earlier age at cardiac NL detection, lower fetal ventricular rates at the time of cardiac NL detection and lower nadir fetal ventricular rate. The frequency of maternal beta agonist exposure was higher in the cardiac dysfunction group, as was in utero DCM. The median fetal severity score was likewise higher. Patients with cardiac dysfunction were more likely to have a pacemaker placed at the time of echo and had a greater length of time paced at the time of echo, number of pacemakers placed and length of time paced with a dual-chamber pacemaker.

In the multivariable logistic regression analysis, female sex was protective, while greater length of time paced was associated with increased odds of cardiac dysfunction. Older age at the time of echocardiogram at age 0–1 year and higher in utero nadir

Table 1 Demographics of overall cardiac NL cohort (N=239)

	Total (N=239)
Demographics of patients with cardiac NL	
Cardiac manifestation, n (%)	
Second-degree heart block	17 (7.1)
Third-degree heart block	216 (90.4)
Isolated extranodal disease	6 (2.5)
Female, n (%)	133 (55.6)
Age at the time of the last available echo (years), mean (SD)	10.8 (9.4)
Time of detection (gestational week), median (IQR) (n=234)	22.4 (20.0–27.9)
In utero ventricular rate at detection (beats/min), mean (SD) (n=153)	66.7 (16.7)
In utero ventricular nadir (beats/min), mean (SD) (n=170)	54.5 (11.2)
Time of delivery (gestational week), mean (SD) (n=231)	37.0 (2.3)
In utero EFE, n (%) (n=184)	19 (10.3)
In utero DCM, n (%) (n=186)	14 (7.5)
In utero hydrops, n (%) (n=181)	9 (5.0)
Any in utero extranodal disease (EFE, DCM or hydrops), n (%) (n=188)	40 (21.3)
Severity score, median (IQR) (n=192)	6.0 (5.0–8.0)
Pacemaker placement, n (%) (n=238)	188 (79.0)
Maternal demographics	
Mother's age at birth, mean (SD) (n=238)	30.4 (4.9)
Mother's diagnosis of SS or SLE at the most recent registry follow-up, n (%) (n=237)	137 (57.8)
Mother's race, n (%) (n=236)	
White	200 (84.7)
Black	12 (5.1)
Other	24 (10.2)
Maternal hydroxychloroquine use during pregnancy, n (%) (n=228)	11 (4.8)
Maternal fluorinated steroids use during pregnancy, n (%) (n=230)	116 (50.4)
Maternal beta agonist use during pregnancy, n (%) (n=228)	37 (16.2)

DCM, dilated cardiomyopathy; EFE, endocardial fibroelastosis;NL, neonatal lupus; SLE, systemic lupus erythematosus; SS, Sjogren's syndrome.

ventricular rates were associated with lower but non-significant odds of dysfunction (table 3A).

In the alternative model (table 3B), older age at the time of echocardiogram and female sex were protective, while length of time with a pacemaker was associated with cardiac dysfunction.

Factors associated with cardiac dysfunction at ages >1–17 years

Among the 176 cases evaluated in the age group >1–17 years, 59 (33.5%) were >1–5 years old, 47 (26.7%) were >5–10 years old and 70 (39.8%) were >10–17 years old at the time of the last analysed echocardiogram. Cardiac dysfunction was present in 26 (14.8%, 95% CI 9.9% to 20.9%) of the 176 children with available echocardiogram reports at ages >1–17 years (figure 1). During childhood, cardiac dysfunction was more common in black individuals compared with other races on bivariate analysis (table 2). Children with cardiac dysfunction at age >1–17 years had lower fetal ventricular rates at the time of detection and a lower fetal nadir rate. The presence of fetal echocardiographic DCM, hydrops and the composite extranodal disease outcome were higher in the cardiac dysfunction group, as was the severity score. Individuals with dysfunction in this age group were more likely to have a pacemaker, and a greater length of time paced, number of pacemakers and length of time biventricularly paced.

In the multivariable model, black race and the length of time with a pacemaker at the time of echocardiogram remained significantly associated with increased odds of cardiac dysfunction. In utero extranodal disease was non-significantly associated with dysfunction (table 4A).

In the alternative model (table 4B), black race, higher fetal severity score and greater length of time with a pacemaker were associated with dysfunction during ages >1–17 years.

Factors associated with cardiac dysfunction at age >17 years

Among the 64 individuals evaluated at age >17 years, 29 (45.3%) were age >17–20 years, 25 (39.0%) were age >20–30 years, and 10 (15.7%) were >30 years. Cardiac dysfunction was documented in 18 cases (28.1%, 95% CI 17.6% to 40.8%) (figure 1). Persistent into adulthood, those with cardiac dysfunction were more likely to be older at the time of echo and to have lower in utero ventricular rates at cardiac NL detection in the bivariate analysis (table 2). The presence of fetal hydrops or any in utero extranodal disease was more common, and the median fetal severity score was higher in individuals older than 17 years with dysfunction. A greater length of time paced, number of pacemakers and cumulative length of time biventricularly paced were associated with dysfunction in this age group.

In the multivariable analysis, greater length of time with a pacemaker remained significantly associated (table 5A).

In the alternative model, greater length of time paced and the severity score were associated with increased odds of cardiac dysfunction in adulthood (table 5B).

Serial evaluation of cardiac dysfunction

A total of 106 children had echocardiograms available both at age 0–1 year (median age 0.43, IQR 0.06–0.79 years) and at age >1–17 years (median 6.1, IQR 2.8–11.5 years). Cardiac dysfunction was noted at age 0–1 year in 16 (15.1%, 95% CI 8.9% to 23.4%) (figure 1). Only seven of these cases (43.8%, 95% CI 19.8% to 70.1%) were also affected at ages >1–17 years, while in the other nine, all prior abnormalities were no longer detected. An additional 8 of the remaining 90 children (8.9%, 95% CI 3.3% to 16.8%) evaluated at both age groups developed new-onset dysfunction during age >1–17 years. Four of these cases were not paced at age 0–1 year but had a pacemaker placed during age >1–17 years, while the other four were paced at both age groups.

There were 34 cases with available echocardiograms at ages >1–17 years (median age 13.0, IQR 6.6–15.6 years) and >17 years (median 19.3, IQR 18.0–20.6 years). Of the three cases (8.8%, 95% CI 1.9% to 23.7%) with dysfunction at age >1–17 years, all remained abnormal afterwards. New cardiac dysfunction developed in adulthood in 2 of the remaining 31 cases (6.5%, 95% CI 0.8% to 21.4%).

Echocardiogram reports were available in all three age groups for 14 individuals. Three cases (21.4%, 95% CI 4.7% to 50.8%) had cardiac dysfunction at age 0–1 year, two of which reverted to normal by age >1–17 years, while one had continued dysfunction through >17 years. No de novo cases of cardiac dysfunction occurred in any cases without abnormalities within 1 year after birth.

Aortic Dilation

(see online supplementary information)

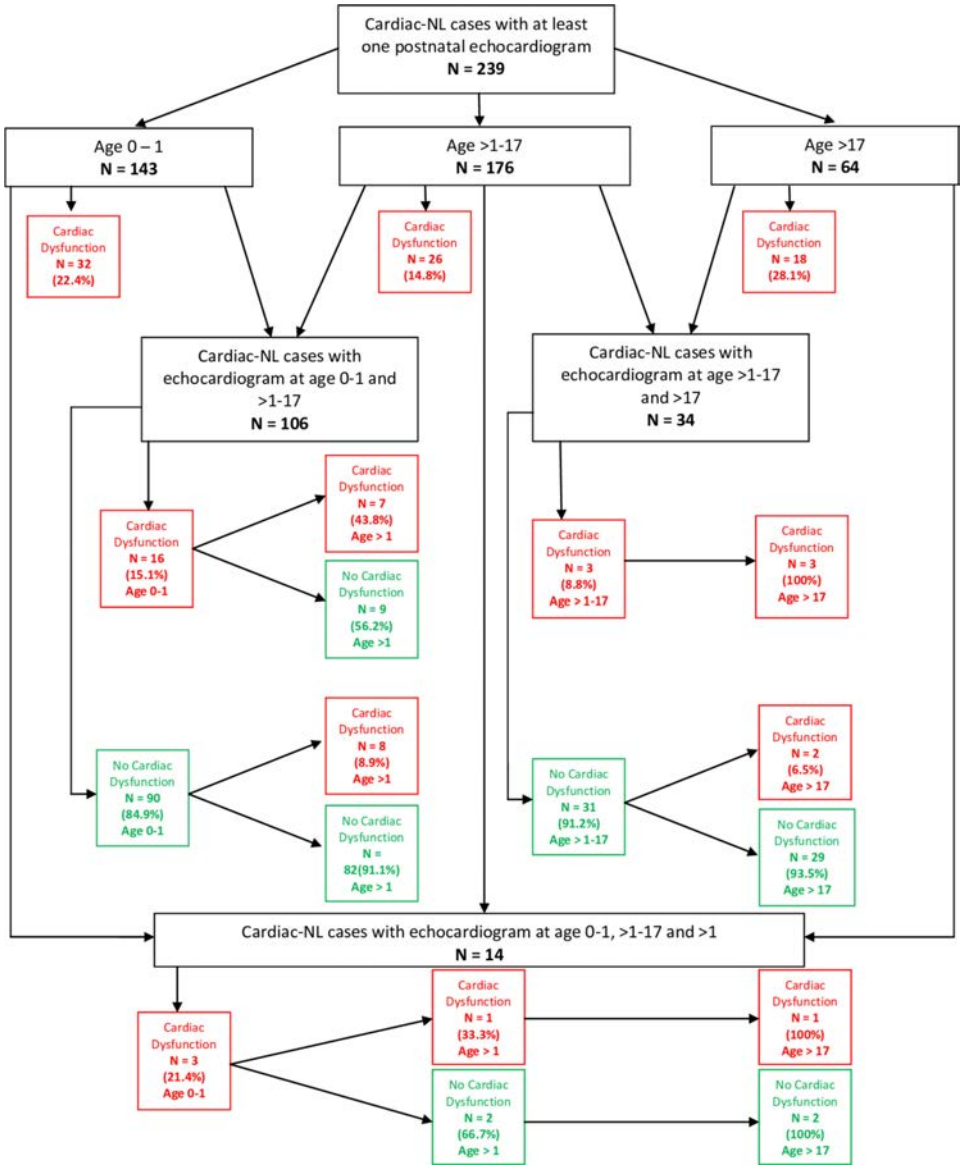

Figure 1 Cardiac dysfunction in NL by age group. NL, neonatal lupus.

DISCUSSION

In this cohort from the US registry of cardiac NL, 239 individuals were evaluated at multiple age groups for postnatal cardiac dysfunction, aortic dilation (online supplementary information) and changes in disease status over time. The prevalence of dysfunction was higher in those aged 0–1 year compared with >1–17 years, although de novo cardiac dysfunction did rarely occur during childhood. The highest rates of dysfunction occurred during adulthood. Risk factors for cardiac dysfunction at different ages included demographics, such as male sex and black race; fetal echocardiographic factors, such as lower in utero heart rates and extranodal disease; and postnatal factors, including length of time paced and total number of pacemakers.

A recent study following 119 patients with CHB from a Swedish population register identified that 16.8% had a diagnosis of heart failure and/or cardiomyopathy based on International Classification of Diseases coding at long-term follow-up.[20] A previous Swedish study evaluated risks of postnatal cardiac dysfunction.[15] LV dysfunction at a single follow-up time point was more common in those with dysfunction on echocardiogram performed before pacemaker implantation, as well as in men, though prenatal factors were not evaluated. In a large French cohort, neonatal DCM was associated with in utero cardiomyopathy and hydrops, while DCM after 28 days was associated with non-European origin, in utero mitral valve insufficiency and pacemaker placement.[16] Given the differing risk factors, the authors hypothesised that neonatal DCM is a continuation of fetal disease, while late-onset cardiomyopathy is a distinct entity. In our US cohort, of 41 available cases with normal LV systolic function at the time of a neonatal echo, 3 (7%) developed late-onset DCM, which were noted on echos evaluated at ages 13.5, 14.6 and 15.4 years. The small sample size precluded evaluation of predictor variables. However, overall several risk factors occurring as early as fetal life can influence cardiac morbidity extending into later childhood and adulthood as described in the >1–17 and >17 age groups.

In the youngest age group, morbidity may result from ongoing inflammation from the fetal life due to continued presence of maternal autoantibodies, or may be secondary to effects of fetal bradycardia causing cardiac enlargement and perhaps damage to an overworked and hypertrophied myocardium. By the childhood years, lower rates of dysfunction and the resolution

Table 2 Bivariate analysis of risk factors associated with cardiac dysfunction at multiple age groups

	Age 0–1 year (n=143)			Age >1–17 years (n=176)			Age >17 years (n=64)		
	Normal (n=111)	Cardiac dysfunction (n=32)	P value	Normal (n=150)	Cardiac dysfunction (n=26)	P value	Normal (n=46)	Cardiac dysfunction (n=18)	P value
Demographic factors									
Female, n (%)	70 (63.1)	15 (46.9)	0.10	89 (59.3)	12 (46.2)	0.21	25 (54.3)	6 (33.3)	0.13
Age at the time of echo, median (IQR)	0.4 (0.0–0.8)	0.4 (0.1–0.6)	0.54	7.3 (3.5–12.9)	7.5 (4.5–14.3)	0.77	19.8 (18.2–24.2)	21.8 (19.8–32.7)	0.058
Mother's age at birth, mean (SD)	31.1 (4.7)	30.8 (4.6)	0.73	30.5 (4.1)	30.9 (5.6)	0.74 (n=175)	29.8 (5.2)	28.6 (4.6)	0.38
Mother's diagnosis of SS or SLE at the most recent registry follow-up, n (%)	56 (50.9)	16 (51.6)	0.94 (n=141)	76 (51.0)	17 (65.4)	0.18 (n=175)	31 (67.4)	16 (88.9)	0.12
Mother's race, n (%)			0.42			**0.005**			0.78
White	90 (81.1)	24 (75.0)		131 (87.3)	21 (80.8)		42 (91.3)	16 (88.9)	
Black	4 (3.6)	3 (9.4)		2 (1.3)	4 (15.4)		1 (2.2)	1 (5.6)	
Other	17 (15.3)	5 (15.6)		17 (11.3)	1 (3.8)		3 (6.5)	1 (5.6)	
Fetal factors									
Time of detection (gestational week), median (IQR)	22.0 (20.0–26.0)	20.4 (19.0–24.0)	0.09 (n=139)	22.6 (20.0–26.1)	21.0 (20.0–24.0)	0.15 (n=173)	26.0 (22.0–32.0)	29.0 (23.3–38.0)	0.22 (n=63)
Gestational week of delivery, mean (SD)	36.8 (2.2)	36.8 (1.9)	0.98 (n=139)	37.0 (2.1)	36.6 (1.8)	0.45 (n=169)	36.8 (2.9)	37.9 (2.7)	0.14 (n=63)
In utero hydroxychloroquine, n (%)	7 (6.7)	0 (0.0)	0.35 (n=135)	8 (5.7)	0 (0.0)	0.36 (n=167)	1 (2.2)	0 (0.0)	1.00 (n=62)
In utero fluorinated steroids, n (%)	60 (56.1)	18 (58.1)	0.84 (n=138)	77 (53.1)	15 (62.5)	0.39 (n=169)	14 (31.1)	4 (23.5)	0.76 (n=62)
In utero beta agonist, n (%)	13 (12.4)	11 (35.5)	**0.003** (n=136)	21 (14.6)	7 (29.2)	0.13 (n=168)	5 (10.9)	3 (17.6)	0.67 (n=63)
In utero ventricular rate at detection (beats/min), mean (SD)	68.5 (17.9)	59.3 (9.8)	**0.003** (n=97)	69.3 (17.1)	61.7 (11.0)	0.09 (n=115)	66.8 (11.1)	55.3 (12.5)	**0.014** (n=40)
In utero ventricular nadir (beats/min), mean (SD)	55.8 (11.9)	47.9 (4.8)	**<0.001** (n=110)	56.4 (11.6)	51.0 (6.2)	**0.004** (n=132)	51.9 (10.8)	48.1 (12.4)	0.40 (n=44)
In utero EFE, n (%)	16 (16.5)	1 (3.7)	0.12 (n=124)	12 (9.8)	1 (5.0)	0.69 (n=143)	0 (0.0)	0 (0.0)	NA (n=42)
In utero DCM, n (%)	2 (2.1)	7 (25.0)	**<0.001** (n=125)	3 (2.4)	5 (22.7)	**0.002** (n=145)	1 (3.0)	2 (22.2)	0.11 (n=42)
In utero hydrops fetalis, n (%)	4 (4.2)	1 (3.7)	1.00 (n=123)	3 (2.4)	4 (21.1)	**0.006** (n=143)	1 (3.1)	2 (25.0)	0.10 (n=40)

Continued

Table 2 Continued

	Age 0–1 year (n=143)			Age >1–17 years (n=176)			Age >17 years (n=64)		
	Normal (n=111)	Cardiac dysfunction (n=32)	P value	Normal (n=150)	Cardiac dysfunction (n=26)	P value	Normal (n=46)	Cardiac dysfunction (n=18)	P value
Any in utero extranodal disease (EFE, DCM or hydrops), n (%)	22 (22.2)	9 (32.1)	0.28 (n=127)	18 (14.5)	9 (40.9)	**0.007** (n=146)	2 (6.1)	3 (33.3)	0.057 (n=42)
Severity score, median (IQR)	6.0 (5.0–8.0)	8.0 (8.0–14.0)	**0.003** (n=129)	5.0 (5.0–8.0)	8.0 (6.0–14.0)	**0.001** (n=148)	5.0 (5.0–6.0)	8.0 (8.0–14.0)	**0.003** (n=43)
Postnatal factors									
Pacemaker at the time of echo, n (%)	39 (35.1)	24 (75.0)	**<0.001**	103 (68.7)	24 (96.0)	**0.005** (n=175)	41 (89.1)	18 (100)	0.31
Length of time with pacemaker at the time of echo (years), median (IQR)	0.0 (0.0–0.1)	0.2 (0.0–0.4)	**<0.001**	2.5 (0.0–9.0)	6.8 (4.4–13.5)	**0.002** (n=175)	17.2 (12.3–18.9)	20.9 (19.1–24.7)	**<0.001**
Number of pacemakers at the time of echo, median (IQR)	0.0 (0.0–1.0)	1.0 (0.0–1.0)	**0.001** (n=140)	1.0 (0.0–2.0)	2.0 (1.0–3.0)	**<0.001** (n=169)	3.0 (2.0–4.0)	5.0 (3.0–5.0)	**0.001** (n=56)
Cumulative length of time RV paced at the time of echo (years), median (IQR)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.886 (n=131)	0.0 (0.0–1.2)	0.0 (0.0–12.2)	0.18 (n=152)	3.2 (0.0–11.5)	0.5 (0.0–14.2)	0.97 (n=41)
Cumulative length of time dual paced at the time of echo (years), median (IQR)	0.0 (0.0–0.0)	0.0 (0.0–0.3)	**0.005** (n=131)	0.0 (0.0–4.7)	2.8 (0.2–6.2)	0.10 (n=154)	5.0 (0.0–11.9)	16.1 (1.3–22.1)	0.19 (n=41)
Cumulative length of time BiV paced at the time of echo (years), median (IQR)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.25 (n=131)	0.0 (0.0–0.0)	0.0 (0.0–0.3)	**<0.001** (n=157)	0.0 (0.0–0.0)	0.0 (0.0–4.2)	**0.001** (n=47)

Bold indicates statistically significant p values (<0.05).
BiV, Biventricular; DCM, dilated cardiomyopathy; EFE, endocardial fibroelastosis; RV, right ventricular; SLE, systemic lupus erythematosus; SS, Sjogren's syndrome.

Table 3 Logistic regression models: cardiac dysfunction at age 0–1 year (N=143, n=32 events)

Predictor	Adjusted OR (95% CI)	P value
(A) Primary model		
Female (ref=male)	0.41 (0.17 to 0.98)	**0.046**
Age at echo at age 0–1 year	0.14 (0.02 to 1.15)	0.067
Mother's race		
Black (ref=white)	3.16 (0.49 to 20.57)	0.23
Other (ref=white)	1.59 (0.47 to 5.39)	0.45
In utero ventricular nadir (beats/min)	0.95 (0.90 to 1.01)	0.077
In utero extranodal disease (ref=no)	1.35 (0.43 to 4.29)	0.61
Length of time with pacemaker at echo (years) at age 0–1 year	10.82 (1.18 to 98.93)	**0.035**
(B) Alternative model		
Female (ref=male)	0.43 (0.18 to 1.02)	0.056
Age at echo at age 0–1 year	0.10 (0.01 to 0.75)	**0.025**
Mother's race		
Black (ref=white)	3.27 (0.52 to 20.54)	0.21
Other (ref=white)	1.59 (0.48 to 5.25)	0.45
Severity score	1.09 (0.95 to 1.25)	0.23
Length of time with pacemaker at echo (years) at age 0–1 year	15.53 (1.88 to 128.23)	**0.011**

Bold indicates statistically significant p values (<0.05).

*Missing data in predictor variables handled using multiple imputation; results based on 40 imputed data sets.

of disease in the majority of serially followed cases may result from clearance of anti-Ro from neonatal circulation and/or initial positive effects of pacing. The highest rates of morbidity occurred in the oldest age group, and an association with fetal severity score remained on multivariable analysis. It is possible that damage from the insult of more severe cardiac NL during initial presentation may predispose to dysfunction later in life. It is known that fibrosis can progress after maternal autoantibody clearance, as evidenced by cases of heart block progression from first to advanced degrees later in life. Furthermore, a 'burned out' myocardium is sometimes seen after childhood insults such as viral myocarditis or anthracycline exposure, with clinical manifestations appreciated only years later.[21 22] In this cohort, small percentages developed new-onset disease after having normal echocardiograms earlier in life, possibly related to the previously reported paradoxical effects from pacing or perhaps due to progression of myocardial damage after the earlier insult.[2]

Table 4 Logistic regression models: cardiac dysfunction at age >1–17 years (N=176, n=26 events)

Predictor	Adjusted OR (95% CI)	P value
(A) Primary model		
Female (ref=male)	0.45 (0.17 to 1.22)	0.12
Age at echo at age >1–17 years	0.83 (0.63 to 1.08)	0.17
Mother's race		
Black (ref=white)	10.28 (1.43 to 73.85)	**0.021**
Other (ref=white)	0.61 (0.07 to 5.29)	0.65
In utero ventricular nadir (beats/min)	0.99 (0.94 to 1.04)	0.56
In utero extranodal disease (ref=no)	3.11 (0.95 to 10.21)	0.061
Length of time with pacemaker at echo (years) at age >1–17 years	1.31 (1.01 to 1.68)	**0.039**
(B) Alternative model		
Female (ref=male)	0.43 (0.16 to 1.15)	0.093
Age at echo at age >1–17 years	0.83 (0.64 to 1.09)	0.18
Mother's race		
Black (ref=white)	11.04 (1.51 to 81.00)	**0.018**
Other (ref=white)	0.57 (0.07 to 4.88)	0.61
Severity score	1.17 (1.00 to 1.36)	**0.048**
Length of time with pacemaker at echo (years) at age >1–17 years	1.30 (1.01 to 1.66)	**0.04**

Bold indicates statistically significant p values (<0.05).
*Missing data in predictor variables handled using multiple imputation; results based on 40 imputed data sets.

Table 5 Logistic regression models: cardiac dysfunction at age >17 years (N=64, n=18 events)

Predictor	Adjusted OR (95% CI)	P value
(A) Primary model		
Age at echo at age >17 years	0.93 (0.79 to 1.08)	0.34
In utero ventricular rate at detection (beats/min)	0.96 (0.89 to 1.03)	0.26
In utero extranodal disease (ref=no)	6.22 (0.50 to 77.25)	0.15
Length of time with pacemaker at echo (years) at age >17 years	1.29 (1.05 to 1.59)	0.018
(B) Alternative model		
Age at echo at age >17 years	1.01 (0.86 to 1.18)	0.94
Severity score	1.42 (1.02 to 1.98)	**0.037**
Length of time with pacemaker at echo (years) at age >17 years	1.28 (1.04 to 1.58)	0.02

Bold indicates statistically significant p values (<0.05).
*Missing data in predictor variables handled using multiple imputation; results based on 40 imputed data sets.

Among demographic risk factors, female sex showed trends towards protection against postnatal cardiac dysfunction. There has been no previous sex difference noted regarding cardiac NL development, recurrence of disease or mortality.[3 4 18 19 23] However, in the Swedish cohort, men had lower heart rates, decreased shortening fraction and higher end-diastolic diameters.[15] While the association in our cohort was not robust, incidence rates and clinical outcomes of DCM in the general population are also more favourable in women.[24 25] Black race is associated with cardiac dysfunction at ages >1–17 years, although overall numbers were small. Minority race has previously been associated with mortality in the RRNL (with the highest percentage in black race), and non-European origin has been associated with both neonatal and late-onset DCM in the French study.[3 16] With multiple cohorts and different outcomes associated with non-white races, a genetic predisposition to severe disease in cardiac NL appears likely.

Pacemaker placement was associated with cardiac dysfunction during childhood, likely reflecting treatment of decreased function in this real-world cohort. The association between dual-chamber pacemakers at age 0–1 year and biventricular pacemakers in older ages is also consistent with standard of care and is not considered to be a causative factor of dysfunction. In contrast to previous reports, length of time RV paced did not associate with cardiac dysfunction.[2 26] The short length of RV pacing in all age groups is again likely due to avoidance of prolonged single chamber pacing given known adverse effects, particularly in autoimmune-mediated cardiac disease.[27] The overall length of time paced and the number of pacemakers were

significantly associated with dysfunction in all age groups. This could be evidence of an adverse effect of pacing, but may alternatively be a marker of more severe fetal or neonatal disease requiring earlier and more frequent intervention.

The primary limitations of this study are inherent in the rare nature of cardiac NL, with low numbers of available patients potentially affecting the power to determine associations. The small sample size in the >17-year group is due to the limited number of registry patients that have reached this age to date and not due to a loss of follow-up or relationship to mortality. The small number of black individuals makes determining the influence of race challenging. Given the nature of the 35-year existence of the RRNL, data have been gathered from multiple centres by many cardiologists over many eras of technology and resources. The RRNL has attempted to review original images when feasible; however, in the majority of cases, only echocardiogram reports and medical records could be evaluated. There is the potential for selection bias in that patients with cardiac dysfunction may seek care more frequently, increasing the rates of observed dysfunction in our cohort. In order to analyse risk factors and outcomes at agnostic time points, data from the last available echo at each age group were evaluated; thus, cardiac dysfunction occurring at other time points may not have been captured. The age ranges were chosen based on clinical and power considerations, and it is recognised that the childhood >1–17 years is a broad sampling. Nevertheless, there were similar percentages of children evaluated in the 0–5, >5–10 and >10–17 years.

This study represents the largest cohort of long-term follow-up in cardiac NL and the first to document outcomes serially and in multiple age ranges. Several factors associated with fetal disease severity remained associated with dysfunction into adulthood. These patients may have early subclinical damage that is more susceptible to future insults or the effects of prolonged pacing. Thus, close monitoring and aggressive treatment of early extranodal disease and bradycardia may have long-term benefit in preventing subsequent morbidity.

Contributors AS designed the study, collected and analysed the data and wrote the manuscript. PMI designed the study, analysed the data and helped write the manuscript. RPB collected the data and helped write the manuscript. RSG collected data and helped write the manuscript. DMF helped design the study, analyse the data and write the manuscript. RE and MYK helped design the study, performed statistical analysis and helped write the manuscript. JPB designed the study, analysed the data and led the study.

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REFERENCES

1 Buyon JP, Friedman DM, Buyon JP, *et al*. Neonatal lupus. In: Lahita RG, Tsokos G, Buyon JP, *et al*, eds. *Systemic lupus erythematosus*. 5th Edition. New York, NY: Academic Press, 2010: 541–71.
2 Moak JP, Barron KS, Hougen TJ, *et al*. Congenital heart block: development of late-onset cardiomyopathy, a previously underappreciated sequela. *J Am Coll Cardiol* 2001;37:238–42.
3 Izmirly PM, Saxena A, Kim MY, *et al*. Maternal and fetal factors associated with mortality and morbidity in a multi-racial/ethnic registry of anti-SSA/Ro-associated cardiac neonatal lupus. *Circulation* 2011;124:1927–35.
4 Eliasson H, Sonesson S-E, Sharland G, *et al*. Isolated atrioventricular block in the fetus: a retrospective, multinational, multicenter study of 175 patients. *Circulation* 2011;124:1919–26.
5 Satoh M, Chan EKL, Ho LA, *et al*. Prevalence and sociodemographic correlates of antinuclear antibodies in the United States. *Arthritis Rheum* 2012;64:2319–27.
6 Brucato A, Frassi M, Franceschini F, *et al*. Risk of congenital complete heart block in newborns of mothers with anti-Ro/SSA antibodies detected by counterimmunoelectrophoresis: a prospective study of 100 women. *Arthritis Rheum* 2001;44:1832–5.
7 Cimaz R, Spence DL, Hornberger L, *et al*. Incidence and spectrum of neonatal lupus erythematosus: a prospective study of infants born to mothers with anti-Ro autoantibodies. *J Pediatr* 2003;142:678–83.
8 Costedoat-Chalumeau N, Amoura Z, Lupoglazoff J-M, *et al*. Outcome of pregnancies in patients with anti-SSA/Ro antibodies: a study of 165 pregnancies, with special focus on electrocardiographic variations in the children and comparison with a control group. *Arthritis Rheum* 2004;50:3187–94.
9 Levesque K, Morel N, Maltret A, *et al*. Description of 214 cases of autoimmune congenital heart block: results of the French neonatal lupus syndrome. *Autoimmun Rev* 2015;14:1154–60.
10 Koh C, Hong W-J, Yung T-C, *et al*. Left ventricular torsional mechanics and diastolic function in congenital heart block with right ventricular pacing. *Int J Cardiol* 2012;160:31–5.
11 Guerra VC, Martins LdeM, Oliveira RM, *et al*. Prevalence of left ventricular dyssynchrony in patients with congenital atrioventricular block and long-term pacing: a three-dimensional echocardiographic study. *Echocardiography* 2015;32:1400–6.
12 Eronen M, Sirèn MK, Ekblad H, *et al*. Short- and long-term outcome of children with congenital complete heart block diagnosed in utero or as a newborn. *Pediatrics* 2000;106:86–91.
13 Udink ten Cate FE, Breur JM, Cohen MI, *et al*. Dilated cardiomyopathy in isolated congenital complete atrioventricular block: early and long-term risk in children. *J Am Coll Cardiol* 2001;37:1129–34.
14 Villain E, Coastedoat-Chalumeau N, Marijon E, *et al*. Presentation and prognosis of complete atrioventricular block in childhood, according to maternal antibody status. *J Am Coll Cardiol* 2006;48:1682–7.
15 Eliasson H, Sonesson S-E, Salomonsson S, *et al*. Outcome in young patients with isolated complete atrioventricular block and permanent pacemaker treatment: a nationwide study of 127 patients. *Heart Rhythm* 2015;12:2278–84.
16 Morel N, Lévesque K, Maltret A, *et al*. Incidence, risk factors, and mortality of neonatal and late-onset dilated cardiomyopathy associated with cardiac neonatal lupus. *Int J Cardiol* 2017;248:263–9.
17 Brix N, Ernst A, Lauridsen LLB, *et al*. Timing of puberty in boys and girls: a population-based study. *Paediatr Perinat Epidemiol* 2019;33:70–8.
18 Buyon JP, Hiebert R, Copel J, *et al*. Autoimmune-associated congenital heart block: demographics, mortality, morbidity and recurrence rates obtained from a national neonatal lupus registry. *J Am Coll Cardiol* 1998;31:1658–66.
19 Saxena A, Izmirly PM, Han SW, *et al*. Serum biomarkers of inflammation, fibrosis, and cardiac function in facilitating diagnosis, prognosis, and treatment of Anti-SSA/Ro-Associated cardiac neonatal lupus. *J Am Coll Cardiol* 2015;66:930–9.
20 Mofors J, Eliasson H, Ambrosi A, *et al*. Comorbidity and long-term outcome in patients with congenital heart block and their siblings exposed to Ro/SSA autoantibodies in utero. *Ann Rheum Dis* 2019;78:696–703.
21 Olivotto I, Cecchi F, Poggesi C, *et al*. Patterns of disease progression in hypertrophic cardiomyopathy: an individualized approach to clinical staging. *Circ Heart Fail* 2012;5:535–46.
22 Lindsey ML, Lange RA, Parsons H, *et al*. The tell-tale heart: molecular and cellular responses to childhood anthracycline exposure. *Am J Physiol Heart Circ Physiol* 2014;307:H1379–89.
23 Llanos C, Izmirly PM, Katholi M, *et al*. Recurrence rates of cardiac manifestations associated with neonatal lupus and maternal/fetal risk factors. *Arthritis Rheum* 2009;60:3091–7.
24 Codd MB, Sugrue DD, Gersh BJ, *et al*. Epidemiology of idiopathic dilated and hypertrophic cardiomyopathy. A population-based study in Olmsted County, Minnesota, 1975-1984. *Circulation* 1989;80:564–72.
25 McNamara DM, Starling RC, Cooper LT, *et al*. Clinical and demographic predictors of outcomes in recent onset dilated cardiomyopathy: results of the IMAC (Intervention in Myocarditis and Acute Cardiomyopathy)-2 study. *J Am Coll Cardiol* 2011;58:1112–8.
26 van Geldorp IE, Delhaas T, Gebauer RA, *et al*. Impact of the permanent ventricular pacing site on left ventricular function in children: a retrospective multicentre survey. *Heart* 2011;97:2051–5.
27 Capone C, Buyon JP, Friedman DM, *et al*. Cardiac manifestations of neonatal lupus: a review of autoantibody-associated congenital heart block and its impact in an adult population. *Cardiol Rev* 2012;20:72–6.

TRANSLATIONAL SCIENCE

Adenosine deaminase 2 as a biomarker of macrophage activation syndrome in systemic juvenile idiopathic arthritis

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ABSTRACT

Objective Macrophage activation syndrome (MAS) is a life-threatening complication of systemic juvenile idiopathic arthritis (sJIA) characterised by a vicious cycle of immune amplification that can culminate in overwhelming inflammation and multiorgan failure. The clinical features of MAS overlap with those of active sJIA, complicating early diagnosis and treatment. We evaluated adenosine deaminase 2 (ADA2), a protein of unknown function released principally by monocytes and macrophages, as a novel biomarker of MAS.

Methods We established age-based normal ranges of peripheral blood ADA2 activity in 324 healthy children and adults. We compared these ranges with 173 children with inflammatory and immune-mediated diseases, including systemic and non-systemic JIA, Kawasaki disease, paediatric systemic lupus erythematosus and juvenile dermatomyositis.

Results ADA2 elevation beyond the upper limit of normal in children was largely restricted to sJIA with concomitant MAS, a finding confirmed in a validation cohort of sJIA patients with inactive disease, active sJIA without MAS or sJIA with MAS. ADA2 activity strongly correlated with MAS biomarkers including ferritin, interleukin (IL)-18 and the interferon (IFN)-γ-inducible chemokine CXCL9 but displayed minimal association with the inflammatory markers C reactive protein and erythrocyte sedimentation rate. Correspondingly, ADA2 paralleled disease activity based on serial measurements in patients with recurrent MAS episodes. IL-18 and IFN-γ elicited ADA2 production by peripheral blood mononuclear cells, and ADA2 was abundant in MAS haemophagocytes.

Conclusions These findings collectively identify the utility of plasma ADA2 activity as a biomarker of MAS and lend further support to a pivotal role of macrophage activation in this condition.

Key messages

What is already known about this subject?

► Macrophage activation syndrome (MAS) is a life-threatening complication of systemic juvenile idiopathic arthritis (sJIA). Because clinical manifestations of active sJIA and MAS overlap, distinguishing biomarkers are needed for rapid diagnosis and treatment.

What does this study add?

► We defined the normal range in peripheral blood of the monocyte/macrophage-secreted protein adenosine deaminase 2 (ADA2) in healthy children and adults.

► We found that ADA2 activity above the upper limit of normal distinguishes MAS in sJIA with high sensitivity and specificity, potentially reflect direct stimulation of monocytes and macrophages by cytokines implicated in MAS.

How might this impact on clinical practice or future developments?

► Our study demonstrates the utility of peripheral blood ADA2 activity as a direct biomarker of macrophage activation to facilitate rapid diagnosis of MAS in sJIA.

INTRODUCTION

Adenosine deaminases (ADA) are a family of enzymes that catalyse the conversion of adenosine to inosine. ADA1 is a ubiquitously expressed intracellular protein that metabolises adenosine and 2′-deoxyadenosine as a crucial step in the purine salvage pathway.[1] Deficiency of ADA1 in humans results in defective lymphocyte development and severe combined immunodeficiency.[2] In contrast, ADA2 is a plasma protein secreted primarily by monocytes and macrophages.[3 4] ADA2 displays much lower substrate affinity compared with ADA1 and its physiological role remains unclear.[5] Supporting a non-redundant role of the ADA isoenzymes, deficiency of ADA2 (DADA2) is a recently described autoinflammatory syndrome characterised by childhood-onset stroke, systemic vasculitis, variable immunodeficiency and haematological defects.[5–7]

In adults, elevated levels of ADA in biological fluids have been described in infections, malignancies, autoimmune diseases and secondary

haemophagocytic syndromes.[3 8–11] However, many early studies focused on ADA1 and little is known about the biology of ADA2. ADA2 levels are higher in children than adults.[7] Whether abnormal ADA2 production is also a hallmark of paediatric inflammatory conditions has not been examined in detail. In this study, we established the normal range of peripheral blood ADA2 activity in healthy children and demonstrated a striking elevation in ADA2 as a sensitive and specific marker of the conversion between active systemic juvenile idiopathic arthritis (sJIA) and sJIA-associated macrophage activation syndrome (MAS), likely reflecting underlying cytokine-driven activation of monocytes and macrophages.

METHODS

Human subjects

Informed consent was provided by participants or legal guardians. Plasma or serum were frozen at –80°C and thawed immediately prior to testing. Demographics of patient groups and diagnostic criteria are provided in online supplementary table S1 and supplemental methods, respectively.

Quantification of ADA2 activity

ADA2 activity was measured in human plasma, serum or cell culture supernatant by modifying a previously described automated spectrophotometric assay,[12–14] which quantifies the adenosine-dependent generation of ammonia in the presence of a selective inhibitor of ADA1, EHNA (erythro-9-Amino-β-hexyl-α-methyl-9H-purine-9-ethanol hydrochloride). All reagents were purchased from Sigma Aldrich (St. Louis, Missouri, USA), and the kinetics of each reaction were analysed using a Synergy Hybrid H1 Microplate Reader (BioTek, Winooski, Vermont, USA). Additional details are provided in supplementary methods.

ELISA, in vitro stimulation, flow cytometry and confocal microscopy

Please refer to supplementary methods for detailed protocol for these studies.

Statistical analysis

Because ADA2 levels in healthy children were not normally distributed (online supplementary figure 1C), non-parametric tests were used for statistical analysis. The differences between two groups were analysed using the Mann-Whitney U test, while comparison of multiple groups was performed using the Kruskal-Wallis test. For each disease studied, 1:1 age-matched controls were randomly assigned from the pool of healthy controls. All tests were two sided, and $p<0.05$ was considered significant. For Bonferroni correction of multiple hypothesis testing, α was adjusted to 0.0014 for the correlation matrix. Statistical analyses were performed using Prism 5.0 software (GraphPad Software, La Jolla, California, USA).

RESULTS

Establishing a reference range of ADA2 activity in healthy children

We first aimed to establish the reference range of plasma ADA2 activity in 324 healthy individuals (174 children and 150 adults). We employed a validated spectrophotometric assay to quantify ADA2 activity in plasma or serum ((online supplementary figure S1A); see Methods). Confirming the specificity of the assay, DADA2 patients with biallelic *ADA2* mutations show a near absence of ADA2 activity, whereas carriers have approximately half-normal plasma ADA2 activity (online supplementary figure S1B). The distribution of plasma ADA2 activity in healthy children (under age 18 years) was skewed towards higher levels (online supplementary figure S1C), with a median of 13.0 U/L (IQR 10.6–16.1). The upper limit of normal (ULN), as defined by the 98th percentile, was 27.8 U/L. Comparison of males and females revealed similar ADA2 levels (online supplementary figure S1D).

Consistent with previous studies,[7 15] plasma ADA2 activity was higher in children than adults (age 18 years and older, 98th percentile 25.7 U/L), with an overall negative correlation with age (figure 1A). Stratification of healthy children into age categories did not reveal differences in ADA2 levels among groups (figure 1B; $p>0.05$ for all comparisons). While all paediatric categories showed significantly higher median levels compared with healthy adults, considerable variability was displayed within each age group.

Evaluation of ADA2 activity in paediatric inflammatory diseases

We compared ADA2 levels in children with various inflammatory conditions with age-matched healthy controls. Demographics of controls and patient groups are provided in online supplementary table S1. We first studied Kawasaki disease (KD), a highly inflammatory childhood vasculitis manifested by skin rash, mucositis, extremity swelling, conjunctivitis and lymphadenopathy. All KD

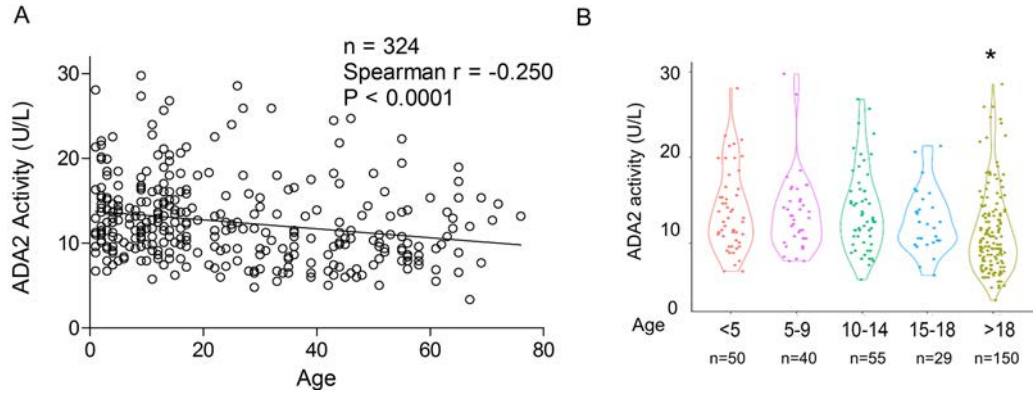


Figure 1 Determination of plasma ADA2 activity in healthy children and adults. (A) Correlation between plasma ADA2 activity and age in healthy children (n=174) and adults (n=150). (B) Violin plot comparing plasma ADA2 activity in healthy individuals stratified by age. *P<0.05 compared with all other groups.

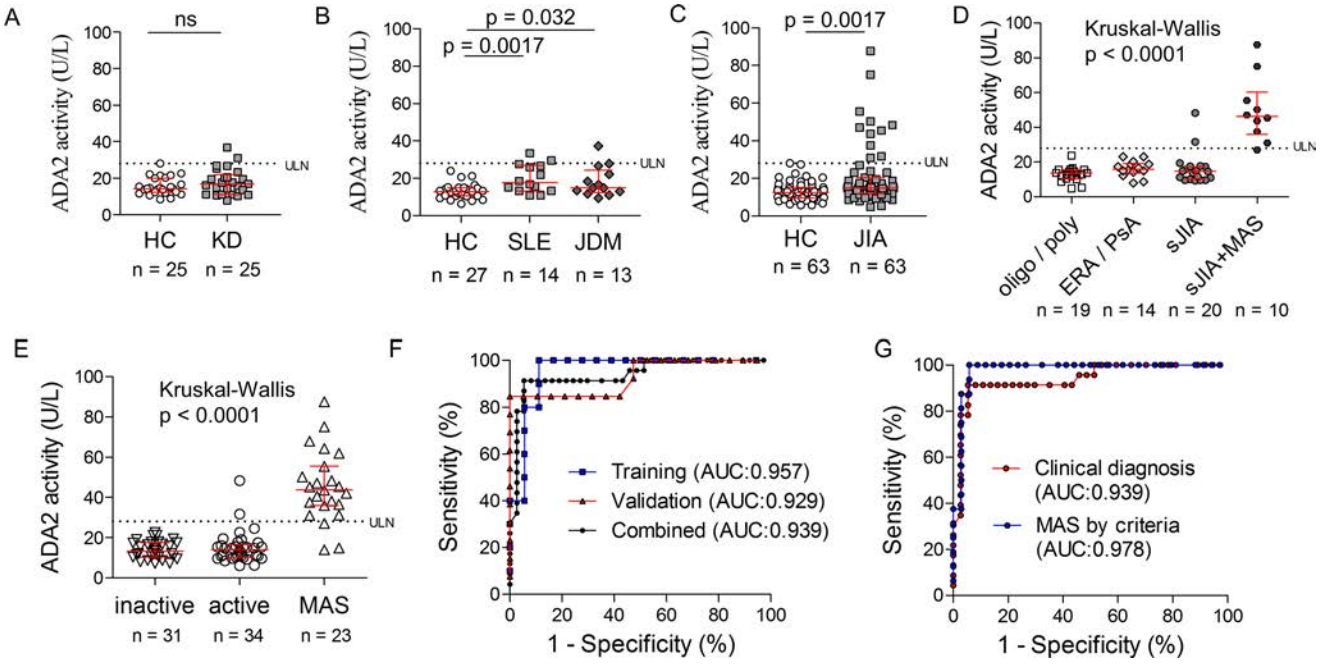


Figure 2 Comparison of ADA2 activity levels in childhood inflammatory diseases. (A) Peripheral blood ADA2 levels in patients with KD during the acute phase of illness and age-matched healthy controls (n=25 per group). (B) Peripheral blood ADA2 levels in patients with pSLE (n=14), patients with JDM (n=13) and age-matched healthy controls (n=27). (C) Peripheral blood ADA2 levels in all patients with JIA and age-matched healthy controls (n=63 per group). (D) Peripheral blood ADA2 levels in patients with JIA stratified by disease category based on the ILAR classification. (E) Stratification of plasma ADA2 levels in sJIA patients by disease activity and MAS (training and validation cohorts combined; n=88). (F) ROC curve of ADA2 in distinguishing active sJIA with or without MAS in training, validation and combined patient cohorts. (G) ROC curve of ADA2 in distinguishing active sJIA with or without MAS diagnosed clinically or MAS diagnosed according to the 2016 MAS classification criteria. Median and IQR are displayed in scatter dot plots. ADA2, adenosine deaminase 2; AUC, area under the curve; ERA, enthesitis-related arthritis; ILAR, International League of Associations for Rheumatology; JDM, juvenile dermatomyositis; JIA, juvenile idiopathic arthritis; KD, Kawasaki disease; MAS, macrophage activation syndrome; PsA, psoriatic arthritis; pSLE, paediatric systemic lupus erythematosus; ROC, receiver operating characteristic; sJIA, systemic juvenile idiopathic arthritis; ULN, upper limit of normal.

samples were collected during the acute phase of disease, prior to treatment. Compared with healthy controls, patients with KD showed similar ADA2 activity (n=25 per group; figure 2A). Elevated C reactive protein (CRP), erythrocyte sedimentation rate (ESR), and white blood cell count in patients with KD did not correlate with ADA2 levels (online supplementary figure S2A), establishing that ADA2 is not a general marker of systemic inflammation.

To examine if ADA2 activity is altered in chronic autoimmune conditions, we studied children with paediatric SLE (pSLE; n=14) and JDM (n=13), most with active disease at the time of sampling (online supplementary table S1). pSLE patients displayed increased plasma ADA2 compared with age-matched controls (figure 2B; 17.6 U/L (13.2–26.8) vs 12.8 U/L (10.5–15.0), p=0.0017), although levels remained predominantly below the ULN. ADA2 levels in patients with JDM (14.9 U/L (12.3–24.4)) were also mildly increased compared with controls (p=0.032) and statistically indistinguishable from pSLE (p=0.50). ADA2 levels did not correlate with markers of disease activity in pSLE (complement C3, C4 and ESR; online supplementary figure S2B) or JDM (aldolase and LDH; online supplementary figure S2C).

Establishing ADA2 as a marker of MAS in sJIA

We next investigated ADA2 levels in patients with JIA. While most JIA patients showed plasma ADA2 activity comparable to age-matched controls, a small subset displayed levels well above the ULN (figure 2C). JIA can be classified into distinct categories based on the International League of Associations for Rheumatology (ILAR) criteria.[16] Stratification by JIA categories revealed ADA2 activity in children with oligoarticular JIA, polyarticular JIA, enthesitis-related arthritis and psoriatic arthritis comparable to healthy controls (figure 2D). All 11 patients with ADA2 levels above the ULN shared the diagnosis of systemic JIA, including 9/10 cases with MAS at the time of sampling.

Distinct from other forms of childhood arthritis, systemic JIA exhibits quotidian fever, lymphadenopathy and systemic inflammation accompanied by variable joint involvement. MAS is a life-threatening complication of sJIA characterised by cytokine storm, haemophagocytosis, cytopaenias, coagulopathy and multiorgan dysfunction. In contrast to the high levels of ADA2 in patients with MAS, most sJIA patients without MAS showed normal levels of ADA2 (figure 2D).

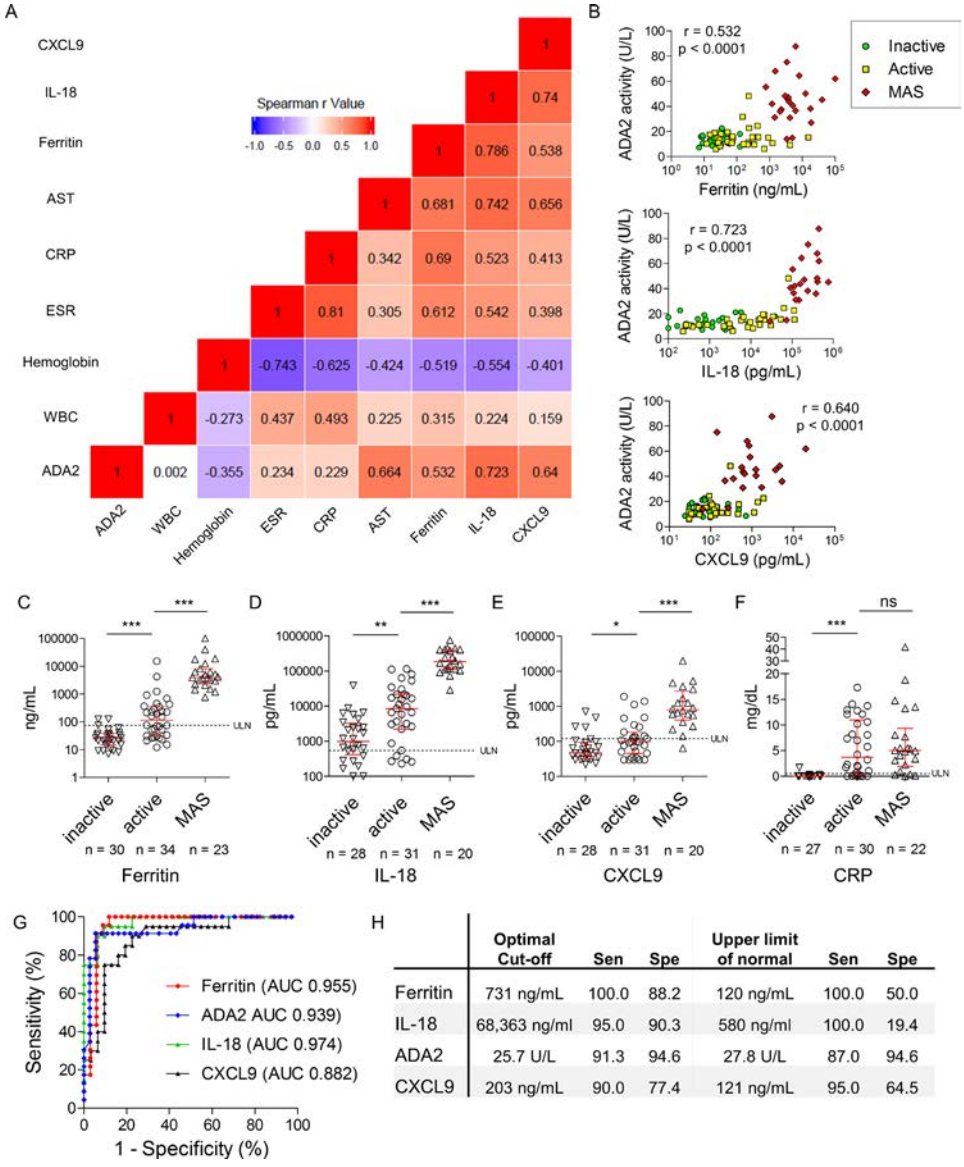
To confirm these findings, we measured ADA2 levels in an independent validation cohort of 58 sJIA patients combined from two other institutions. Consistent with our initial observation, patients with MAS displayed significantly higher levels of ADA2 compared with those without MAS, regardless of sJIA disease activity (online supplementary figure S3A). Combined analysis of both sJIA cohorts showed that ADA2 levels beyond the ULN distinguished cases of MAS and active sJIA with a sensitivity of 86% and specificity of 94% (figure 2E). The utility of ADA2 as a biomarker of MAS was supported by receiver operating characteristic (ROC) curve with area under the curve (AUC) of 0.939 (95% CI 0.87 to 1.00; figure 2F).

The diagnosis of MAS in these patients was determined clinically by the treating physicians. Review of laboratory data revealed that 15 of the 23 cases fulfilled the 2016 Classification Criteria for MAS.[17] The remaining cases all had hyperferritinaemia (>684 ng/mL) but either did not meet at least two of the criteria (n=4; 'No' group) or met one of the minor criteria but did not have complete laboratory parameters to apply the full criteria (n=4; 'Partial' group). Notably, patients who did not meet MAS criteria displayed significantly lower ADA2 levels compared with those who fully or partially met the criteria (online supplementary figure S3B). The performance of ADA2 on ROC was enhanced when the MAS group was filtered for patients fulfilling the formal classification criteria (AUC=0.978; figure 2G).

ADA2 measurements from healthy controls, and all patient groups are displayed in online supplementary file 1 to allow direct comparison. While MAS can also occur with other inflammatory diseases, we did not identify any case of MAS in our KD, SLE or JDM cohorts. Taken together, these data demonstrate the utility of ADA2 as a biomarker for MAS associated with sJIA.

Comparison of ADA2 with other markers of MAS

Next, we compared ADA2 activity in sJIA patients with serological parameters of inflammation and biomarkers of MAS including ferritin, interleukin (IL)-18[18 19] and the interferon γ-inducible chemokine CXCL9,[20] combining training and validation cohorts. A correlation matrix was created based on the Spearman r values



	Optimal Cut-off	Sen	Spe	Upper limit of normal	Sen	Spe
Ferritin	731 ng/mL	100.0	88.2	120 ng/mL	100.0	50.0
IL-18	68,363 ng/ml	95.0	90.3	580 ng/ml	100.0	19.4
ADA2	25.7 U/L	91.3	94.6	27.8 U/L	87.0	94.6
CXCL9	203 ng/mL	90.0	77.4	121 ng/mL	95.0	64.5

Figure 3 Comparison of ADA2 and other biomarkers of MAS. (A) Correlation matrix of ADA2 activity with laboratory parameters in sJIA. Heat-map displays the strength of correlation based on Spearman's rank correlation coefficient. (B) Correlations of ADA2 with serum ferritin, IL-18 and CXCL9. All patients with sJIA (inactive, active and MAS groups) were included for calculation of Spearman's rank correlation coefficient. (C–F) Comparison of peripheral blood ferritin, IL-18, CXCL9 and CRP levels in sJIA patient with inactive disease, active disease or MAS. Median and IQR are displayed in scatter dot plots. (G) ROC curves of ferritin, ADA2, IL-18 and CXCL9. (H) Sensitivity (SEN) and specificity (SPE) of MAS biomarkers using the optimised cut-off or upper limit of normal as cut-off. *P<0.05, **p<0.001, ***p<0.0001. ADA2, adenosine deaminase 2; AST, aspartate aminotransferase; AUC, area under the curve; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; MAS, macrophage activation syndrome; ROC, receiver operating characteristic; sJIA, systemic juvenile idiopathic arthritis; ULN, upper limit of normal; WBC, white blood cell count.

of the cross comparisons (figure 3A). Individual comparisons showed that ADA2 levels correlated well with ferritin (r=0.532; p<0.0001), IL-18 (r=0.723; p<0.0001) and CXCL9 (r=0.640; p<0.0001; figure 3B). These correlations remained statistically significant after Bonferroni correction. ADA2 activity also correlated well with increased aspartate aminotransferase levels in MAS (online supplementary figure S4A). By contrast, ADA2 activity did not correlate with conventional markers of inflammation including ESR and CRP after adjusting for multiple comparisons (online supplementary figure S4B, C).

Unlike the specific elevation of ADA2 in MAS (figure 2E), ferritin levels were normal in inactive sJIA and displayed a stepwise increase in active sJIA and MAS groups (figure 3C). As described by previous studies,[18 19] IL-18 was elevated in the majority of patients with sJIA, including many with inactive disease (figure 3D). Higher levels of IL-18 were observed with active disease, and log-fold increases were seen the MAS group. CXCL9 was significantly elevated in the MAS group compared with the active sJIA group (figure 3E), matching the observations by Bracaglia and colleagues.[20] Only a small difference in CXCL9 levels was observed between the inactive and active sJIA groups.

Despite the different patterns displayed by these markers, all of them were sensitive and specific in discriminating MAS from active sJIA (figure 3G). Unlike other markers, ADA2 effectively distinguished MAS from active sJIA using the ULN as cut-off, without the need to optimise the cut-off value (figure 3H online supplementary tables S2 and S3). The recently described ferritin-to-ESR ratio[21] also correlated with ADA2 levels and performed well as an MAS biomarker (online supplementary figure 4D-F). In contrast, CRP could differentiate active versus inactive disease in sJIA but performed poorly as a biomarker of MAS (figure 3F).

Because features of MAS including ferritin levels may be influenced by biological therapy,[22] we evaluated the relationship between ADA2 activity and treatment choices in MAS. More than half of patients in the combined MAS cohort received IL-1 blockade (anakinra or canakinumab); one patient received tocilizumab, and three patients received combined IL-1 and IL-6 blockade. Overall, we did not find differences in ADA2 activity related to biological therapy (online supplementary figure S4G).

Longitudinal evaluation of ADA2 in patients with recurrent MAS

Serial samples were available from seven MAS patients, with MAS classification criteria data at each time point. Consistent with our cross-sectional analysis, ADA2 levels were generally higher during confirmed MAS episodes compared with time points without MAS (figure 4A). As illustrated by a patient with recurrent MAS episodes, longitudinal measures of ADA2 activity trended closely with serum ferritin (figure 4B).

Notably, patients with recurrent MAS episodes exhibited ADA2 levels near the ULN even in the absence of MAS (figure 4A). Expanding on this observation, we asked whether a history of MAS was associated with differences in ADA2 activity in the inactive and active sJIA groups (without MAS at the time of sampling). Among the 65 patients in these two groups (figure 2E), 21 had a history of at least one MAS episode; these patients collectively displayed significantly higher levels of ADA2 activity compared with the group without a history of MAS (figure 4C; median 17.5 U/L vs 11.8 U/L, p=0.005).

Mechanism and cellular source of ADA2 production

The pathophysiology of MAS is reminiscent of primary haemophagocytic lymphohistiocytosis, wherein the inability to remove activated leucocytes results in a vicious cycle of reciprocal immune activation by NK/T cells and macrophages.[23] Cytokine storm is a hallmark of MAS, and repeated stimulation by Toll-like receptor ligands (TLR) causes excess cytokine production and a MAS-like disease in mice.[24] To understand what drives the expression of ADA2 in MAS, we stimulated peripheral blood mononuclear cells (PBMCs) from healthy donors with selected cytokines and TLR ligands. Stimulation with IFNγ, IL-12, IL-18 and TNF-α increased ADA2 activity in the supernatant (figure 5A). Many of these cytokines, especially IFN-γ (as measured by its proxy CXCL9) and IL-18, are highly elevated in MAS.[18 20] Combining IL-18 and IFN-γ did not further enhance ADA2 production (online supplementary figure S5A). In contrast, ADA2 levels were not altered by IL-1β, IL-4, IL-6, IL-10, IFN-α or transforming growth factor β (TGF-β). While plasma IL-10 and TGF-β levels were elevated in a subset of patients with MAS and the two cytokines correlated with each other, they did not correlate with ADA2 levels (online supplementary figure S5B, C). Neither M1/M2 polarisation of

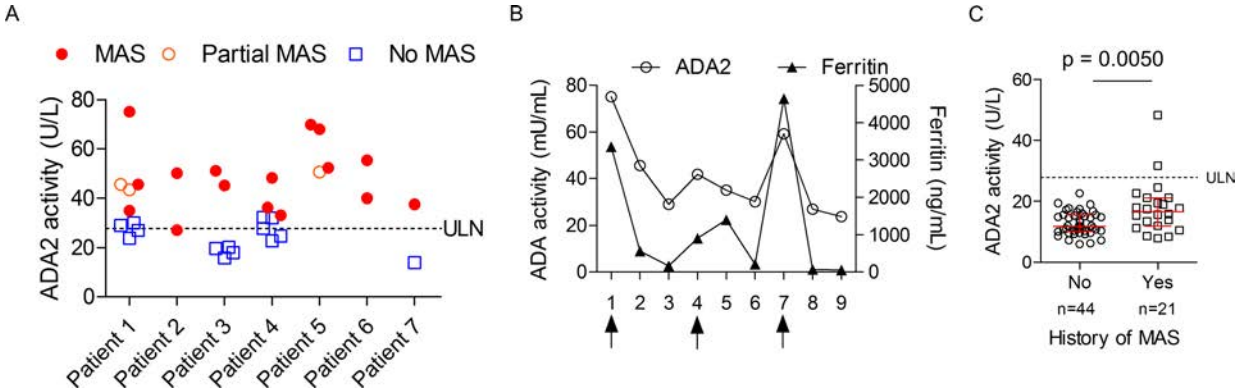


Figure 4 Longitudinal analysis of ADA2 levels in patients with sJIA-associated MAS. (A) Display of ADA2 activity measurements from multiple visits in seven sJIA patients with recurrent MAS. Laboratory parameters from each time point were reviewed to determine the presence of MAS based on the 2016 MAS classification criteria. (B) Longitudinal display of ADA2 activity and serum ferritin levels in a patient with recurrent MAS. Arrows indicate confirmed MAS episodes based on the classification criteria. (C) ADA2 activity in patients with inactive and active sJIA (without active MAS) stratified by history of MAS. Median and IQR are displayed in panel C. ADA2, adenosine deaminase 2; MAS, macrophage activation syndrome; sJIA, systemic juvenile idiopathic arthritis; ULN: upper limit of normal.

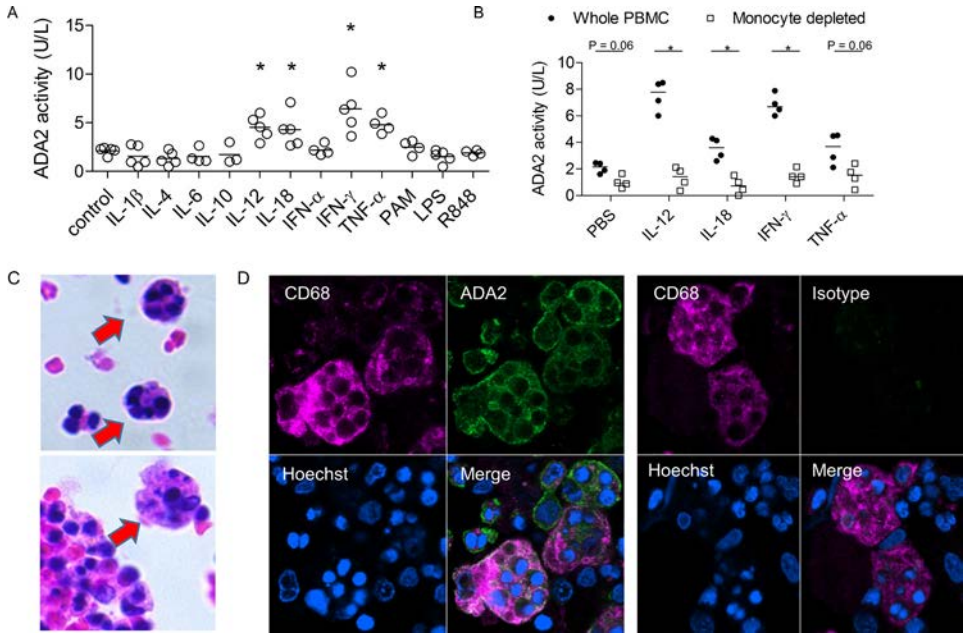


Figure 5 Mechanism and source of ADA2 production in MAS. (A) ADA2 activity in the supernatant of healthy donor PBMC stimulated with cytokines or TLR ligands for 5 days. (B) ADA2 activity in the supernatant of total donor PBMC or monocyte-depleted PBMC following cytokine stimulation for 5 days. Dots represent results from 3 to 5 healthy donors per condition. *P<0.05 compared with unstimulated control (panel A) or compared with whole PBMC (panel B). (C) H&E staining of bone marrow aspirate illustrating the presence of haemophagocytes. (D) Confocal microscopy of ADA2 staining and isotype staining in $CD68^+$ haemophagocytes. ADA2, adenosine deaminase 2; MAS, macrophage activation syndrome; PBMC, peripheral blood mononuclear cell; TLR, Toll-like receptor ligand.

macrophages nor stimulation with TLR ligands affected ADA2 activity (figure 5A and online supplemental figure S5D), further showing that ADA2 release is closely regulated.

Early studies found that monocytes and macrophages are primary sources of ADA2.[3 4] We performed both monocyte depletion and enrichment studies and confirmed that ADA2 production by PBMC was primary derived from monocytes, with or without cytokine stimulation (figure 5B and online supplementary figure S5E). Thus, ADA2 activity represents a functional readout of monocyte/macrophage activation by cytokines implicated in the pathogenesis of MAS.

Haemophagocytosis by activated macrophages is a hallmark histological finding of MAS.[23] To investigate whether these tissue macrophages represent a source of ADA2, we performed confocal microscopy of bone marrow from a patient with adult onset Still's disease and overt MAS. An abundance of haemophagocytes engulfing other leukocytes was evident (H&E staining; figure 5C). Remarkably, by confocal microscopy, haemophagocytes expressing the macrophage marker CD68 revealed particularly strong expression of ADA2 using polyclonal anti-ADA2 antibodies (figure 5D). Isotype staining confirmed the specificity of ADA2 staining, and similar results were found using a monoclonal antibody for ADA2 (figure 5D and online supplementary figure S6). These data support monocytes and macrophages, including haemophagocytes, as likely sources of ADA2 in MAS.

Discussion

MAS is a life-threatening complication that occurs in sJIA and other inflammatory conditions. Unopposed activation of immune cells and excess cytokine production results in a vicious cycle of inflammation that can lead to rapid clinical deterioration. Biomarkers of MAS could assist with early detection and therapeutic monitoring of this dangerous complication. Our study now establishes ADA2 as a novel biomarker of MAS in patients with sJIA.

Hallmark findings of MAS include hyperferritinaemia, cytopaenias, transaminase elevation and coagulopathy. Activation of T cells, NK cells and myeloid cells in MAS is reflected in markers such as soluble IL-2 receptor (CD25), soluble CD163, IL-18 and the IFN-γ-induced chemokines CXCL9 and CXCL10. In two cohorts of sJIA patients, we found that ADA2 levels were largely normal in both inactive and active sJIA as long as MAS was absent. Using the ULN established in healthy children, we found that ADA2 is a sensitive and specific marker that distinguishes MAS from active sJIA. While elevation of ferritin, IL-18 and CXCL9 are all indicative of MAS, cut-off values well above the ULN are required to distinguish MAS from active sJIA.

ADA2 levels are also increased in the setting of certain infections, including HIV and tuberculosis,[8 25] perhaps in part due to the production of IL-18 and IFN-γ. While ADA2 is a specific marker of MAS in children with sJIA, it remains to be determined whether it can distinguish MAS from infection. Importantly, ADA2 levels were not increased in acute KD, confirming that ADA2 is not simply a marker of systemic inflammation. ADA2 levels were mildly increased in patients with pSLE and JDM compared with healthy controls. The increased ADA2 levels may reflect abnormal monocyte/macrophage activation and cytokine production associated with these diseases, which can also be complicated by MAS.[26–28]

The factors that regulate ADA2 production have not been studied.[1 4] We showed monocytes to be the major producers of ADA2 in PBMC. Using confocal microscopy, we also showed abundant expression of ADA2 in MAS haemophagocytes. Furthermore, we found that stimulation with a specific panel of cytokines increased the production and release of ADA2. Extremely high levels of these cytokines, especially IL-18 and IFN-γ, provides a potential mechanistic explanation for the elevated plasma ADA2 level in sJIA-associated MAS.[28]

Whether ADA2 contributes to the pathophysiology of MAS is not clear. The physiological function of ADA2 remains to be determined. Features of inflammatory vasculitis in patients with DADA2 suggests an immunomodulatory function of ADA2 as well as a role in preventing vasculopathy. Depending on context and receptor utilisation, the ADA substrate adenosine can be proinflammatory or anti-inflammatory.[29] The reaction product inosine also has immunomodulatory functions.[30] The role of adenosine and inosine in MAS is a topic of interest for future study.

In summary, we establish the normal range of peripheral blood ADA2 activity in children and demonstrate that levels above this range are sensitive and specific for MAS associated with sJIA. The monocyte/macrophage origin of ADA2 and induction by IL-18 and IFN-γ lend further support to the key role of macrophage activation in this life-threatening complication of sJIA.

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Contributors PL and PAN conceived and designed the study. PL, YH, JS, YL, RB, AW and TD performed the experiments and acquired data. PL, GS, SC, KJH, OH, MHC, FD, EM, MSL, RS, ETR, JN, MBS, LAH and PAN recruited patients and collected samples. MSH provided the method for measuring ADA2 activity and in some cases confirmed results. PL, JS, YL and PAN analysed the data. PYL and PAN drafted the manuscript and all authors edited the manuscript.

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Competing interests None declared.

Patient and public involvement statement This research was done without direct patient involvement beyond sample collection. Patientsdid not participate in designing the study, analyzing the data, or drafting the manuscript.

Patient consent for publication Not required.

Ethics approval These studies were approved by the Institutional Review Boards at Boston Children's Hospital (P00005723), Cincinnati Children's Hospital Medical Center (2016-2234), University of Pittsburg (PRO16120025) and Brigham and Women's Hospital (P000664). Informed consent was provided by participants or legal guardians.

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REFERENCES

1 Ungerer JP, Oosthuizen HM, Bissbort SH, *et al*. Serum adenosine deaminase: isoenzymes and diagnostic application. *Clin Chem* 1992;38:1322–6.
2 Giblett ER, Anderson JE, Cohen F, *et al*. Adenosine-Deaminase deficiency in two patients with severely impaired cellular immunity. *Lancet* 1972;2:1067–9.
3 Ratech H, Martiniuk F, Borer WZ, *et al*. Differential expression of adenosine deaminase isozymes in acute leukemia. *Blood* 1988;72:1627–32.
4 Iwaki-Egawa S, Yamamoto T, Watanabe Y. Human plasma adenosine deaminase 2 is secreted by activated monocytes. *Biol Chem* 2006;387:319–21.
5 Schrader WP, Pollara B, Meuwissen HJ. Characterization of the residual adenosine deaminating activity in the spleen of a patient with combined immunodeficiency disease and adenosine deaminase deficiency. *Proc Natl Acad Sci U S A* 1978;75:446–50.
6 Navon Elkan P, Pierce SB, Segel R, *et al*. Mutant adenosine deaminase 2 in a polyarteritis nodosa vasculopathy. *N Engl J Med* 2014;370:921–31.
7 Zhou Q, Yang D, Ombrello AK, *et al*. Early-Onset stroke and vasculopathy associated with mutations in ADA2. *N Engl J Med* 2014;370:911–20.
8 Gakis C, Calia G, Naitana A, *et al*. Serum adenosine deaminase activity in HIV positive subjects. A hypothesis on the significance of ADA2. *Panminerva Med* 1989;31:107–13.
9 Stancíková M, Lukác J, Istok R, *et al*. Serum adenosine deaminase activity and its isoenzyme pattern in patients with systemic lupus erythematosus. *Clin Exp Rheumatol* 1998;16:583–6.
10 Sari RA, Taysi S, Yilmaz O, *et al*. Correlation of serum levels of adenosine deaminase activity and its isoenzymes with disease activity in rheumatoid arthritis. *Clin Exp Rheumatol* 2003;21:87–90.
11 Chen W, Zhang S, Zhang W, *et al*. Elevated serum adenosine deaminase levels in secondary hemophagocytic lymphohistiocytosis. *Int J Lab Hematol* 2015;37:544–50.
12 Lee PY, Huang Y, Zhou Q, *et al*. Disrupted N-linked glycosylation as a disease mechanism in deficiency of ADA2. *J Allergy Clin Immunol* 2018;142:e1368:1363–5.
13 Muraoka T, Katsuramaki T, Shiraishi H, *et al*. Automated enzymatic measurement of adenosine deaminase isoenzyme activities in serum. *Anal Biochem* 1990;187:268–72.
14 Slaats EH, Asberg EG, van Keimpema AR, *et al*. A continuous method for the estimation of adenosine deaminase catalytic concentration in pleural effusions with a Hitachi 705 discrete analyser. *J Clin Chem Clin Biochem* 1985;23:677–82.
15 Nanthapisal S, Murphy C, Omoyinmi E, *et al*. Deficiency of adenosine deaminase type 2: a description of phenotype and genotype in fifteen cases. *Arthritis Rheumatol* 2016;68:2314–22.
16 Petty RE, Southwood TR, Manners P, *et al*. International League of associations for rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton, 2001. *J Rheumatol* 2004;31:390–2.
17 Ravelli A, Minoia F, Davi S, *et al*. 2016 classification criteria for macrophage activation syndrome complicating systemic juvenile idiopathic arthritis: a European League against Rheumatism/American College of Rheumatology/Paediatric rheumatology international trials organisation collaborative initiative. *Arthritis Rheumatol* 2016;68:566–76.
18 Shimizu M, Yokoyama T, Yamada K, *et al*. Distinct cytokine profiles of systemic-onset juvenile idiopathic arthritis-associated macrophage activation syndrome with particular emphasis on the role of interleukin-18 in its pathogenesis. *Rheumatology* 2010;49:1645–53.
19 Weiss ES, Girard-Guyonvarc'h C, Holzinger D, *et al*. Interleukin-18 diagnostically distinguishes and pathogenically promotes human and murine macrophage activation syndrome. *Blood* 2018;131:1442–55.
20 Bracaglia C, de Graaf K, Pires Marafon D, *et al*. Elevated circulating levels of interferon-γ and interferon-γ-induced chemokines characterise patients with macrophage activation syndrome complicating systemic juvenile idiopathic arthritis. *Ann Rheum Dis* 2017;76:166–72.
21 Eloseily EMA, Minoia F, Crayne CB, *et al*. Ferritin to erythrocyte sedimentation rate ratio: simple measure to identify macrophage activation syndrome in systemic juvenile idiopathic arthritis. *ACR Open Rheuma* 2019;34.
22 Schulert GS, Minoia F, Bohnsack J, *et al*. Effect of biologic therapy on clinical and laboratory features of macrophage activation syndrome associated with systemic juvenile idiopathic arthritis. *Arthritis Care Res* 2018;70:409–19.
23 Al-Samkari H, Berliner N, Lymphohistiocytosis H. Hemophagocytic lymphohistiocytosis.. *Annu Rev Pathol* 2018;13:27–49.
24 Behrens EM, Canna SW, Slade K, *et al*. Repeated TLR9 stimulation results in macrophage activation syndrome-like disease in mice. *J Clin Invest* 2011;121:2264–77.
25 Ungerer JP, Oosthuizen HM, Retief JH, *et al*. Significance of adenosine deaminase activity and its isoenzymes in tuberculous effusions. *Chest* 1994;106:33–7.
26 Amerio P, Frezzolini A, Abeni D, *et al*. Increased IL-18 in patients with systemic lupus erythematosus: relations with Th-1, Th-2, pro-inflammatory cytokines and disease activity. IL-18 is a marker of disease activity but does not correlate with pro-inflammatory cytokines. *Clin Exp Rheumatol* 2002;20:535–8.
27 Li Y, Lee PY, Reeves WH. Monocyte and macrophage abnormalities in systemic lupus erythematosus. *Arch Immunol Ther Exp* 2010;58:355–64.
28 Schulert GS, Grom AA. Pathogenesis of macrophage activation syndrome and potential for cytokine- directed therapies. *Annu Rev Med* 2015;66:145–59.
29 Haskó G, Linden J, Cronstein B, *et al*. Adenosine receptors: therapeutic aspects for inflammatory and immune diseases. *Nat Rev Drug Discov* 2008;7:759–70.
30 Haskó G, Sitkovsky MV, Szabó C. Immunomodulatory and neuroprotective effects of inosine. *Trends Pharmacol Sci* 2004;25:152–7.

CLINICAL SCIENCE

In an early SLE cohort the ACR-1997, SLICC-2012 and EULAR/ACR-2019 criteria classify non-overlapping groups of patients: use of all three criteria ensures optimal capture for clinical studies while their modification earlier classification and treatment

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ABSTRACT

Objectives Classification criteria are biased towards classifying long-standing disease. We compared the European League Against Rheumatism (EULAR)/American College of Rheumatology (ACR)-2019, Systemic Lupus International Collaborating Clinics (SLICC)-2012 and ACR-1997 criteria in an early (median 48 months) systemic lupus erythematosus (SLE) cohort.

Methods Patients diagnosed with SLE (n=690) or control diseases (n=401). Sensitivity, specificity of the criteria and time-to-classification were calculated. Modified classification algorithms were derived from a random 80% and validated in the remaining 20% of the dataset running multiple iterations.

Results At last assessment, sensitivities of ACR-1997, SLICC-2012 and EULAR/ACR-2019 criteria were 85.7%, 91.3% and 88.6%, with specificities 93.0%, 93.8% and 97.3%, respectively. Both SLICC and EULAR/ACR enabled earlier classification. Only 76.7% of patients with SLE met all three criteria suggesting non-overlapping groups. Notably, unclassified patients had high prevalence of British Isles Lupus Assessment Group moderate/severe manifestations (43.3%–60%) and SLICC/ACR organ damage (30%–50%). At diagnosis, criteria missed 25.6%–30.5% of patients. Modification of EULAR/ACR and SLICC algorithms to include hypocomplementaemia and/or positive anti-phospholipid antibodies as alternative entry criterion, and/or allow classification with fewer clinical criteria from multiple organs, increased their sensitivity at diagnosis (median 82.0% and 86.2%) and overall (93.7% and 97.1%) with modest decreases in specificity. Importantly, patients who were still missed by the modified criteria had lower incidence of major organ involvement, use of immunosuppressive/biological therapies and organ damage.

Conclusions The SLICC and EULAR/ACR are more sensitive than the ACR and the EULAR/ACR criteria have superior specificity in early SLE, although patients with significant disease can be missed. Combination and/or modification of the classification algorithms may enhance their sensitivity, allowing earlier classification and treatment of more patients with high disease burden.

Key messages

What is already known about this subject?

- Systemic lupus erythematosus (SLE) classification criteria have been developed to classify homogeneous patient groups in epidemiological studies and clinical trials but they are often used to aid disease diagnosis.

What does this study add?

- Both the European League Against Rheumatism (EULAR)/American College of Rheumatology (ACR) and the Systemic Lupus International Collaborating Clinics (SLICC) criteria have increased sensitivity with superior specificity of the EULAR/ACR criteria in early SLE and enable earlier classification as compared with the ACR criteria.
- All three sets of criteria may miss or delay the classification of a proportion of patients with SLE with moderate/severe disease, particularly cases of neurological-dominant lupus.
- The criteria may classify non-overlapping groups of patients with SLE; their combination ensures maximum patients capture in clinical studies.
- Modification of the EULAR/ACR and SLICC criteria by lowering the classification thresholds may enhance their sensitivity both at early disease stages and later.

How might this impact on clinical practice or future developments?

- Use of all three existing sets of criteria may ensure maximum capture of more representative groups of patients with SLE for clinical studies.
- Pending validation in prospective studies, modification of the SLE classification algorithms may improve their performance especially in early disease.

INTRODUCTION

Classification criteria for systemic lupus erythematosus (SLE) have been developed to ensure the inclusion of homogeneous groups of patients in clinical studies.[1] Nonetheless, these criteria are often used in clinical practice to aid diagnosis. In this regard, the Systemic Lupus International Collaborating Clinics (SLICC) 2012 criteria[2] were reported to have increased sensitivity[3–5] and capture more patients at the population level,[6 7] as compared with the American College of Rheumatology (ACR) 1997 criteria.[8] Still, clinical diagnosis may precede classification,[9 10] suggesting that especially at early stages, not all individuals with SLE will fulfil the criteria. Moreover, organ-dominant forms may occur imposing further classification challenges.

Recently, the European League Against Rheumatism (EULAR) jointly with the ACR have introduced new classification criteria,[11 12] which are based on two novel concepts, namely antinuclear antibodies(ANA) as an entry criterion coupled with variably weighed features.[13] Whether the new criteria have higher accuracy and allow for earlier classification merits investigation across different cohorts. The prognostic implications of classifying or not patients with SLE with the existing criteria is also not known.

We compared the three classification criteria in a large cohort of patients with early diagnosis of SLE or other rheumatological diseases, spanning from the community to tertiary care. Sensitivity was determined at the time of diagnosis and also, at last follow-up; we also examined which criteria enabled earlier classification. Guided by our observation that criteria classify non-overlapping patient groups, we compared the phenotypic characteristics and outcomes among patients who are unclassified by each criterion. Based on our sample characteristics, we propose modifications in the classification algorithms to assure the highest combination of sensitivity and specificity, thus allowing earlier classification and treatment of patients with potentially high disease burden.

METHODS

Setting and participants

We performed a retrospective observational study in two centres. The Department of Rheumatology, University Hospital of Heraklion, serves as referral centre for autoimmune diseases on the island of Crete (population 0.6M), connects to a cooperative network of private rheumatologists and general physicians, and provides inpatient and outpatient services from primary to tertiary level.[9] The Rheumatology Clinic, Attikon University Hospital, covers 1.7 M citizens in Western Attica.[14] Both centres have established SLE registries and use homogenised, structured forms for collecting detailed demographics, clinical characteristics (including classification criteria), use of treatments and disease outcomes.[9] We included consecutively registered cases diagnosed during 01/2005-12/2016 with SLE or other rheumatological diseases by consultant rheumatologists with ≥5 years clinical practice (online supplementary methods, online supplementary figure S1). We selected this diagnosis time interval to ensure data completeness and reduce possible information/classification bias. Additional inclusion criteria were as follows: sufficient patient identification and clinical data, age of diagnosis ≥16 years, known ANA status and follow-up ≥6 months to confirm the diagnosis. The study was approved by the local Ethics Committees.

Data collection, criteria and attribution

For each patient, demographics, rheumatological disease and date of diagnosis, presence and date of earliest reported occurrence of the items from all three classification criteria sets,[2 8 11 12] and date of last follow-up visit/assessment were extracted from medical charts. Neuropsychiatric manifestations were assessed through multidisciplinary approach[15] and ascertained by the use of the Italian Study Group attribution model[16] (online supplementary methods). Attribution of the criteria items to SLE or not was arbitrated by rheumatologists (DB, GB and AF) with special interest and experience in the disease, and according to the EULAR/ACR attribution process[11 12] The date of appearance of each item was considered either the date of the visit that this was first documented. A few patients with no documentation of immunological tests were considered negative for these items. Patients with unknown ANA were excluded (online supplementary figure S1).

Disease outcomes

Presence and corresponding date of each item of the SLICC/ACR damage index[17] was monitored. Severity of disease manifestations was classified with the British Isles Lupus Assessment Group (BILAG) system.[18] Use of immunosuppressive/biological treatments and physician global assessment of SLE severity were collected[9 19] (online supplementary methods)

Statistical analysis

Sensitivity of the criteria was assessed against physician diagnosis, both at the time of diagnosis (extended by 3 months to allow completion of diagnostic work-up) and at last patient visit/assessment (overall sensitivity). Patients with unverified date of appearance of any criteria item were included only in the overall sensitivity analysis. Specificity was determined against patients with other rheumatological diseases. We produced a likelihood ratios graph of the three sets of criteria.[20] In separate analysis, we calculated the earliest date of fulfilment of each set of criteria and the time elapsed since the date of the earliest item. Hazard analysis was used to determine the median (95% CI) time-to-classification for each set of criteria. Between-groups comparisons were performed by the McNemar's test or linear mixed model analysis for partially paired samples. All analyses were performed using SPSS V.24.0.

RESULTS

The EULAR/ACR and SLICC criteria have increased sensitivity and enable earlier classification

We assessed the performance of the three classification criteria in patients diagnosed with SLE (n=690) or other rheumatological diseases (n=401) (online supplementary table S1). Both the EULAR/ACR and SLICC had higher sensitivity (88.6% and 91.3%, respectively) than the ACR criteria (85.7%), with the EULAR/ACR having higher specificity than the other two sets (97.3% vs 93.0%–93.8%) (figure 1A). Accordingly, the EULAR/ACR criteria had lower false-positive rate, whereas the SLICC had higher true-positive rate (figure 1B). Only 2.9% of SLE cases were missed by all three criteria, suggesting that their combination enables the classification of the vast majority of patients encountered in clinical practice.

By analysing patients with disease duration <3 years, we found significantly increased sensitivity of the EULAR/ACR (87.3%) and SLICC (91.4%) as compared with the ACR criteria (79.9%, $p<0.01$ and $p<0.001$, respectively) (figure 1C). In this group, the median (95% CI) time interval between the earliest item and fulfilment of the criteria was shorter for the EULAR/ACR (9.1 (6.5–11.8) months) and SLICC (9.1 (6.9–11.3) months) than the ACR (12.1 (9.6–14.7) months, $p=0.043$ and $p=0.001$, respectively) criteria (figure 1D). Together, both the EULAR/ACR and SLICC criteria have increased sensitivity in early SLE and enable earlier classification than the ACR criteria.

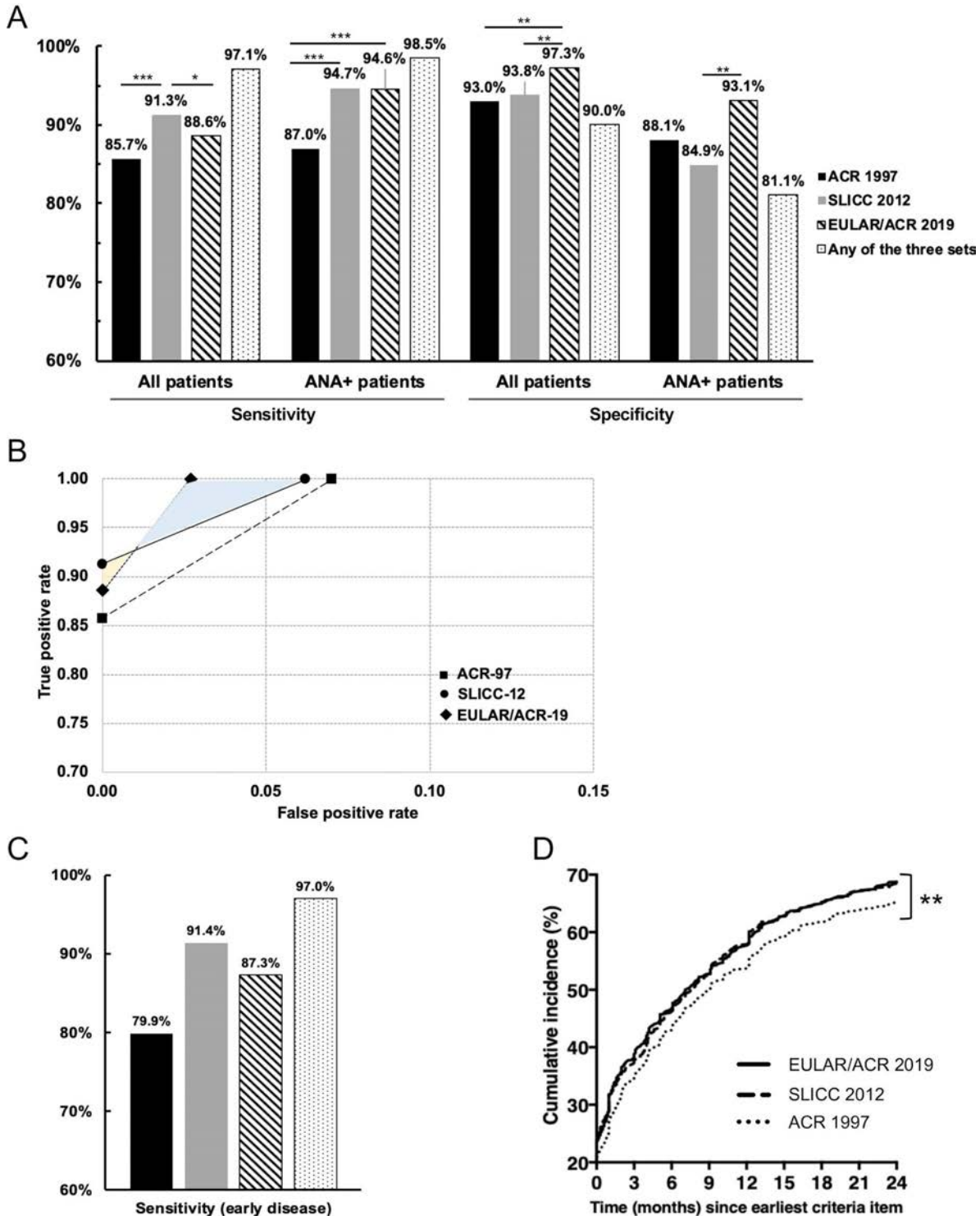

Figure 1 The EULAR/ACR 2019 and SLICC 2012 criteria have increased sensitivity and allow earlier classification in patients with SLE as compared with the ACR 1997 criteria. (A) Sensitivity and specificity (assessed at the time of last patient assessment/visit) of the EULAR/ACR 2019, SLICC 2012 and ACR 1997 criteria as well as their combination, both in the total of patients with SLE (n=690) and disease controls (n=401) and within the respective ANA-positive subgroups (n=646 and n=159, respectively). (B) Modified plot of true-positive rate and false-positive rate[20] for the EULAR/ACR 2019, SLICC 2012 and ACR 1997 criteria. The blue *shaded* region corresponds to superior performance of the EULAR/ACR 2019 over SLICC 2012 criteria in confirming the absence of SLE (ie, increased specificity). the yellow shaded region corresponds to superior performance of the SLICC 2012 over EULAR/ACR 2019 criteria in confirming the presence of SLE (ie, increased sensitivity). The lower-right position of the ACR 1997 plot corresponds to its inferior classification performance as compared with the SLICC 2012 and EULAR/ACR 2019 criteria. (C) Sensitivity of the three classification criteria within the subgroup of patients with SLE with early disease (duration <3 years). (D) Time-to-classification (time elapsing between the date of first appearance of the earliest criteria (belonging to any of the three sets) until the earliest date of fulfilment of each set of criteria) analysis for each set of classification criteria within the subgroup of patients with SLE with early disease (as in B). *p<0.05; **p<0.01; ***p<0.001. ACR, American College of Rheumatology; EULAR, European League Against Rheumatism; SLE, systemic lupus erythematosus; SLICC, Systemic Lupus International Collaborating Clinics.

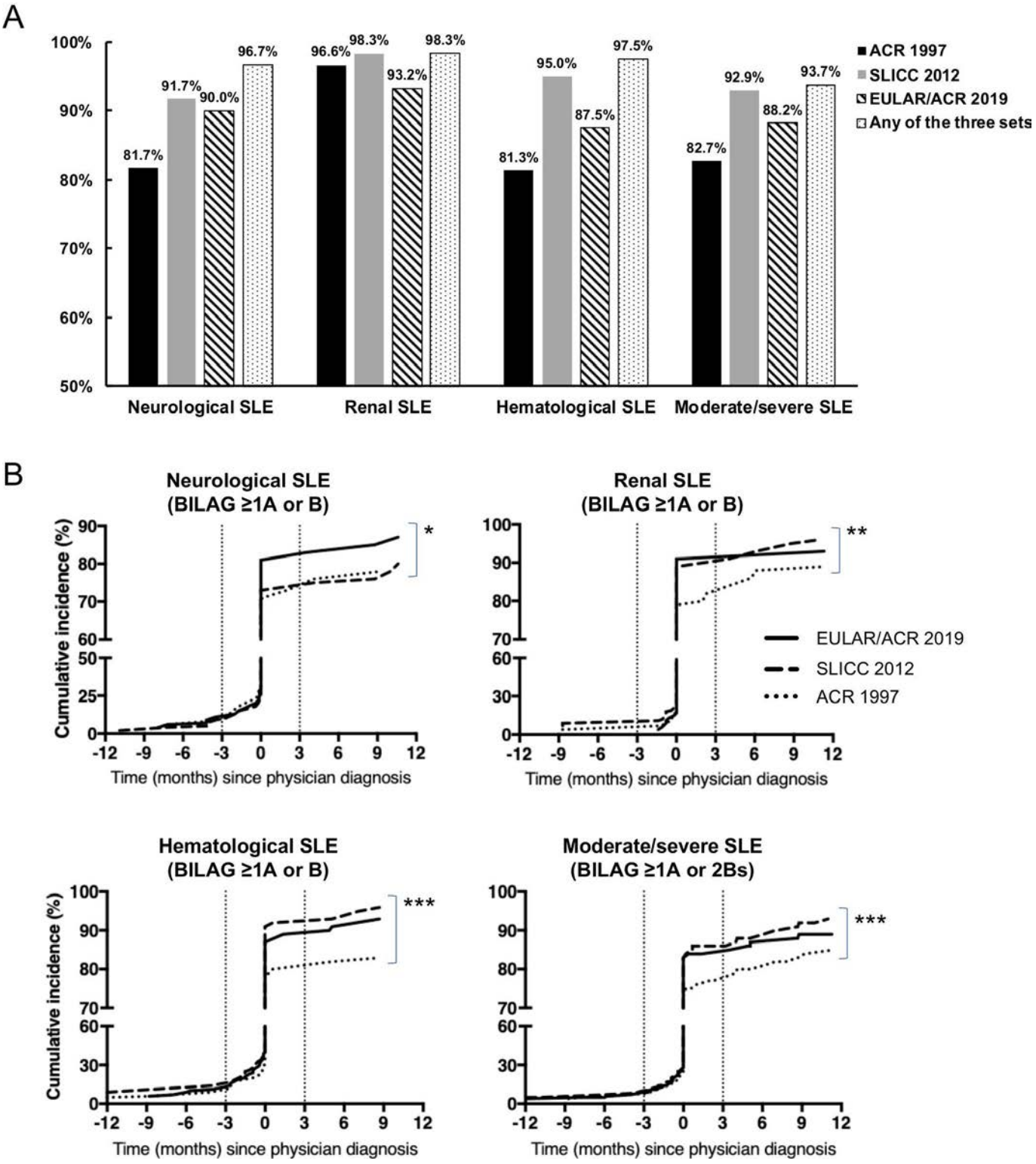

Figure 2 Existing criteria may miss or delay the classification of patients with SLE with major organ disease especially neurological SLE. (A) Sensitivity of the EULAR/ACR 2019, SLICC 2012 and ACR 1997 criteria as well as their combination across subgroups of patients with SLE with major organ disease. Neurological, renal and haematological disease were defined as history of activity from the respective organs/domains classified as BILAG B or A. Moderate/severe SLE was defined as history of at least 1 BILAG A or 2 BILAG Bs active manifestations (from any organ/domain). (B) Timing of SLE classification (according to the EULAR/ACR 2019, SLICC 2012, ACR 1997 criteria) in reference to physician diagnosis within the subgroups of patients with SLE with neurological, renal, haematological and overall moderate/severe disease (defined as above). The x-axis represents the difference (in months) in the time of criteria classification compared with diagnosis. *p<0.05 for the comparison of the EULAR/ACR 2019 versus ACR 1997 criteria (Wilcoxon signed-rank test); **p<0.01 for the comparison of the EULAR/ACR 2019 versus ACR 1997 and the SLICC 2012 versus ACR 1997 criteria (Wilcoxon signed-rank test); ***p<0.001 for the comparison of the EULAR/ACR 2019 versus ACR 1997 and the SLICC 2012 versus ACR 1997 criteria (Wilcoxon signed-rank test). ACR, American College of Rheumatology; BILAG, British Isles Lupus Assessment Group; EULAR, European League Against Rheumatism; SLE, systemic lupus erythematosus; SLICC, Systemic Lupus International Collaborating Clinics.

Table 1 Prevalence of clinical and immunological features (SLICC 2012 criteria) across groups of patients with SLE who were not classified by the classification criteria

	Missed by the					
	ACR 1997 criteria	SLICC 2012 criteria	EULAR/ACR 2019 criteria	All three criteria		
SLICC 2012 items Groups:	A (n=99)	B (n=60)	C (n=79)	D (n=20)	P value†	Group (pairwise) comparisons
Acute cutaneous lupus	49 (49.5%)	48 (80.0%)	57 (72.2%)	12 (60.0%)	**<0.001**	A versus B/C***
Chronic cutaneous lupus	10 (10.1%)	5 (8.3%)	14 (17.7%)	1 (5.0%)	0.055	–
Non-scarring alopecia	42 (42.4%)	22 (36.7%)	50 (63.3%)	9 (45.0%)	**<0.001**	A versus C**; B versus C***
Mucosal ulcers	12 (12.1%)	14 (23.3%)	41 (51.9%)	3 (15.0%)	**<0.001**	A/B/D versus C***
Mucocutaneous domain (any)	72 (72.7%)	53 (88.3%)	73 (92.4%)	16 (80.0%)	**<0.001**	A versus B*; A versus C***
Synovitis	69 (69.7%)	52 (86.7%)	59 (74.7%)	13 (65.0%)	**<0.001**	A versus B**; B versus C/D*
Serositis	6 (6.1%)	6 (10.0%)	10 (12.7%)	2 (10.0%)	0.363	–
Renal disorder	3 (3.0%)	2 (3.3%)	5 (6.3%)	2 (10.0%)	0.136	–
Neurological disorder	8 (8.1%)	4 (6.7%)	5 (6.3%)	4 (20.0%)	0.058	–
Haemolytic anaemia	3 (3.0%)	0 (0.0%)	2 (2.5%)	0 (0.0%)	0.216	–
Leucopaenia	20 (20.2%)	9 (15.0%)	22 (27.8%)	2 (10.0%)	**0.016**	B versus C*; C versus A*
Thrombocytopaenia	18 (18.2%)	4 (6.7%)	10 (12.7%)	2 (10.0%)	**0.036**	A versus B*
Haematol. domain (any)	29 (29.3%)	12 (20.0%)	27 (34.2%)	4 (20.0%)	**0.034**	B versus C*
Haematol. domain (≥2 items)	12 (12.1%)	1 (1.7%)	7 (8.9%)	0 (0.0%)	**0.002**	A versus B*; A versus D**; C versus B/D*
Anti-DNA Ab	18 (18.2%)	2 (3.3%)	0 (0.0%)	0 (0.0%)	**<0.001**	A versus B**; A versus B/C***
Anti-Sm Ab	2 (2.0%)	0 (0.0%)	1 (1.3%)	0 (0.0%)	0.224	–
Anti-phospholipid Ab	18 (18.2%)	1 (1.7%)	9 (11.4%)	1 (5.0%)	**<0.001**	A versus B***; B versus C*
Autoantibodies (any)	26 (26.3%)	3 (5.0%)	10 (12.7%)	1 (5.0%)	**<0.001**	A versus B***; A versus C*; A versus C**
Low complement	43 (43.4%)	2 (3.3%)	17 (21.5%)	1 (5.0%)	**<0.001**	A versus B/C/D***; B versus C***; C versus D**
Coombs test	5 (5.1%)	0 (0.0%)	2 (2.5%)	0 (0.0%)	0.057	–
ANA	84 (84.8%)	34 (56.7%)	35 (44.3%)	10 (50.0%)	**<0.001**	A versus B/C/D***

*p<0.05; **p<0.01; ***p<0.001.
†Linear mixed model analysis was used to consider for partially paired samples included in the four groups (A–D). Repeated covariance type was set to scale identity to avoid inflated type I error. Main effects were tested by robust estimation method. Pairwise effects were adjusted for multiple comparisons (sequential Bonferroni method).
ACR, American College of Rheumatology; EULAR, European League Against Rheumatism; SLE, systemic lupus erythematosus; SLICC, Systemic Lupus International Collaborating Clinics.

Existing criteria may miss or delay classification in a proportion of patients especially with neurological SLE

Patients with SLE with major organ disease often require immunosuppressive therapy and accrue more damage. All three criteria had lower sensitivity for classifying neurological versus renal SLE (81.7%–91.7% vs 93.0%–96.5%, respectively) (figure 2A, online supplementary table S2). The SLICC criteria had increased sensitivity for haematological manifestations. Depending on the criteria set, 7.1%–17.3% of patients with moderately severe or severe disease (BILAG) were not classified. Notably, the combination of all three criteria ensured the maximum capture of major disease subgroups (93.7%–98.5%) (figure 2A).

We also compared the timing of classification against physician diagnosis within classified patients with SLE. Although classification concurred with or antedated diagnosis in the majority of cases, still, diagnosis predated classification by >3 months in 17.3%–19.9% (online supplementary table S3). In agreement with sensitivity, increased rates of delay between classification and diagnosis were noted for neurological (20.0%–26.8%) versus renal (7.7%–14.8%) SLE (online supplementary table S3, figure 2B). Across all patient subgroups with major disease, the SLICC and EULAR/ACR enabled earlier classification than the ACR criteria (figure 2B).

The three sets of criteria classify non-overlapping groups of patients with SLE

Only 76.7% of patients with SLE met all three classification criteria, suggesting non-overlapping patient groups. To decipher the ability of criteria to classify distinct disease phenotypes, we compared patients who were missed by each one of the criteria sets and by all three of them (table 1). Patients who did not meet the ACR criteria had significantly higher prevalence of haematological and immunological features. Patients who were unclassified by the EULAR/ACR had increased rates of mucocutaneous disease and leucopaenia, whereas those missed by the SLICC criteria had predominant skin and joints disease. Among patients who were not classified by any of the criteria, 20% had neurological manifestations, whereas the prevalence of immunological features was lower. Together, existing criteria capture non-identical groups of patients with SLE.

Patients with SLE missed by the classification criteria may exhibit high disease burden

Next, we asked whether patients who are missed by the criteria differ in terms of disease outcomes. To this end, we assessed the severity of lupus manifestations and irreversible organ damage across the aforementioned groups of patients. Irrespective of the criteria used, a significant proportion (43.3%–55.7%) of non-classified patients had moderate/severe manifestations which required immunosuppressive or biological treatment (figure 3A). Concordantly, organ damage developed in 30.0%–40.5% of unclassified patients (figure 3B). Notably, patients missed by all three criteria also exhibited significant disease burden (45.0% severe manifestations, 50.0% with organ damage). These results suggest that despite their high sensitivity, classification criteria may miss patients with potentially severe disease.

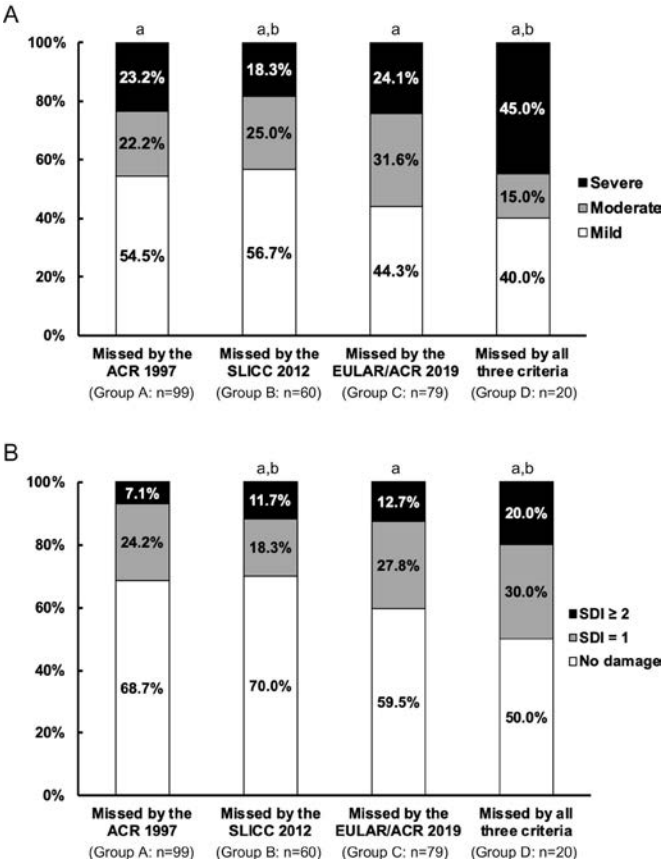

Figure 3 Patients with SLE who are missed by the classification criteria exhibit a high disease burden with high rates of moderate/severe disease and organ damage. (A) Distribution of disease severity (physician global assessment: mild, moderate, severe) across groups of patients with SLE who are not classified by the ACR 1997 (Group A), SLICC 20012 (Group B), EULAR/ACR 2019 (Group C) and all three classification criteria (Group D). Between-group comparisons were performed with linear mixed models to account for partially paired data. [a] Statistically significant difference in prevalence of severe manifestations between group D and groups A, B, C ($p<0.05$: group D vs groups A, C; $p=0.001$: group D vs group B); [b] $p=0.037$ for the difference in disease severity (moderate/severe manifestations) between group B and group D. (B) Organ damage accrual based on the SDI (assessed at last patient visit/assessment) across the aforementioned patient groups. [a,b] statistically significant difference in prevalence of organ damage (SDI>0) between group B and groups C ($p=0.008$) and group D ($p=0.015$). ACR, American College of Rheumatology; EULAR, European League Against Rheumatism; SDI, SLICC damage index; SLE, systemic lupus erythematosus; SLICC, Systemic Lupus International Collaborating Clinics.

Modified classification algorithms show enhanced accuracy for SLE classification

In view of the above, we explored alternative classification algorithms based on the existing criteria to enhance their accuracy. We focused on the EULAR/ACR and SLICC criteria as they displayed the highest sensitivity, and extracted a random 80% (*derivation*) set of our total sample (SLE and controls) to identify subgroups of patients who failed classification and assess the frequency of individual items over the entire observation period (online supplementary figure S2). Patients unclassified by the EULAR/ACR criteria included primarily cases who (a) did not meet the entry criterion of positive ANA, yet they had hypocomplementemia and/or positive anti-phospholipid antibodies and (b) met the entry criterion, scored ≥6 from the clinical domains/criteria but their aggregate clinical and immunology score was eight or 9, thus falling short of the classification threshold.[13] In the case of the SLICC criteria, two major groups of non-classified patients included those with (a) single immunological criterion and two clinical criteria from different organs/domains and (b) no immunological criteria but ≥4 clinical criteria from ≥2 different organs/domains. Most of the patients in these two groups had ACR-defined photosensitivity, which was included as additional clinical criterion to preserve the sensitivity/specificity ratio.

By amending the classification algorithms to include the aforementioned patient groups, the sensitivity of the 'modified' EULAR/ACR and SLICC criteria in the derivation set increased by 5.5 and 6.1 percent units, respectively, with modest decreases in specificity (online supplementary table S6). We tested the modified algorithms in the remaining 20% of the sample running 100 iterations to account for patient heterogeneity. The median sensitivity of the modified EULAR/ACR and SLICC criteria in the validation sets were 93.7% and 97.1%, respectively, with corresponding specificities 94.9% and 90.2% (figure 4A, online supplementary table S6).

At the time of physician diagnosis (extended by 3 months for completion of work-up, 't_0'), the sensitivity of the criteria ranged 69.5%–75.4% (online supplementary table S7). By applying the same modified EULAR/ACR and SLICC classification algorithms at 't_0', we confirmed increases in sensitivity by 7.3% and 12.2%, with reductions in specificity by 1.3% and 2.5%, respectively (figure 4B, online supplementary table S7). Collectively, modifications of the classification algorithms may enhance their accuracy although vigilance will be needed to avoid misclassification of patients with non-lupus.

The modified criteria classify more patients with SLE with early disease progression

Our results demonstrated high disease burden among patients who were missed by the classification criteria (figure 3). We therefore asked whether the modified classification algorithms had increased sensitivity over the original versions in capturing severe forms of the disease. Initially, we focused on early disease stages and grouped patients according to whether they fulfilled or not the original and modified versions of SLICC and EULAR/ACR criteria at t_0. These groups were then monitored for new-onset adverse outcomes (BILAG A/B activity from the renal, neurological or haematological domains, use of immunosuppressive or biological treatments, organ damage) during the first 6–24 months since diagnosis. We observed a high incidence of adverse disease outcomes in patients missed by the original criteria at t_0, although lower compared with classified patients (figure 5). Rates of all three outcomes were significantly lower (by 17% to 44%, $p\leq0.017$) among patients who were missed by the modified than the original EULAR/ACR and SLICC criteria. Similarly, the modified criteria captured significantly more patients with moderate/severe SLE and organ damage as compared with the original versions (online supplementary figure S3A,B). Accordingly, modified algorithms show increased sensitivity for classifying severe/progressive forms of SLE and likewise, patients who are unclassified by the modified criteria manifest milder disease.

DISCUSSION

We compared the EULAR/ACR and previous classification criteria against clinical SLE diagnosis, focusing also on the prognostic implications of classification. Our results demonstrate

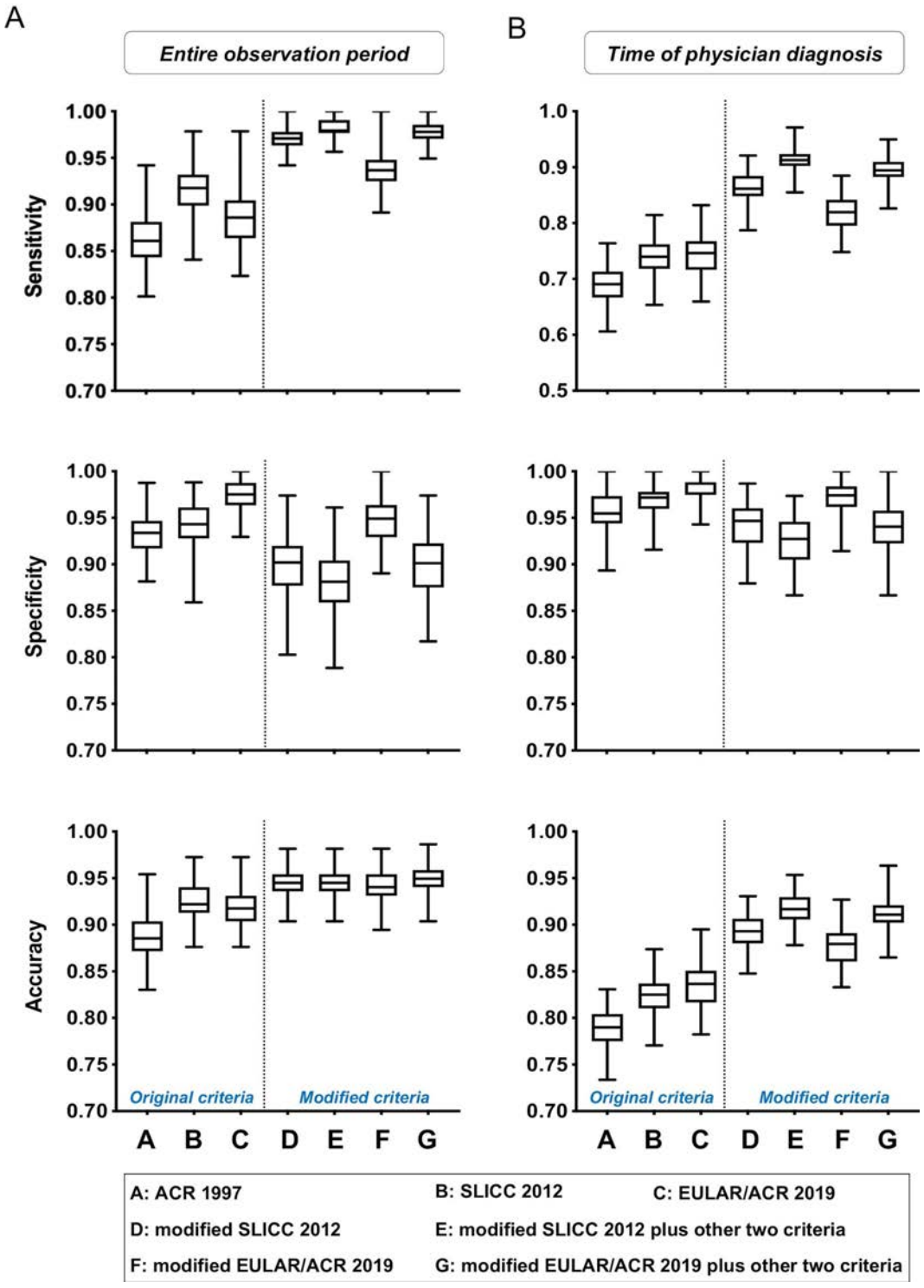

Figure 4 Modified classification algorithms based on the SLICC 2012 and EULAR/ACR 2019 criteria have increased sensitivity and accuracy for classifying SLE. (A) Box-plot of the sensitivity (*upper panel*), specificity (*middle panel*) and accuracy (*lower panel*) of the original and modified versions of the classification criteria assessed over the entire observation period. results are derived from 100 random iterations in the validation datasets (20% of total sample of patients with SLE and disease controls). Horizontal lines represent median values. See the Methods section for more details. modified SLICC 2012 classification: (a) ≥5 clinical criteria (including the SLICC 2012 clinical criteria and ACR 1997-defined photosensitivity) and NO immunological criterion; (b) ≥3 clinical criteria (including the SLICC 2012 clinical criteria and ACR 1997-defined photosensitivity) and ≥1 immunological criterion; modified EULAR/ACR 2019 classification: (a) ANA test is negative but there is hypocomplementaemia (low C3 and/or) C4 and/or positive anti-phospholipid antibodies (alternative entry criterion) and EULAR/ACR 2019 clinical score is ≥10; (b) ANA-positive, negative for other immunological tests (EULAR/ACR 2019 immunological score=0), EULAR/ACR 2019 clinical score is ≥8 and ACR 1997-defined photosensitivity is present; (c) ANA-positive, EULAR/ACR 2019 immunological score is ≥2 and EULAR/ACR 2019 clinical score is ≥6. (B) Box-plot of the sensitivity (upper panel), specificity (middle panel) and accuracy (lower panel) of the original and modified versions of the classification criteria assessed at the time of physician diagnosis (extended by 3 months) (100 iterations, validation sets). ACR, American College of Rheumatology; EULAR, European League Against Rheumatism; SLE, systemic lupus erythematosus; SLICC, Systemic Lupus International Collaborating Clinics.

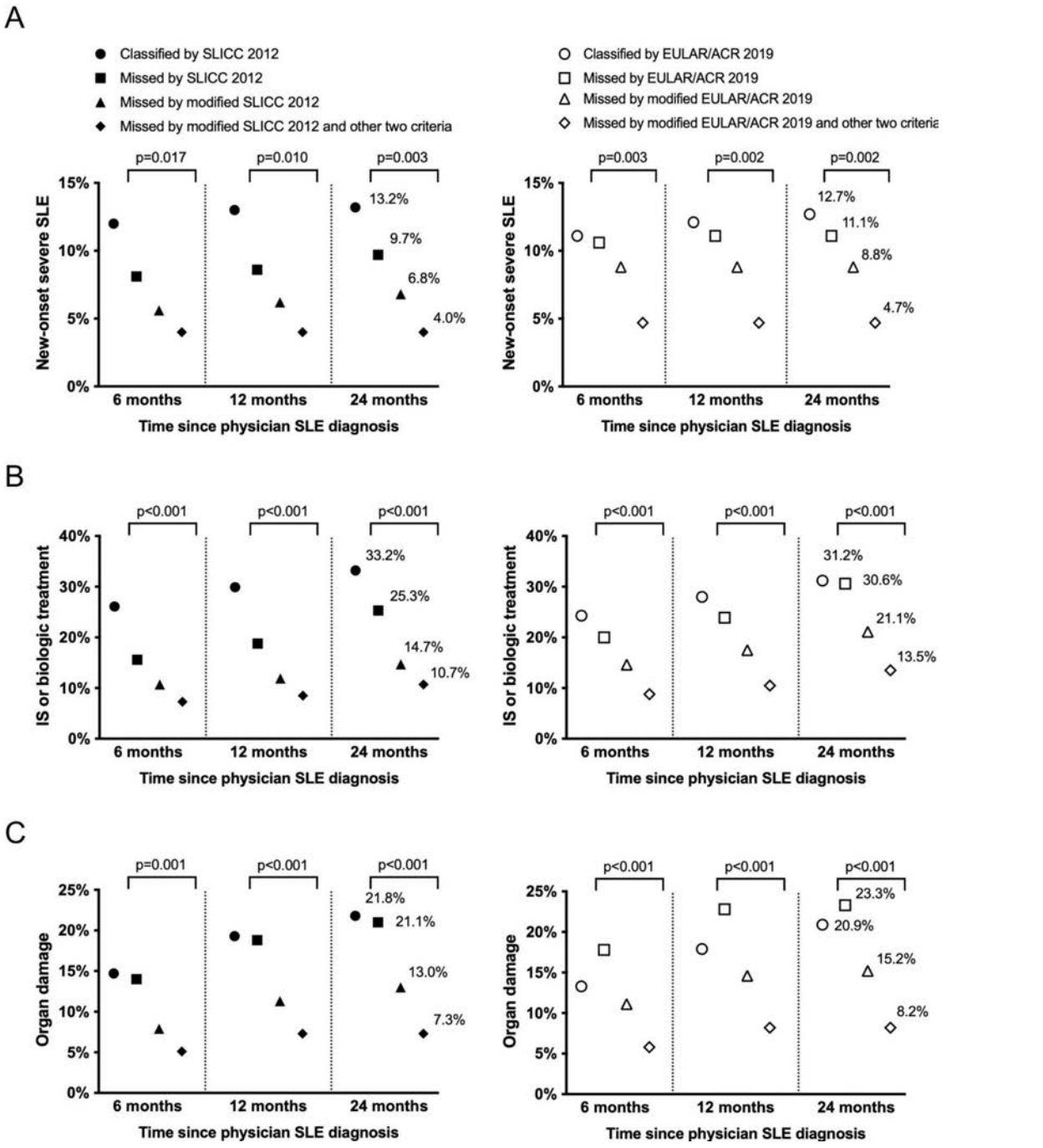

Figure 5 Patients with SLE missed by the modified versions of the SLICC 2012 and EULAR/ACR 2019 classification criteria demonstrate significantly lower incidence of adverse disease outcomes at early stages. (A) Incidence of BILAG B or BILAG A activity from the neurological, renal and/or haematological domains in patients with SLE who—at the time of physician diagnosis—are as follows: (i) classified (*circle*) or not (*square*) by the original EULAR/ACR 2019 (or SLICC 2012) criteria, (ii) are missed by the modified version of the EULAR/ACR 2019 (or SLICC 2012) criteria (*triangle*) and (iii) are missed by both the modified version of the EULAR/ACR 2019 (or SLICC 2012) criteria and the remaining two criteria (original versions) (*diamond*). modified SLICC 2012 classification: (a) ≥5 clinical criteria (including the SLICC 2012 clinical criteria and ACR 1997-defined photosensitivity) and NO immunological criterion; (b) ≥3 clinical criteria (including the SLICC 2012 clinical criteria and ACR 1997-defined photosensitivity) and ≥1 immunological criterion; modified EULAR/ACR 2019 classification: (a) ANA test is negative but there is hypocomplementemia (low C3 and/or) C4 and/or positive anti-phospholipid antibodies (*alternative entry criterion*) and EULAR/ACR 2019 clinical score is ≥10; (b) ANA-positive, negative for other immunological tests (EULAR/ACR 2019 immunological score=0), EULAR/ACR 2019 clinical score is ≥8 and ACR 1997-defined photosensitivity is present; (c) ANA-positive, EULAR/ACR 2019 immunological score is ≥2 and EULAR/ACR 2019 clinical score is ≥6. (B) Incidence of use of high-potency immunosuppressive and/or biological treatments due to active SLE in the same patient groups as in A. (C) Incidence of organ damage accrual (SDI>0) in the same patient groups as in a. statistical comparisons in panels A–C are performed by linear mixed model (repeated measures) analysis for partially matched data across the groups missed by the original criteria, missed by the modified version of the criteria, and missed by the modified version and the other two (original) sets of criteria. ACR, American College of Rheumatology; BILAG, British Isles Lupus Assessment Group; EULAR, European League Against Rheumatism; SDI, SLICC damage index; SLE, systemic lupus erythematosus; SLICC, Systemic Lupus International Collaborating Clinics.

that despite improved sensitivity of the EULAR/ACR and SLICC criteria, classification may be missed or delayed in some patients, including patients with high disease burden. Criteria classify non-overlapping patient groups suggesting that their combinatorial use may ensure maximum capture of patients with SLE with diverse presentations. By adjusting the classification thresholds, the sensitivity and accuracy of the criteria for classifying SLE—including severe/progressive forms of the disease—is enhanced.

Classification criteria enable the highest possible inclusion of patients with confirmed disease in clinical studies. Previous reports suggested increased sensitivity of the SLICC—with comparable or lower specificity—than the ACR criteria.[4 5 10 21 22] We found greater sensitivity of both SLICC and EULAR/ACR over the ACR criteria, with higher specificity of the EULAR/ACR criteria. The superior performance of SLICC and EULAR/ACR was more evident within patients with early disease, which agrees with published results comparing the SLICC against the ACR criteria.[5] This was further corroborated by our time-to-classification analysis showing earlier classification with the EULAR/ACR and SLICC criteria.

Differences between our results with those in other cohorts might be due to various factors such as the ethnic/demographic background of patients, disease duration and inclusion of disease controls. Our study included exclusively Caucasians with average disease duration <5 years, which is lower than other studies.[5] Moreover, the frequency of ANA was 93.6%, which is similar to that in a contemporary international SLE cohort (92.3%),[23] yet lower than the estimates of a large meta-analysis of epidemiological studies (97.8%, 95% CI: 96.8% to 98.5%).[24] This might be explained by our cohort characteristics such as the short disease duration, white/Caucasian ethnicity and older age of onset (median 42 years).[23 25] Due to high clinical suspicion, 39 out of 44 ANA-negative patients had been repeatedly tested by standard indirect Hep-2 immunofluorescence (1:80 dilution). Within ANA-positive patients, our sensitivity of the EULAR/ACR criteria was higher (94.6%) and similar to the SLICC criteria (94.7%) (figure 1A), which agrees with the results from the EULAR/ACR validation cohort.[11 12]

In patients with major organ involvement, early treatment initiation is critical to ensure good outcomes.[26–29] Notably, all three criteria had lower sensitivity for neurological than renal SLE. Likewise, the delay between classification and physician diagnosis was greater in neurological disease. It has long been recognised that some patients with lupus may present with organ-dominant/limited disease particularly involving the nervous system, kidneys or blood,[1 30] and before sufficient number of criteria accrue to meet classification. In recognition of this, the SLICC first introduced the renal stand-alone criterion for classifying renal-dominant lupus,[2] and the EULAR/ACR have introduced higher-weighted items to enable classification with fewer number of afflicted organs.[13] To this end, the EULAR/ACR criteria enabled earlier classification of neurological SLE (figure 2B), which represents a step forward this unmet need.

The three sets of criteria vary in the number and diversity of included manifestations, their definition, weighing score and the algorithms to qualify for classification. Only 76.7% of patients with SLE met all three criteria and likewise, the combination of all three criteria enabled maximum capture of patients. By comparing patients who were missed by each of the three criteria, significant differences were noted in prevalence of individual features especially from the mucocutaneous, musculoskeletal, haematological and immunological domains (table 1), suggesting that criteria may classify non-overlapping groups. This might have implications in clinical trial design as it is possible that different manifestations may respond differently to therapeutic agents.[31]

In agreement with previous reports,[10] we noted that at the time of clinical diagnosis, the sensitivity of the criteria was modest. Detailed analysis revealed that physicians often relied on only a few features to secure diagnosis at early stages. This prompted us to devise 'lower threshold' EULAR/ACR and SLICC classification algorithms by introducing alternative 'entry criterion' in the case that ANA test is negative, and/or allowing the classification of patients with fewer criteria from multiple organs. The new algorithms exhibited enhanced sensitivity with modest decreases in specificity for classifying SLE both at early stage and later during the disease course. Importantly, patients who were missed by the modified criteria exhibited lower rates of adverse outcomes. These findings raise the possibility that modifications of the classification algorithms could be exploited as putative tools in clinical practice. Pending verification, classification criteria can only aid in the diagnosis of SLE and judgement by an experienced SLE specialist is typically required.

Our study is limited by its retrospective design and data extraction from medical records; accordingly, some clinical information may have been missed or underestimated. Nonetheless, both centres maintain detailed patient registries and use structured forms for collecting clinical data, which helps to reduce possible information/data completeness bias. Direct Coombs was not routinely performed before the publication of the SLICC criteria[2] (unless in suspected autoimmune haemolysis), which could have underestimated the sensitivity/specificity of SLICC. By analysing patients diagnosed since 2012 (n=354 SLE, n=202 controls), we obtained comparable results with those in the total cohort (SLICC sensitivity: 92.7%, specificity: 93.2%).

In summary, the rheumatologic community has gradually refined the classification criteria taking into consideration caveats in their use. The ACR-1997 criteria acknowledged that ANA positivity was not essential for the classification but some organs/domains were over-represented. The SLICC-2012 introduced the concept of 'organ-dominant' disease in the case of nephritis. More recently, the EULAR/ACR-2019 criteria have remedied the over-representation of some organs/domains by introducing the weighting of various manifestations, thus gaining in specificity.

Herein, we have evaluated the performance of the new compared with the previous SLE classification criteria in a large patient cohort. The EULAR/ACR and SLICC criteria have increased sensitivity but classification may be missed or delayed especially at early stages and in cases of neurological lupus. Modification of the classification algorithms may enhance their sensitivity, potentially allowing earlier classification and treatment of patients with potentially severe disease.

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Contributors CA, DN and AF collected data from patient medical charts and also performed data entry. I. Genitsaridi performed part of the statistical analyses (time-to-classification, 80/20 validation of the modified criteria). AB organised the RedCap database and assisted in data analyses. EK and EP contributed in the maintenance of the Cretan Lupus Registry and assisted in data collection. I. Gergianaki contributed in the establishment of the Cretan Lupus Registry and the selection of candidate study participants. PS assisted in patient recruitment and reviewed the manuscript. DB and GB conceived and supervised the study. GB performed statistical analyses. CA and GB drafted the manuscript.

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Competing interests None declared.

Patient consent for publication Not required.

Ethics approval The study was approved by the Ethics Committee of the University Hospital of Iraklio (protocol no. 13960/10-10-2018) and the Ethics Committee of the 'Attikon' University Hospital of Athens.

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REFERENCES

1 Bertsias GK, Pamfil C, Fanouriakis A, *et al*. Diagnostic criteria for systemic lupus erythematosus: has the time come? *Nat Rev Rheumatol* 2013;9:687–94.
2 Petri M, Orbai A-M, Alarcón GS, *et al*. Derivation and validation of the systemic lupus international collaborating clinics classification criteria for systemic lupus erythematosus. *Arthritis Rheum* 2012;64:2677–86.
3 Fonseca AR, Gaspar-Elsas MIC, Land MGP, *et al*. Comparison between three systems of classification criteria in juvenile systemic lupus Erythematous. *Rheumatology* 2015;54:241–7.
4 Ighe A, Dahlström Örjan, Skogh T, *et al*. Application of the 2012 systemic lupus international collaborating clinics classification criteria to patients in a regional Swedish systemic lupus erythematosus register. *Arthritis Res Ther* 2015;17.
5 Inês L, Silva C, Galindo M, *et al*. Classification of systemic lupus erythematosus: systemic lupus international collaborating clinics versus American College of rheumatology criteria. A comparative study of 2,055 patients from a real-life, International systemic lupus erythematosus cohort. *Arthritis Care Res* 2015;67:1180–5.
6 Izmirly PM, Wan I, Sahl S, *et al*. The incidence and prevalence of systemic lupus erythematosus in New York County (Manhattan), New York: the Manhattan lupus surveillance program. *Arthritis Rheumatol* 2017;69:2006–17.
7 Ungprasert P, Sagar V, Crowson CS, *et al*. Incidence of systemic lupus erythematosus in a population-based cohort using revised 1997 American College of rheumatology and the 2012 systemic lupus international collaborating clinics classification criteria. *Lupus* 2017;26:240–7.
8 Hochberg MC. Updating the American College of rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1725;1997.
9 Gergianaki I, Fanouriakis A, Repa A, *et al*. Epidemiology and burden of systemic lupus erythematosus in a southern European population: data from the community-based lupus Registry of Crete, Greece. *Ann Rheum Dis* 2017;76:1992–2000.
10 Pons-Estel GJ, Wojdyla D, McGwin G, *et al*. The American College of rheumatology and the systemic lupus international collaborating clinics classification criteria for systemic lupus erythematosus in two multiethnic cohorts: a commentary. *Lupus* 2014;23:3–9.
11 Aringer M, Costenbader K, Daikh D, *et al*. European League against Rheumatism/American College of rheumatology classification criteria for systemic lupus erythematosus. *Arthritis Rheumatol* 2019;2019:1400–12.
12 Aringer M, Costenbader K, Daikh D, *et al*. European League against Rheumatism/American College of rheumatology classification criteria for systemic lupus erythematosus. *Ann Rheum Dis* 2019;2019:1151–9.
13 Tedeschi SK, Johnson SR, Boumpas D, *et al*. Developing and refining new candidate criteria for systemic lupus erythematosus classification: an international collaboration. *Arthritis Care Res* 2018;70:571–81.
14 Nikolopoulos D, Pieta A, Kostopoulou M, *et al*. Trends of severity, progression and burden of disease in the "Attikon" Systemic Lupus Erythematosus (SLE) cohort: evidence for the rule of "one third" in disease severity. *Ann Rheum Dis* 2019;78.
15 Papadaki E, Fanouriakis A, Kavroulakis E, *et al*. Neuropsychiatric lupus or not? cerebral hypoperfusion by perfusion-weighted MRI in normal-appearing white matter in primary neuropsychiatric lupus erythematosus. *Ann Rheum Dis* 2018;77:441–8.
16 Bortoluzzi A, Scirè CA, Bombardieri S, *et al*. Development and validation of a new algorithm for Attribution of neuropsychiatric events in systemic lupus erythematosus. *Rheumatology* 2015;54:891–8.
17 Gladman D, Ginzler E, Goldsmith C, *et al*. The development and initial validation of the systemic lupus international collaborating clinics/American College of rheumatology damage index for systemic lupus erythematosus. *Arthritis Rheum* 1996;39:363–9.
18 Isenberg DA, Rahman A, Allen E, *et al*. BILAG 2004. development and initial validation of an updated version of the British Isles lupus assessment group's disease activity index for patients with systemic lupus erythematosus. *Rheumatology* 2005;44:902–6.
19 Gergianaki I, Fanouriakis A, Adamichou C, *et al*. Is systemic lupus erythematosus different in urban versus rural living environment? data from the Cretan lupus epidemiology and surveillance registry. *Lupus* 2019;28:104–13.
20 Biggerstaff BJ. Comparing diagnostic tests: a simple graphic using likelihood ratios. *Stat Med* 2000;19:649–63.
21 Amezcua-Guerra LM, Higuera-Ortiz V, Arteaga-García U, *et al*. Performance of the 2012 systemic lupus international collaborating clinics and the 1997 American College of rheumatology classification criteria for systemic lupus erythematosus in a real-life scenario. *Arthritis Care Res* 2015;67:437–41.
22 Oku K, Atsumi T, Akiyama Y, *et al*. Evaluation of the alternative classification criteria of systemic lupus erythematosus established by systemic lupus international collaborating clinics (SLICC). *Mod Rheumatol* 2018;28:642–8.
23 Choi MY, Clarke AE, St. Pierre Y, *et al*. Antinuclear Antibody–Negative systemic lupus erythematosus in an international inception cohort. *Arthritis Care Res* 2019;71:893–902.
24 Leuchten N, Hoyer A, Brinks R, *et al*. Performance of antinuclear antibodies for classifying systemic lupus erythematosus: a systematic literature review and meta-regression of diagnostic data. *Arthritis Care Res* 2018;70:428–38.
25 Cervera R, Khamashta MA, Font J, *et al*. Systemic lupus erythematosus: clinical and immunologic patterns of disease expression in a cohort of 1,000 patients. The European Working Party on systemic lupus erythematosus. *Medicine* 1993;72:113–24.
26 Esdaile JM, Mackenzie T, Barré P, *et al*. Can experienced clinicians predict the outcome of lupus nephritis? *Lupus* 1992;1:205–14.
27 Faurschou M, Dreyer L, Kamper A-L, *et al*. Long-Term mortality and renal outcome in a cohort of 100 patients with lupus nephritis. *Arthritis Care Res* 2010;62:873–80.
28 Lu X, Gu Y, Wang Y, *et al*. Prognostic factors of lupus myelopathy. *Lupus* 2008;17:323–8.
29 Oglesby A, Korves C, Laliberté F, *et al*. Impact of early versus late systemic lupus erythematosus diagnosis on clinical and economic outcomes. *Appl Health Econ Health Policy* 2014;12:179–90.
30 Calvo-Alén J, Bastian HM, Straaton KV, *et al*. Identification of patient subsets among those presumptively diagnosed with, referred, and/or followed up for systemic lupus erythematosus at a large tertiary care center. *Arthritis Rheum* 1995;38:1475–84.
31 Fanouriakis A, Adamichou C, Koutsoviti S, *et al*. Low disease activity—irrespective of serologic status at baseline—associated with reduction of corticosteroid dose and number of flares in patients with systemic lupus erythematosus treated with belimumab: a real-life observational study. *Semin Arthritis Rheum* 2018;48:467–74.

TRANSLATIONAL SCIENCE

Transcriptome reprogramming and myeloid skewing in haematopoietic stem and progenitor cells in systemic lupus erythematosus

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ABSTRACT

Objectives Haematopoietic stem and progenitor cells (HSPCs) are multipotent cells giving rise to both myeloid and lymphoid cell lineages. We reasoned that the aberrancies of immune cells in systemic lupus erythematosus (SLE) could be traced back to HSPCs.

Methods A global gene expression map of bone marrow (BM)-derived HSPCs was completed by RNA sequencing followed by pathway and enrichment analysis. The cell cycle status and apoptosis status of HSPCs were assessed by flow cytometry, while DNA damage was assessed via immunofluorescence.

Results Transcriptomic analysis of Lin$^-$Sca-1$^+$c-Kit$^+$ haematopoietic progenitors from diseased lupus mice demonstrated a strong myeloid signature with expanded frequencies of common myeloid progenitors (CMPs)—but not of common lymphoid progenitors—reminiscent of a 'trained immunity' signature. CMP profiling revealed an intense transcriptome reprogramming with suppression of granulocytic regulators indicative of a differentiation arrest with downregulation trend of major regulators such as *Cebpe*, *Cebpd* and *Csf3r*, and disturbed myelopoiesis. Despite the differentiation arrest, frequencies of BM neutrophils were markedly increased in diseased mice, suggesting an alternative granulopoiesis pathway. In patients with SLE with severe disease, haematopoietic progenitor cells (CD34$^+$) demonstrated enhanced proliferation, cell differentiation and transcriptional activation of cytokines and chemokines that drive differentiation towards myelopoiesis, thus mirroring the murine data.

Conclusions Aberrancies of immune cells in SLE can be traced back to the BM HSPCs. Priming of HSPCs and aberrant regulation of myelopoiesis may contribute to inflammation and risk of flare.

Trial registration number 4948/19-07-2016.

Key messages

What is already known about this subject?

► Most cells participating in the pathogenesis of systemic lupus erythematosus (SLE) originate from bone marrow (BM) haematopoietic stem and progenitor cells (HSPCs). HSPCs actively respond to inflammatory stimuli by myeloid skewing, but this may lead to exhaustion, decreased function, increased risk for inflammation, decreased adaptive immunity and increased cardiovascular mortality.

What does this study add?

► In SLE, there is evidence of deregulation of haematopoiesis with skewing towards the myeloid lineage at the expense of lymphopoiesis and priming of HSPCs that exhibit a 'trained immunity' signature; this may contribute to inflammation and risk of flare.

How might this impact on clinical practice or future developments?

► Abnormalities of immune cells in SLE can be traced back in the BM HSPCs, a disease where stem cell therapy has been considered for refractory cases. Re-establishment of the appropriate myeloid versus lymphoid balance and alleviation of cell exhaustion may improve transplantability of HSPCs and may restore immune function. This could also decrease risk of infection and atherosclerosis and attenuate inflammation, decreasing the risk of flare.

INTRODUCTION

In systemic lupus erythematosus (SLE), an interplay between environmental, genetic and epigenetic factors leads to perturbation of complex biological networks culminating into diverse clinical phenotypes. In this disease, interferon-alpha (IFN-α)-driven immunological alterations result in persistent immune responses against autologous nucleic acids, mimicking a sustained antiviral response. Intractable tissue damage caused by autoantibodies or immune-complex depositions affects several organs, leading to significant morbidity.[1 2]

Haematopoietic stem and progenitor cells (HSPCs) represent the most primitive multipotent population giving rise to all blood cell types.[3] HSPCs reside in the bone marrow (BM) niche and remain in a quiescent state. Under stress or inflammatory conditions, they respond by proliferating and differentiating to replenish any progeny needed.[4–6]

A key observation in SLE is that most cells participating in its pathogenesis, such as lymphocytes, monocytes and neutrophils, originate from HSPCs. In a congenic strain of lupus mice, the function of

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hematopoietic stem cells is altered by both genetic and inflammatory factors with evidence of increased self-renewal and resistance to stress.[7] In patients with SLE with cytopenias, BM exhibits necrosis, stromal alterations, hypocellularity, dyspoiesis and distortion of normal architecture with abnormal localisation of immature precursors aggregates.[8] Gene expression studies from our group have demonstrated upregulation of genes involved in cell death and granulopoiesis, providing further evidence of the role of apoptosis and granulocytes in its pathogenesis.[9]

Within the BM niche, inflammatory cytokines and myeloid-specific growth factors, including interleukin (IL)-1 and granulocyte macrophage growth factor,[10] drive the reprogramming of HSPCs towards myeloid lineage by epigenetic modifications and induction of lineage-specific transcription factors. These alterations increase their adaptation to inflammatory and haematopoietic stress, promoting the generation of myeloid cells that confer protection to secondary infection, in a phenomenon termed 'trained innate immunity' or 'innate immune memory'.[11 12] Perturbed immunological imprinting (mediated by hyperactive and myeloid priming) could be detrimental, exaggerating immune responses in autoimmune and inflammatory diseases such as arthritis,[13 14] SLE[15] or atherothrombosis.[16–18]

We reasoned that the fundamental molecular aberrations in SLE (genetic or epigenetic) may be traced back in the HSPCs within the BM. To this end, we used the NZBW/F1 mouse model of SLE to investigate the lupus transcriptome of HSPCs and compared it with transcriptomic data from human SLE $CD34^+$ cells. Herein, we report reprogramming of HSPCs towards myeloid lineage—with evidence of a 'trained immunity' signature—and propose that this may contribute to exaggerated immune responses and flares in SLE.

METHODS

Mice

C57BL/6 mice were purchased from the Jackson Laboratory. NZB/OlaHsd and NZW/OlaHsd mice were purchased from Envigo. NZBW/F1 mice were considered diseased, when exhibiting ≥100 ng/dL of urine protein after the completion of 6 months of their life.[19] Age-matched female mice were used (B6-young (B6-Y)/F1-prediseased (F1-P): 12 and B6-old (B6-O)/F1-lupus (F1-L): 28–36 weeks old). All animals were maintained in the BRFAA animal facility.

Flow cytometry and cell sorting

Single-cell suspensions were prepared from BM, peripheral blood mononuclear cells (PBMCs) or spleen and were stained with conjugated antibodies. $CD11b^-Gr\text{-}1^-Ter119^-B220^-CD16/32^-Sca\text{-}1^+c\text{-}Kit^+$ ($Lin^-Sca\text{-}1^+c\text{-}Kit^+$ (LSK)) and $CD11b^-Gr\text{-}1^-Ter119^-B220^-CD16/32^-CD34^+Sca\text{-}1^+c\text{-}Kit^+$ (common myeloid progenitor (CMP)) cells were isolated from BM (tibia, femur and brachial) and sorted on a FACS-ARIA-III (Becton Dickinson Biosciences). Cell purity was ≥95%. Data were analysed with FlowJo.

Human subjects

BM aspirates and PBMCswere obtained from SLE and gender matched healthy controls (HCs). Patients met the 1999 American College of Rheumatology revised criteria for the classification of SLE.[20] Patients' clinical and serological characteristics are summarised in online supplementary tables 1 and 5.

Mononuclear cell isolation and processing

Bone marrow mononuclear cells (BMMCs) were isolated using Histopaque 1077 (Sigma-Aldrich). BMMCs were washed, and erythrocytes were lysed with red blood cell buffer (420301, Biolegend) and stained with conjugated antibodies. $CD34^+$ cells were isolated from BMMCs using magnetic beads (18056, StemCell Technologies).

RNA sequencing

LSKs and CMPs were sorted from BM of NZBW/F1 and C57BL/6 mice. $CD34^+$ cells were isolated from BMMCs. Genes with a false discovery rate of ≤0.05 and a fold change of >1.5 were considered statistically significantly upregulated/downregulated, respectively. For the human–mouse comparison, human genes were converted to mouse orthologos and pathways were compared based on their ID.

Statistics

Statistical analyses were performed using unpaired two-tailed Student's t-test, while Mann-Whitney U test was used for the comparison of two groups. Data are presented as mean±SD. Differences were considered statistically significant at $p<0.05$. All data were analysed using GraphPad Prism V.5 software.

Study approval

Informed consent was obtained from all patients and HC prior to sample collection (Athens, Greece, protocol 10/22-6-2017). All procedures in mice were in accordance with institutional guidelines and were reviewed and approved by the Greek Federal Veterinary Office (Athens, Greece).

RESULTS

The transcriptional profile of murine lupus HSPCs demonstrates myeloid skewing

To study whether HSPCs in SLE exhibit transcriptional alterations, we used the spontaneous mouse model NZBW/F1[21–23] at two time points: *preclinical* stage (F1-P) and *clinical stage*, defined as the point with proteinuria of >100 ng/dL (F1-L). Age-matched female C57BL/6 mice served as controls (B6-Y and B6-O, respectively). Gene profiling was performed in murine LSK compartment—representing HSPCs in mice—sorted by flow cytometry from BM of NZBW/F1 lupus and C57BL/6 control mice (figure 1A). A total of 758 differentially expressed genes (DEGs) between F1-P and F1-L mice were identified (figure 1B and online supplementary table 2), including enriched GO terms per cluster: haematopoietic cell lineage, neutrophil degranulation and cell adhesion (red); and lymphocyte activation, extracellular region and immunoglobulin heavy chain variable region genes (IGVH) repertoire (green/blue). Gene set enrichment analysis (GSEA) showed a positive correlation with signatures related to inflammatory response, activation of innate immune response and platelet degranulation (figure 1C and online supplementary table 3) in F1-L mice. These categories are crucial for the adaptation of stem cell phenotype to inflammation with studies showing expansion of stem cell-like megakaryocyte committed cells within HSPCs under inflammatory conditions.[24] *Gene ontology and pathway analysis* demonstrated DEGs implicated in myeloid leukocyte-mediated immunity, cytokine secretion, granulocyte/neutrophil activation and migration in F1-L mice (online supplementary figure 1A). Notably, F1-L LSK demonstrated increased proliferation and strong myeloid signature (figure 1D). IFN-associated genes (*Gbp6* and *Ciita*) were upregulated in F1-L LSK, showing a strong type I

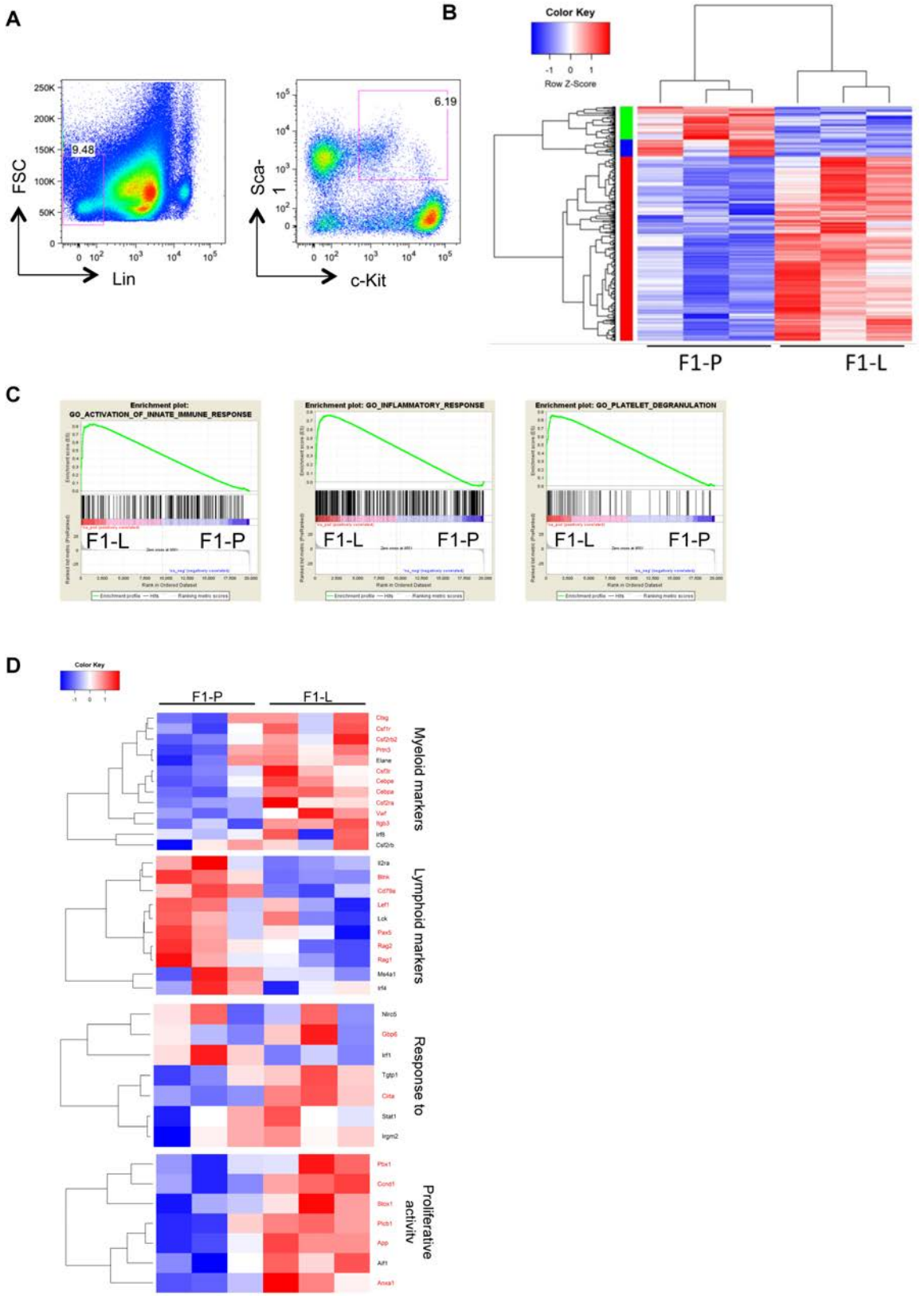

Figure 1 Transcriptional profiling of murine LSK by RNA sequencing demonstrates myeloid skewing. (A) Representative fluorescence-activated cell sorting plots for the identification of haematopoietic stem and progenitor cells. After gating for Lin^- cells, LSK were characterised as $c\text{-}Kit^+Sca\text{-}1^+$. (B) Heatmap of DEGs (|FC|>1.5, FDR<0.05) in BM-derived LSK between F1-P and F1-L mice (n=3 replicates per group). (C) GSEA plot showing the enrichment of 'GO activation of innate immune response' (NES 1.62, FDR 0.002), 'GO inflammatory response' (NES 1.57, FDR 0.022) and 'GO platelet degranulation' (NES 1.60, FDR 0.009) gene sets in LSK F1-L mice. (D) Heatmaps of genes related to myelopoiesis, lymphopoiesis, IFN response and enriched GSEA term 'GO positive regulation of cell cycle phase transition' (FDR 0.16) in BM-derived LSK F1-P and F1-L mice. Genes with p<0.05 are marked in red. BM, bone marrow; DEG, differentially expressed gene; FC, fold change; FDR, false discovery rate; FSC, forward scatter; F1-L, F1-lupus; F1-P, F1-prediseased; GSEA, gene set enrichment analysis; IFN, interferon; LSK, $Lin^-Sca\text{-}1^+c\text{-}Kit^+$; NES, normalised enrichment score.

IFN signature, a hallmark of active SLE[25] (figure 1D). Comparison with publicly available gene sets—encompassing CMP[26] and granulocyte–macrophage progenitor (GMP)[27] signatures from wild-type mice as reference—demonstrated positive enrichment in F1-L LSK for CMP and GMP signatures (online supplementary figure 1B) indicative of priming towards this direction. Together, these transcriptomic data indicate increased proliferation and strong differentiation of F1-L LSK towards myeloid/granulocytic lineage.[28]

Active proliferation and replicative stress of murine lupus HSPCs cells

To validate transcriptomic data, we analysed the BM LSK compartment and its subpopulations. LSK were increased by almost twofold in F1-L mice compared with F1-P (figure 2A,B). Within the LSK compartment, short-term HSCs and multipotent progenitor cells (MPPs) had higher frequency in F1-L mice (figure 2A,C). Compared with their control counterparts, we found enhanced frequency of circulating LSK in the peripheral blood—but not in the spleen—of F1-L mice (online supplementary figure 1C,D), suggesting that BM-derived LSK may be activated, may exit the niche and then migrate to the periphery.

During steady state, HSPCs are relatively quiescent, maintaining a low number of cycling cells that will differentiate into mature blood cells.[29] Cell cycle analysis of lupus LSK revealed increased proliferation with fewer F1-L LSK in G0 phase compared with B6-O control (figure 2D). The frequency of apoptotic LSK from NZBW/F1 mice was also increased compared with B6 with no significant difference in F1-P versus F1-L stage (online supplementary figure 1E). In view of the enhanced proliferation, γ-H2AX was measured as a marker of proliferative stress. Lupus LSK exhibited increased double-strand DNA breaks compared with B6 (figure 2E), suggesting that LSK in lupus mice is under replicative stress (see discussion).

Differentiation arrest of CMPs with lupus disease progression

To delineate the haematopoietic differentiation after the LSK stage, we characterised the progenitors of each lineage. CMPs were increased by 2.5-fold (**$p \leq 0.01$), while GMPs were 2-fold (***$p \leq 0.001$) reduced in F1-L versus F1-P mice (figure 3A). There was no significant difference in common lymphoid progenitors (online supplementary figure 2A). Both LSK and CMP frequencies were increased in F1-L mice, a profile reminiscent of emergency granulopoiesis.[30]

To further investigate the regulation of myeloid differentiation, we performed transcriptional analysis. Most of 721 DEGs were downregulated at F1-L CMPs (figure 3B and online supplementary table 2), including enriched GO terms per cluster: response to IFN-beta and nucleotide signalling (green); immune response/immunoglobulins (blue); and cytokine signalling, neutrophil degranulation and haematopoietic cell lineage (red). Myeloid markers were downregulated and proliferation markers were not differentially expressed (figure 3C). DEGs were involved in pathways related to myeloid-mediated immunity, granulocyte activation, neutrophil migration and complement activation (online supplementary figure 2B). Thus, we checked the expression of specific granulocytic markers (figure 3D). Chemokines and regulators of IL-1 family[11 31 32] were downregulated in F1-L CMPs. Indicatively, the expression of main regulators such as *Cebpe*, *Cebpd*, *Csf3r* and *Csf2rα* was dampened in CMPs of F1-L stage (figure 3D). Collectively, these results suggest differentiation arrest at the level of myeloid progenitors.

Increased neutrophils in the lupus BM: evidence of 'granulocytic priming'

In view of the differentiation arrest, we assumed that terminally differentiated cells may be decreased. However, neutrophils exhibited a 1.6-fold increase in the F1-L mice compared with F1-P, while there were comparable monocyte levels in BM (figure 4A,B). Ageing accounted for only an increase of 1.16-fold in neutrophils of control mice. In contrast, there was marked decrease of neutrophils in blood and spleen of F1-L mice (figure 4C,D, respectively), while monocytes did not differ significantly in the periphery (online supplementary figure 3A,B, respectively). Together these data suggest priming in the lupus BM towards neutrophils.

Deregulation of differentiation of primed HSPCs indicates an alternative granulopoiesis pathway in lupus mice

To investigate how 'granulocytic priming' evolves during differentiation of haematopoiesis, we performed a comparative analysis between LSK and CMP transcriptomes. We used Regulatory Network Enrichment Analysis (RNEA) algorithm[33] to report enrichment of transcription factors and regulators by combining previous studies with our data. We identified 13 common differentially expressed transcription factors and regulators (online supplementary figure 4A), predominantly downregulated in the F1-L CMP stage (online supplementary figure 4B), mainly of myeloid and granulocytic differentiation. Therefore, we looked into expression of major regulators of granulocytic and neutrophilic differentiation, such as *Cebpα*, *Cebpe*, *Irf8*, *Mpo* and *Elane*, and found them upregulated in F1-L LSK while downregulated in F1-L CMPs (online supplementary figure 4C). Collectively, these data indicate a deregulation of differentiation at the CMP level and 'priming' of LSK towards granulocytes, indicative of an *alternative granulopoiesis pathway*.

Human SLE CD34$^+$ transcriptome demonstrates active proliferation and myeloid skewing

We next asked whether we could trace the 'lupus LSK signature' in human disease. To this end, we purified CD34$^+$ cells from BM of female patients with SLE [34 35] (online supplementary tables 1 and 5) and HC. In humans, the CD34$^+$ compartment comprises a cluster of 0.5%–2.0% of BM, which encompasses both stem and progenitor cells of different lineages.[36] We identified 2364 DEGs between patients with SLE and HC, which contained 832 upregulated and 1532 downregulated genes (online supplementary table 2). Enriched GO terms per cluster include extracellular vesicle-mediated signalling in recipient (blue); myeloid leucocyte migration and chemokine signalling pathway (turquoise); integrin-mediated signalling and regulation of cell–cell adhesion (red); antigen processing and presentation, autoimmunity and abnormal inflammatory response (green); and cell surface receptor signalling pathway (yellow) (figure 5A). Lymphoid markers were downregulated in SLE, while expression of myeloid markers exhibited considerable variation within the patients. Combining these two panels, we found that early haematopoiesis in humans is characterised by skewing towards myeloid lineage (figure 5B). SLE CD34$^+$ cells exhibited enhanced proliferation (figure 5B), while GSEA indicates a positive correlation with 'activation of ATR in response to replication stress', 'cell cycle' and 'DNA-dependent DNA replication' sets (figure 5C and online supplementary table 4). In view of enhanced proliferation,[37] γ-H2AX was assessed to check if CD34$^+$ cells are in proliferative stress; indeed, SLE HSPCs exhibited increased double-strand DNA breaks (figure 5D). Collectively, CD34$^+$ cells of patients with SLE exhibited enhanced proliferation

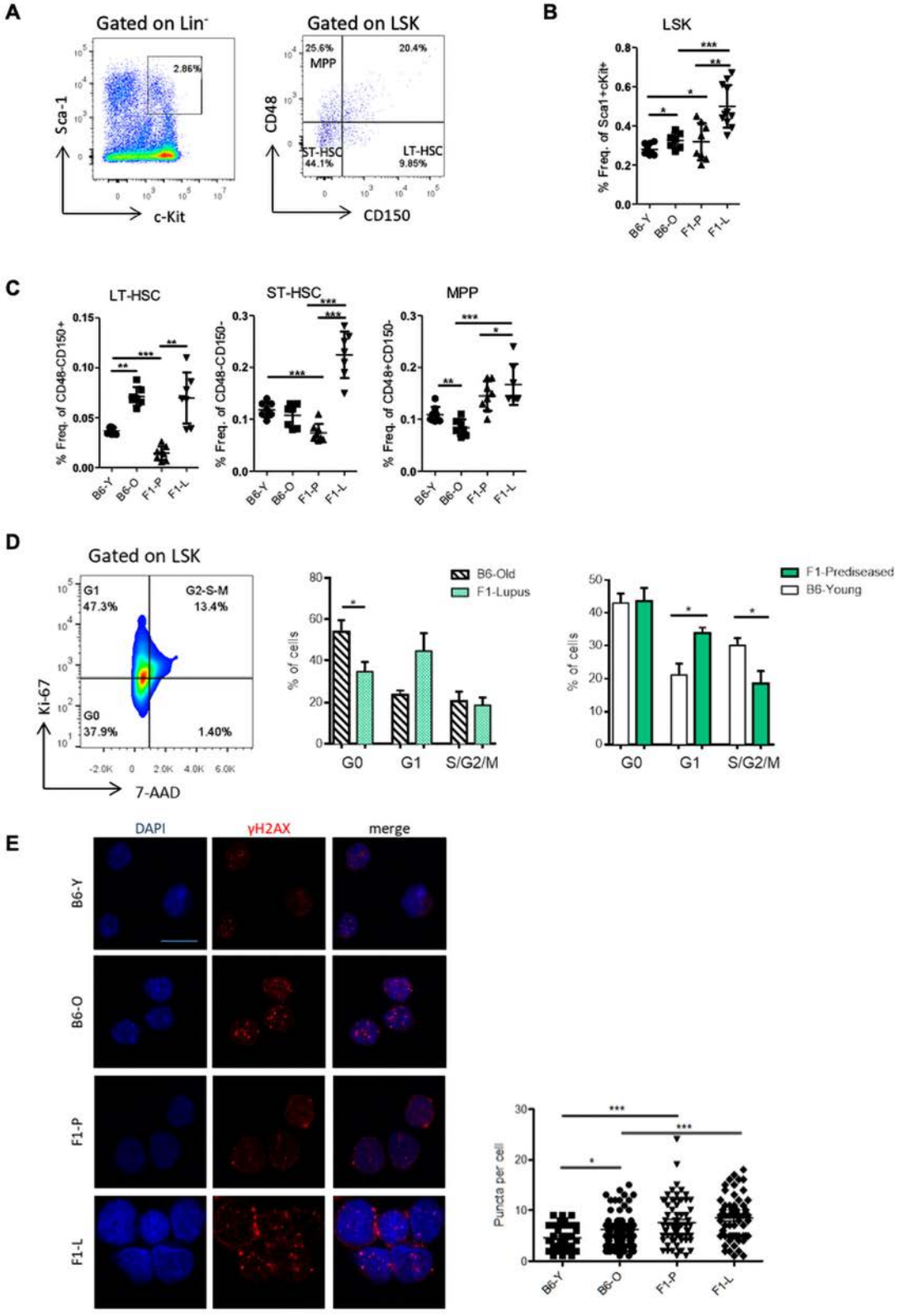

Figure 2 Phenotypical analysis of murine LSK by flow cytometry demonstrates enhanced proliferation. (A) Representative flow cytometry analysis within LSK compartment. (B) Frequencies of LSK in BM of F1-P, F1-L and their age-matched C57BL/6 control mice (n=7–10). (C) LT-HSCs, ST-HSCs and MPP in BM of F1-P, F1-L and their age-matched C57BL/6 control mice (n=7–10). (D) Representative flow cytometry plot of BM-derived LSK cell cycle analysis using Ki-67/7-AAD marker and frequencies of cells in each different phase of cell cycle (G0, G1 and S/G2/M) (n=4–6). (E) Representative confocal microscopy images for γ-H2AX (red) and DAPI (blue), and γ-H2AX puncta/cell in sorted LSK from BM of F1-P, F1-L and their age-matched C57BL/6 control mice (n=3–4, Leica TCS SP5 63x, scale bar: 10 µM). One representative experiment of four is shown. Results are mean±SEM. Statistical significance was obtained by unpaired Student's t-test (*p≤0.05, **p≤0.01, ***p≤0.001). 7-AAD, 7-Aminoactinomycin D; BM, bone marrow; DAPI, 4′,6-diamidino-2-phenylindole; F1-L, F1-lupus; F1-P, F1-prediseased; LSK, $Lin^{-}Sca\text{-}1^{+}c\text{-}Kit^{+}$; LT-HSC, Long Term-Hematopoietic Stem Cell; MPP, multipotent progenitor cell; ST-HSC, short term hematopoietic stem cell.

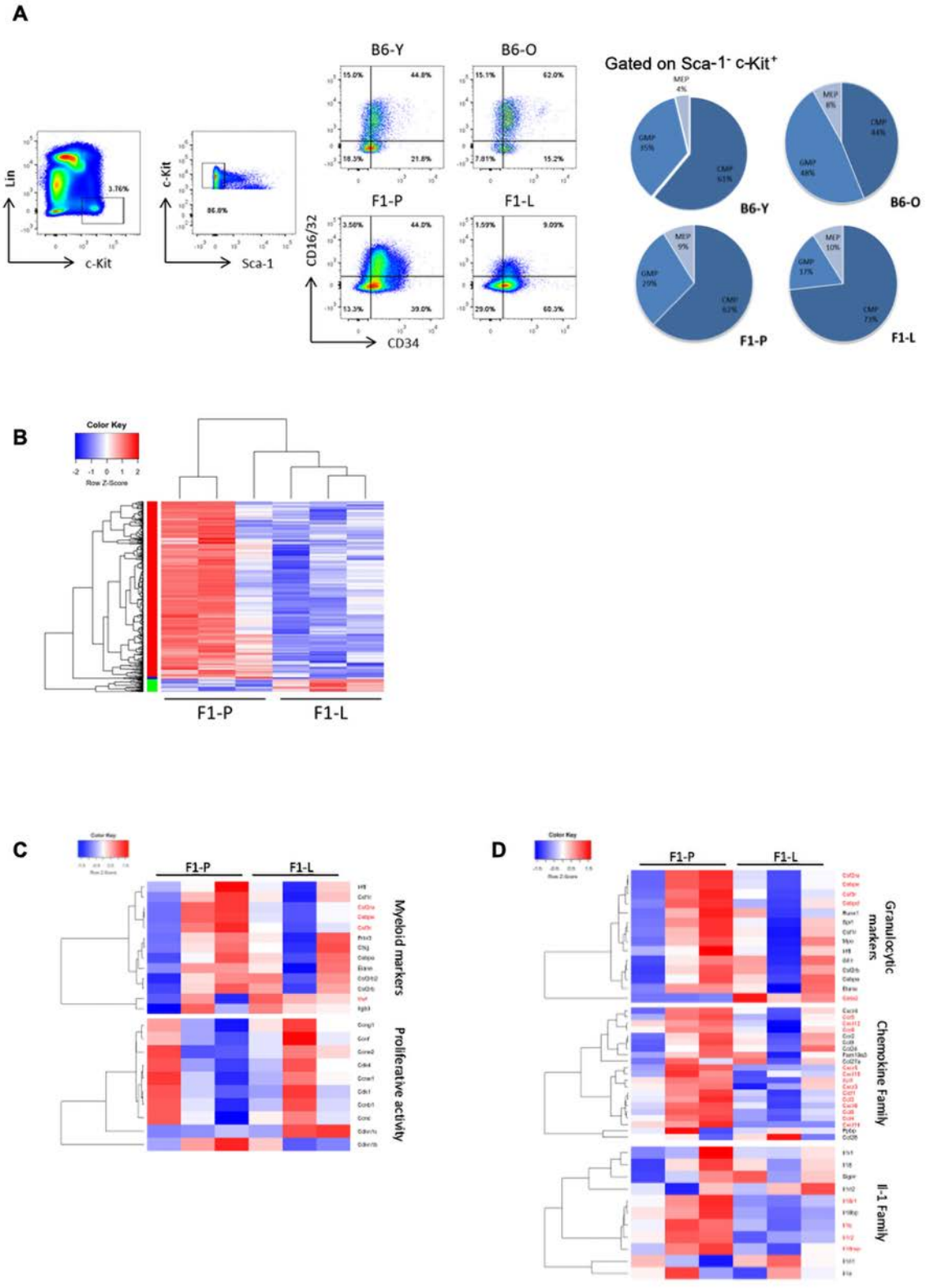

Figure 3 Attenuation of murine lupus CMP differentiation with the progression of the disease. (A) Representative flow cytometry analysis and frequencies of BM-derived CMPs ($CD34^+CD16/32^-$) and GMPs ($CD34^+CD16/32^+$) of F1-P, F1-L and their age-matched C57BL/6 control mice (n=8–10). (B) Heatmap of DEGs (|FC|>1.5, FDR<0.05) in BM-derived CMP cells between F1-P and F1-L mice (n=4 per replicate). (C) Heatmaps of genes related to myelopoiesis and proliferation in BM-derived CMP F1-P and F1-L mice. Genes with p<0.05 are marked in red. (D) Heatmaps of genes related to granulopoiesis, chemokine-related and IL-1-related factors in BM-derived CMP of F1-P and F1-L mice. Genes with p<0.05 are marked in red. BM, bone marrow; CMP, committed myeloid progenitor; FC, fold change; FDR, false discovery rate; F1-L, F1-lupus; F1-P, F1-prediseased; GMP, granulocyte–macrophage progenitor; IL, interleukin.

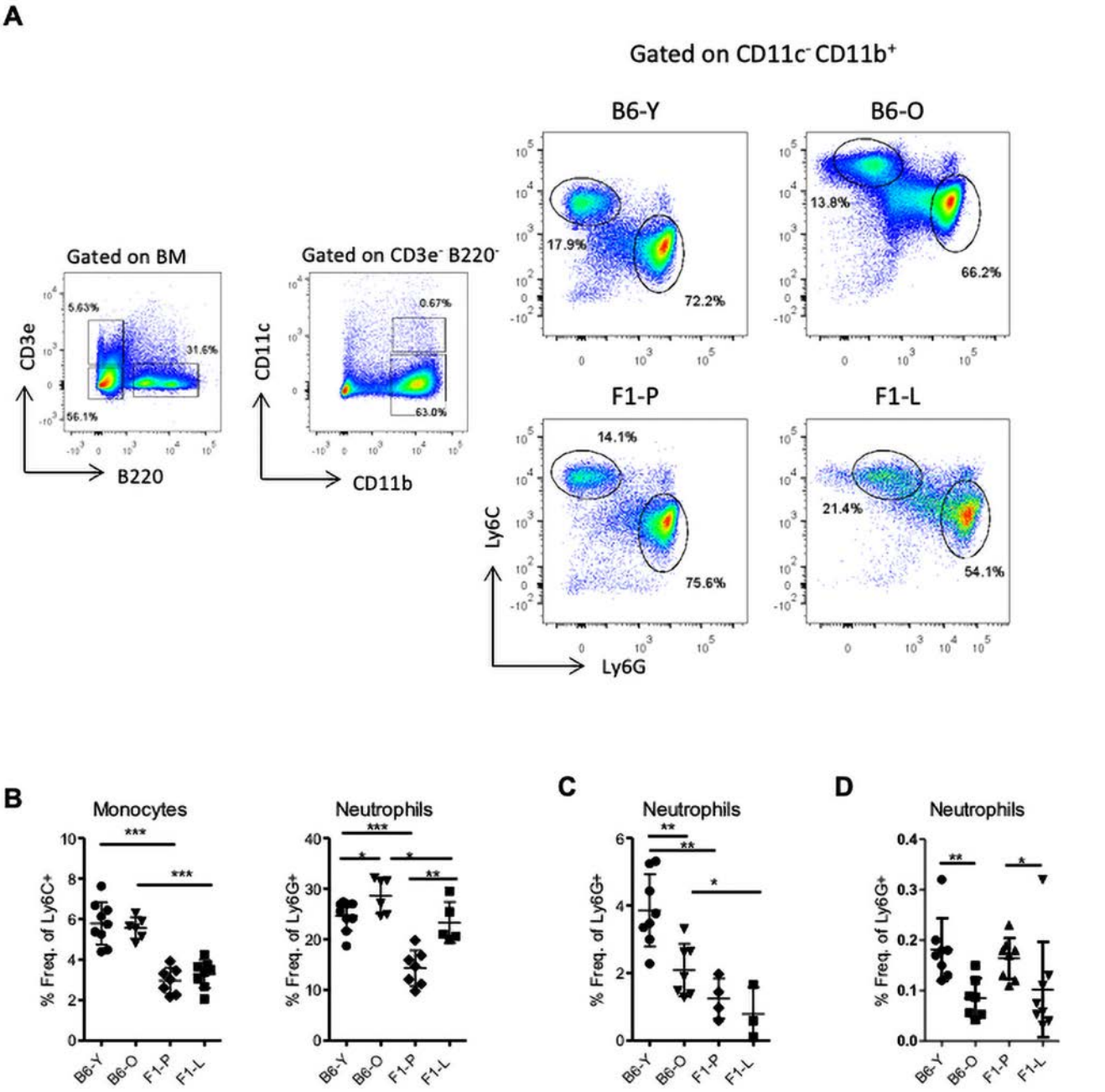

Figure 4 Neutrophils increase in the BM but decrease in the periphery of lupus mice. (A) Representative flow cytometry analysis of monocytes ($CD3e^-$ $B220^-$ $CD11b^+$ $Ly6C^+$) and neutrophils ($CD3e^-$ $B220^-$ $CD11b^+$ $Ly6G^+$) in BM of F1-P, F1-L and their age-matched C57BL/6 control mice. (B) Frequencies of monocytes and neutrophils in BM of F1-P, F1-L mice and their age-matched C57BL/6 control mice (n=6–11). (C) Frequencies of neutrophils in peripheral blood (n=3–8) and (D) spleen of F1-P, F1-L and their age-matched C57BL/6 control mice (n=6–10; *p≤0.05, **p≤0.01, ***p≤0.001). BM, bone marrow; F1-L, F1-lupus; F1-P, F1-prediseased.

with increased DNA damage and unbalanced differentiation towards myeloid axis.

Subgroup analysis showed that patients with SLE with both severe and moderate disease (online supplementary figure 6) showed variable expression of myeloid markers (figure 5E). Notably, expression of specific markers for granulopoiesis, such as *CEBPZ*, *CEBPD*, *GATA2*, was increased in a subgroup of patients with severe SLE compared with those with moderate disease (figure 5E), a result consistent with our findings in murine lupus LSK. GSEA revealed a positive correlation with 'cytokine–cytokine receptor interaction' and 'positive regulation of locomotion' sets in patients with severe SLE (figure 5F and online supplementary table 4). Enrichment analysis using upregulated genes in severe SLE revealed an over-representation of chemotaxis and migration of granulocytes and neutrophilic GO terms (figure 5G). Together, HSPCs are activated and more proliferative in patients with SLE compared with HC with a distinct transcriptional differentiation profile in patients with severe disease.

Comparison of human lupus $CD34^+$ with murine lupus CMP transcriptome reveals common attributes with evidence of arrest at the progenitor stage

Next, we compared human $CD34^+$ transcriptome to LSK and CMP data.[38] We found a significant overlap of LSK DEGs

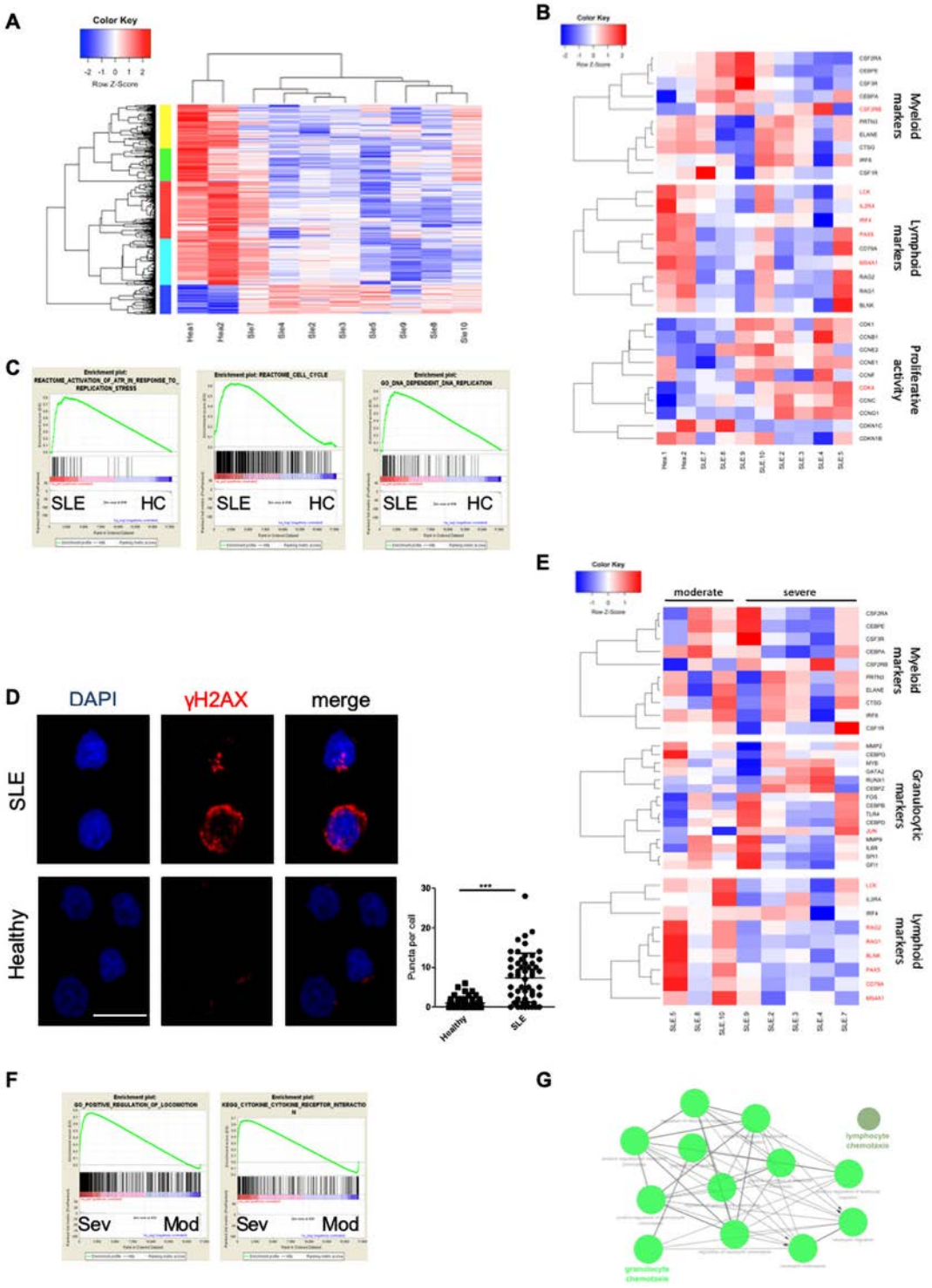

Figure 5 RNA sequencing of human CD34$^+$ cells in patients with SLE suggests active proliferation with myeloid skewing. (A) Heatmap of DEGs in CD34$^+$ cells isolated from BM of patients with SLE (n=8) and HC (n=2). (B) Heatmaps of genes related to myelopoiesis, lymphopoiesis and proliferation in patients with SLE and HC. Genes with p<0.05 are marked in red. (C) GSEA plot showing the enrichment of 'reactome: activation of ATR in response to replication stress' (NES 1.73, FDR 0.056), 'reactome: cell cycle' (NES 1.81, FDR 0.002), 'GO DNA dependent DNA replication' (NES 2.03, FDR<0.001) gene sets in CD34$^+$ patient with SLE samples. (D) Representative confocal microscopy images for γ-H2AX (red) and DAPI (blue), and γ-H2AX puncta/cell in CD34$^+$ cells from BM of patients with SLE (n=3) and HC (n=2) (Leica TCS SP5 63x, Scale bar: 10 μM). One representative experiment is shown. Results are mean±SEM. Statistical significance was obtained by unpaired Student's t-test (*p≤0.05, **p≤0.01, ***p≤0.001). (E) Heatmaps of genes associated with myeloid, lymphoid and granulocytic markers in CD34$^+$ cells isolated from BM of SLE with Mod (n=3) and Sev (n=5) disease. Genes with p<0.05 are marked in red. (F) GSEA plot showing the enrichment of 'GO Positive regulation of locomotion' (NES 1.58, FDR 0.0053) and 'KEGG cytokine-cytokine receptor interaction' (NES 1.30, FDR 0.042) gene sets in CD34$^+$ SLE Sev patients. (H) Network of the upregulated genes associated with migration and chemotaxis of granulocytes and neutrophils using ClueGo plug-in in Cytoscape. BM, bone marrow; DAPI, 4',6-diamidino-2-phenylindole; DEG, differentially expressed gene; FDR, false discovery rate; GSEA, gene set enrichment analysis; HC, healthy control; Mod, moderate; Sev, severe; SLE, systemic lupus erythematosus.

(6.7%) and CMPs DEGs (8.5%) with the human CD34$^+$ DEGs (representation factor (RF)=3.2, $p<2.7\times10^{-29}$ and RF=4, $p<4.7\times10^{-18}$, respectively) (figure 6A,B). The majority of shared DEGs (26) between mouse LSK and human CD34$^+$ cells were downregulated in patients with human SLE, but not in murine lupus (figure 6C). However, the expression of common DEGs (54) between mouse CMPs and human CD34$^+$ cells exhibited commonalities (figure 6D). Thus, the SLE CD34$^+$ transcriptomic profile is more reminiscent of murine lupus CMPs than murine lupus LSK. This is likely due to the fact that the haematopoietic stem cell signature might be diluted within the heterogeneous CD34$^+$ population in humans.[39]

At pathway level, the overlap between mouse and human data was more prominent. Compared with gene-level results, there was a fivefold higher representation of the enriched pathways in LSK and a twofold higher representation of the enriched pathways in CMPs (RF=81, $p<6.8\times10^{-172}$, and RF=43.3, $p<4.8\times10^{-178}$, respectively), which were also found enriched in human HSPCs (figure 6A,B). Key pathways such as cell activation, regulation of cell differentiation, immune system development and leucocyte migration were shared. Using RNEA, we found 15 suppressed regulators in both lupus CMPs and CD34$^+$ SLE cells. Among them, there were significant disease-specific effectors such as IFNγ and IL-6, as well as regulators for the homeostasis–differentiation balance of stem cells like Lif, Ets1 and Pax5 (figure 6E).

Collectively, human CD34$^+$ cells from patients with SLE display more commonalities in their transcripthmic profile with murine lupus CMPs rather than LSK. This comparison indicates an arrest at the progenitor level both in murine and human lupus haematopoiesis as important regulators for terminal differentiation are downregulated.

DISCUSSION

Blood and immune cells derive from HSPCs, which reside in the BM in quiescent state, being ready to respond to stress, such as severe infection, systemic inflammation or iatrogenic myeloablation.[40] Recent data suggest significant heterogeneity within the HSPCs with evidence of early lineage segregation, lineage-biassed existence and containment of lineage-restricted progenitors.[41] These lineage-biassed and lineage-restricted cells within the phenotypical HSPC compartment might serve as an emergency backup for stress conditions, capable of efficiently and specifically counterbalance the sudden loss of a particular lineage. Herein, we provide evidence of dysregulated differentiation during haematopoiesis in SLE. Transcriptomic data demonstrate enhanced activation and differentiation preference towards myeloid/granulocytic lineage after disease onset. NZBW/F1 exhibit detectable levels of type I IFN compared with other SLE mouse models.[42] We show that, indeed, IFN signature is present in the NZBW/F1 model and that lupus HSPCs can sense and respond to IFN. Chronic activation of the IFN-α pathway in HSPCs impairs their function, whereas acute IFN-α treatment promotes the proliferation of dormant HSPCs.[43]

Our flow cytometric analysis revealed enhanced proliferation of LSK in lupus mice and increase in their subpopulations. This finding is consistent with findings by Niu *et al*[7] in a congenic lupus model where they found a genetic polymorphism on the *Cdkn2c* gene related to cell cycle. In the context of stem cell proliferation and activation, Walter *et al*[37] showed direct response of HSPCs by exiting quiescence with concomitant DNA damage. In agreement to this, lupus LSK had more DNA damage compared with their controls, which could be detrimental for their maintenance and self-renewal. Pronounced cell cycle entry and consequent proliferative stress may result in impaired HSC self-renewal potential.[44 45]

To confirm the transcriptomic results on LSK differentiation, we profiled CMPs. Physiological myelopoiesis evolves through MPPs to lineage-restricted CMPs and then converges to GMPs.[46] Phenotypical analysis of progenitors showed increased frequency of CMPs but decreased frequency of GMPs, as evidenced in RNA sequencing by 'silencing' of differentiation after the CMP stage. Myeloid skewing is, in part, expected due to inflammation and ageing,[47] both operant in our model. Our results suggest 'priming' of LSK with a pronounced 'myeloid/granulocytic signature' but downregulation as the differentiation evolves towards canonical myelopoiesis (figure 6F).

Increased neutrophils in lupus BM suggest deregulation of homeostatic mechanisms in the level of CMPs with priming of LSK towards the granulocytic differentiation at the expense of lymphopoiesis. These results are consistent with our earlier findings of strong granulopoiesis signature in the BM by using DNA arrays.[9] Priming of LSK highly correlates with the signature of HSPCs after 'training' with β-glucan[11] (online supplementary figure 3), strongly indicating that 'SLE inflammatory milieu' promotes the immune training memory of BM progenitor cells. Accordingly, we found differentially methylated regions from lupus HSPCs overlapping with transcription factor binding sites relevant to haematopoietic development, including *Cebpα* (*data not shown*). Innate immune memory, while beneficial to host defence against pathogens, could also lead to maladaptation of the immune system in chronic inflammation, leading to perpetuation of chronic inflammatory disorders and predisposing to flares in response to environmental stimuli such as infections or stress.[48]

Myeloid cells are crucial for disease progression. In the periphery of lupus mice, we found increased circulating LSK but decreased neutrophils. This could be due to either extensive destruction of neutrophils in the periphery or migration to target tissues. This might act as a positive feedback loop where an inflammatory environment triggers priming and exit of HSPCs to periphery, driving them to increased myeloid output, which in turn circulates and perpetuates the inflammation as proposed by Oduro *et al*[14] in an arthritis mouse model. It is conceivable that neutrophils may migrate to the inflamed tissues, hence their relative paucity in the periphery. The release of neutrophil extracellular traps represents a novel neutrophil effector function contributing to thromboinflammation and fibrosis in SLE.[49]

It has been assumed that various blood cell lineages arise via a hierarchical scheme—starting with HSPCs—and that their differentiation potential becomes increasingly restricted through oligopotent and then unipotent progenitors. However, recent work suggests a developmental shift to an adult 'two-tier' hierarchy whereby HSPCs can generate restricted subsets of terminally differentiated progeny, bypassing the stepwise progression through common progenitor stage.[50] Yammamoto *et al*[51] proposed a revised model of haematopoietic differentiation with the existence of progenitors within the HSPC compartment, mostly myeloid-committed ones. Our results are in agreement with this model. It is reasonable to assume that SLE LSK are already predefined to differentiate towards granulocytic lineage, creating an alternative granulopoiesis pathway in the haematopoietic tree (figure 6F). In parallel, differentiation arrest at the intermediate stage of CMPs blocks flow of haematopoiesis towards GMPs.

In summary, we have presented evidence for deregulation of granulopoiesis and priming of HSPCs, which may contribute

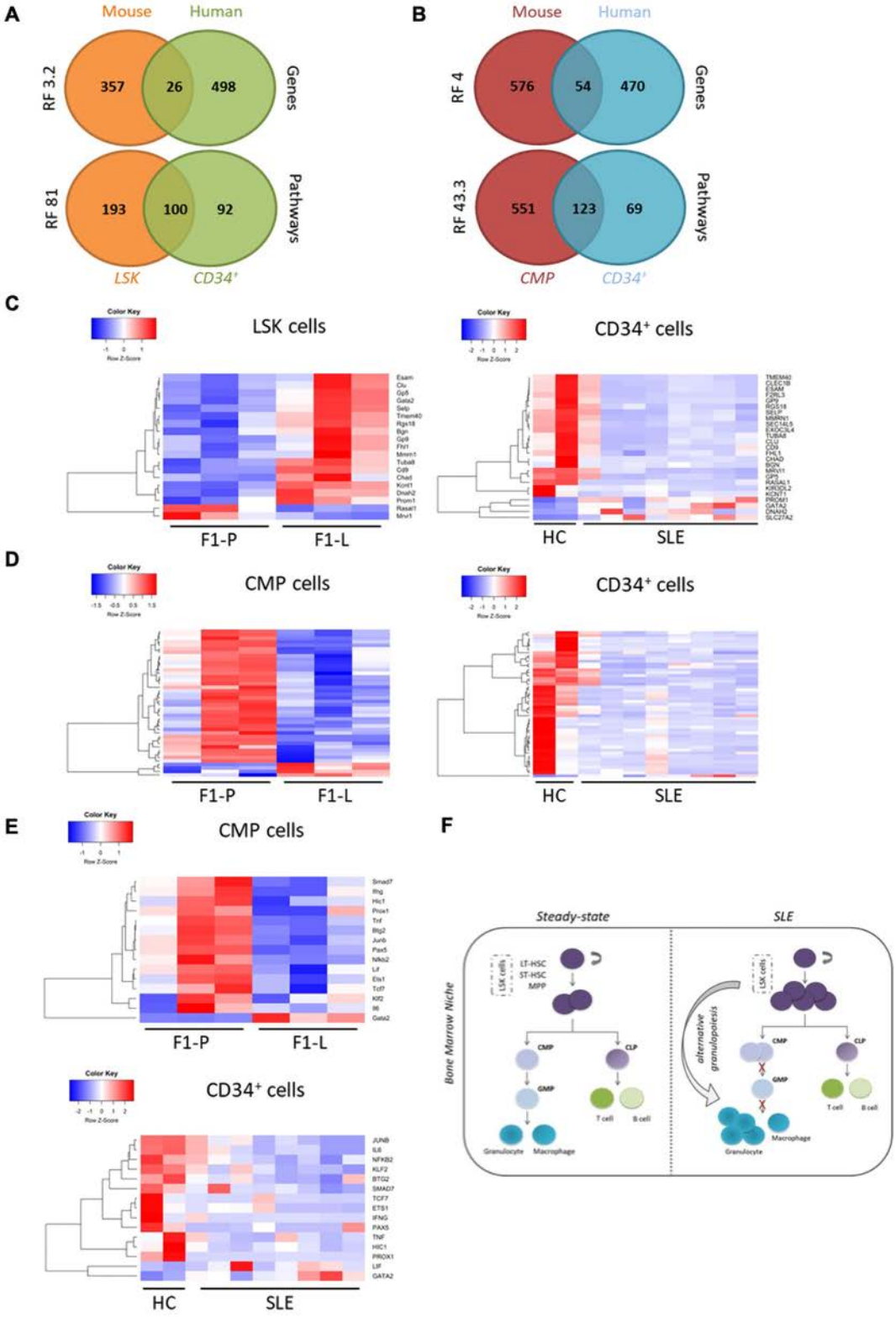

Figure 6 Comparison at the transcriptome level of human lupus CD34$^+$ with murine lupus LSK and CMP reveals common attributes with evidence of arrest at the progenitor stage. (A) Venn diagrams showing the overlap between significant DEGs and the respective enriched GO terms and pathways in human CD34$^+$ and mouse LSK. (B) Venn diagrams showing the overlap between significantly DEGs and the respective enriched GO terms and pathways in human CD34$^+$ and mouse CMP cells. (C) Heatmaps of 26 common DEGs in murine LSK (left panel) and human CD34$^+$ cells (right panel). (D) Heatmaps of 54 common DEGs in murine CMP cells (left panel) and human CD34$^+$ cells (right panel). (E) Heatmaps of the enriched transcription factors and regulators in murine CMP cells (left panel) and CD34$^+$ cells based on RNEA algorithm (right panel). (F) Proposed model of alternative granulopoiesis in SLE. LSK in SLE are predefined to differentiate towards the granulocytic lineage by skipping the CMP and GMP stages of the haematopoietic lineage. CLP, common lymphoid progenitor; CMP, committed myeloid progenitor; DEG, differentially expressed gene; HC, healthy control; LSK, Lin$^-$Sca-1$^+$c-Kit$^+$; LT-HSC, long erm-hematopoietic stem cell; tSLE, systemic lupus erythematosus; MPP, multipotent progenitor cell; RNEA, Regulatory Network Enrichment AnalysisST-HSC, short term-hematopoietic stem cell.

to persistent inflammation in SLE and risk of flare once the disease is in remission. Myeloid skewing of HSPCs, associated with epigenetic tinkering, is also typical of HSPCs during ageing,[51 52] contributing to decreased adaptive immunity and enhanced cardiovascular mortality of the elderly population.[53–55] Re-establishment of the appropriate lymphoid versus myeloid balance in systemic autoimmune diseases may improve immune function, decreasing risk of infection or atherosclerosis and resolution of inflammation.

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Contributors MG and AB designed and performed the experiments, analysed data, generated figures and wrote the manuscript. AF and PP analysed the data and generated the figures. SG, VK, DN and AP performed clinical evaluation of patients, provided human specimens and interpreted the human data. GB, PV and IM participated in the design, the interpretation of data and the editing of the manuscript. DTB supervised the study and the writing of the manuscript.

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REFERENCES

1 Tsokos GC. Systemic lupus erythematosus. *N Engl J Med* 2011;365:2110–21.
2 Bertsias GK, Salmon JE, Boumpas DT. Therapeutic opportunities in systemic lupus erythematosus: state of the art and prospects for the new decade. *Ann Rheum Dis* 2010;69:1603–11.
3 King KY, Goodell MA. Inflammatory modulation of HSCs: viewing the HSC as a foundation for the immune response. *Nat Rev Immunol* 2011;11:685–92.
4 Baldridge MT, King KY, Goodell MA. Inflammatory signals regulate hematopoietic stem cells. *Trends Immunol* 2011;32:57–65.
5 Trumpp A, Essers M, Wilson A. Awakening dormant haematopoietic stem cells. *Nat Rev Immunol* 2010;10:201–9.
6 Zhao JL, Baltimore D. Regulation of stress-induced hematopoiesis. *Curr Opin Hematol* 2015;22:286–92.
7 Niu H, Fang G, Tang Y, *et al*. The function of hematopoietic stem cells is altered by both genetic and inflammatory factors in lupus mice. *Blood* 2013;121:1986–94.
8 Voulgarelis M, Giannouli S, Tasidou A, *et al*. Bone marrow histological findings in systemic lupus erythematosus with hematologic abnormalities: a clinicopathological study. *Am J Hematol* 2006;81:590–7.
9 Nakou M, Knowlton N, Frank MB, *et al*. Gene expression in systemic lupus erythematosus: bone marrow analysis differentiates active from inactive disease and reveals apoptosis and granulopoiesis signatures. *Arthritis Rheum* 2008;58:3541–9.
10 Mitroulis I, Kalafati L, Hajishengallis G, *et al*. Myelopoiesis in the context of innate immunity. *J Innate Immun* 2018;10:365–72.
11 Mitroulis I, Ruppova K, Wang B, *et al*. Modulation of myelopoiesis progenitors is an integral component of trained immunity. *Cell* 2018;172:e12:147–61.
12 Kaufmann E, Sanz J, Dunn JL, *et al*. BCG Educates hematopoietic stem cells to generate protective innate immunity against tuberculosis. *Cell* 2018;172:e19:176–90.
13 Dragoljevic D, Kraakman MJ, Nagareddy PR, *et al*. Defective cholesterol metabolism in haematopoietic stem cells promotes monocyte-driven atherosclerosis in rheumatoid arthritis. *Eur Heart J* 2018;39:2158–67.
14 Oduro KA, Liu F, Tan Q, *et al*. Myeloid skewing in murine autoimmune arthritis occurs in hematopoietic stem and primitive progenitor cells. *Blood* 2012;120:2203–13.
15 Maeda K, Malykhin A, Teague-Weber BN, *et al*. Interleukin-6 aborts lymphopoiesis and elevates production of myeloid cells in systemic lupus erythematosus-prone B6.Sle1.Yaa animals. *Blood* 2009;113:4534–40.
16 Christ A, Günther P, Lauterbach MAR, *et al*. Western diet triggers NLRP3-Dependent innate immune reprogramming. *Cell* 2018;172:e14:162–75.
17 Murphy AJ, Akhtari M, Tolani S, *et al*. ApoE regulates hematopoietic stem cell proliferation, monocytosis, and monocyte accumulation in atherosclerotic lesions in mice. *J Clin Invest* 2011;121:4138–49.
18 Yvan-Charvet L, Pagler T, Gautier EL, *et al*. ATP-Binding cassette transporters and HDL suppress hematopoietic stem cell proliferation. *Science* 2010;328:1689–93.
19 Vlachou K, Mintzas K, Glymenaki M, *et al*. Elimination of granulocytic myeloid-derived suppressor cells in lupus-prone mice linked to reactive oxygen species-dependent extracellular trap formation. *Arthritis Rheumatol* 2016;68:449–61.
20 Hochberg MC. Updating the American College of rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997;40:1725.
21 Theofilopoulos AN, Dixon FJ. Murine models of systemic lupus Erythematosus1. In: *Advances in immunology*. Elsevier, 1985: 269–390.
22 Dixon FJ, Andrews BS, Eisenberg RA, *et al*. Etiology and pathogenesis of a spontaneous lupus-like syndrome in mice. *Arthritis Rheum* 1978;21:S64–7.
23 Perry D, Sang A, Yin Y, *et al*. Murine models of systemic lupus erythematosus. *J Biomed Biotechnol* 2011;2011:271694:1–19.
24 Haas S, Hansson J, Klimmeck D, *et al*. Inflammation-Induced emergency megakaryopoiesis driven by hematopoietic stem cell-like megakaryocyte progenitors. *Cell Stem Cell* 2015;17:422–34.
25 Crow MK. Type I interferon in the pathogenesis of lupus. *J Immunol* 2014;192:5459–68.
26 Klimmeck D, Cabezas-Wallscheid N, Reyes A, *et al*. Transcriptome-Wide profiling and posttranscriptional analysis of hematopoietic stem/progenitor cell differentiation toward myeloid commitment. *Stem Cell Reports* 2014;3:858–75.
27 Rundberg Nilsson A, Soneji S, Adolfsson S, *et al*. Human and murine hematopoietic stem cell aging is associated with functional impairments and intrinsic megakaryocytic/erythroid bias. *PLoS One* 2016;11:e0158369.
28 Forsberg EC, Prohaska SS, Katzman S, *et al*. Differential expression of novel potential regulators in hematopoietic stem cells. *PLoS Genet* 2005;1:e28.
29 Pietras EM, Warr MR, Passegué E. Cell cycle regulation in hematopoietic stem cells. *J Cell Biol* 2011;195:709–20.
30 Manz MG, Boettcher S. Emergency granulopoiesis. *Nat Rev Immunol* 2014;14:302–14.

31 Hérault A, Binnewies M, Leong S, *et al*. Myeloid progenitor cluster formation drives emergency and leukaemic myelopoiesis. *Nature* 2017;544:53–8.
32 Pietras EM, Mirantes-Barbeito C, Fong S, *et al*. Chronic interleukin-1 exposure drives haematopoietic stem cells towards precocious myeloid differentiation at the expense of self-renewal. *Nat Cell Biol* 2016;18:607–18.
33 Chouvardas P, Kollias G, Nikolaou C. Inferring active regulatory networks from gene expression data using a combination of prior knowledge and enrichment analysis. *BMC Bioinformatics* 2016;17:181.
34 Ortona E, Pierdominici M, Maselli A, *et al*. Sex-based differences in autoimmune diseases. *Ann Ist Super Sanita* 2016;52:205–12.
35 Christou EAA, Banos A, Kosmara D, *et al*. Sexual dimorphism in SLE: above and beyond sex hormones. *Lupus* 2019;28:3-10.
36 Strobl H, Takimoto M, Majdic O, *et al*. Myeloperoxidase expression in CD34+ normal human hematopoietic cells. *Blood* 1993;82:2069–78.
37 Walter D, Lier A, Geiselhart A, *et al*. Exit from dormancy provokes DNA-damage-induced attrition in haematopoietic stem cells. *Nature* 2015;520:549–52.
38 Ntougkos E, Chouvardas P, Roumelioti F, *et al*. Genomic responses of mouse synovial fibroblasts during tumor necrosis factor-driven Arthritogenesis greatly mimic those in human rheumatoid arthritis. *Arthritis Rheumatol* 2017;69:1588–600.
39 Karamitros D, Stoilova B, Aboukhalil Z, *et al*. Single-cell analysis reveals the continuum of human lympho-myeloid progenitor cells. *Nat Immunol* 2018;19:85–97.
40 Mendelson A, Frenette PS. Hematopoietic stem cell niche maintenance during homeostasis and regeneration. *Nat Med* 2014;20:833–46.
41 Carrelha J, Meng Y, Kettyle LM, *et al*. Hierarchically related lineage-restricted fates of multipotent haematopoietic stem cells. *Nature* 2018;554:106–11.
42 Zhuang H, Szeto C, Han S, *et al*. Animal models of interferon signature positive lupus. *Front Immunol* 2015;6:291.
43 Essers MAG, Offner S, Blanco-Bose WE, *et al*. IFNalpha activates dormant haematopoietic stem cells in vivo. *Nature* 2009;458:904–8.
44 Moehrle BM, Geiger H. Aging of hematopoietic stem cells: DNA damage and mutations? *Exp Hematol* 2016;44:895–901.
45 Behrens A, van Deursen JM, Rudolph KL, *et al*. Impact of genomic damage and ageing on stem cell function. *Nat Cell Biol* 2014;16:201–7.
46 Akashi K, Traver D, Miyamoto T, *et al*. A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. *Nature* 2000;404:193–7.
47 Kovtonyuk LV, Fritsch K, Feng X, *et al*. Inflamm-Aging of hematopoiesis, hematopoietic stem cells, and the bone marrow microenvironment. *Front Immunol* 2016;7:502.
48 Penkov S, Mitroulis I, Hajishengallis G, *et al*. Immunometabolic crosstalk: an ancestral principle of trained immunity? *Trends Immunol* 2019;40:1–11.
49 Frangou E, Chrysanthopoulou A, Mitsios A, *et al*. REDD1/autophagy pathway promotes thromboinflammation and fibrosis in human systemic lupus erythematosus (SLE) through NETs decorated with tissue factor (TF) and interleukin-17A (IL-17A). *Ann Rheum Dis* 2019;78:238–48.
50 Notta F, Zandi S, Takayama N, *et al*. Distinct routes of lineage development reshape the human blood hierarchy across ontogeny. *Science* 2016;351:aab2116.
51 Yamamoto R, Morita Y, Ooehara J, *et al*. Clonal analysis unveils self-renewing lineage-restricted progenitors generated directly from hematopoietic stem cells. *Cell* 2013;154:1112–26.
52 Dykstra B, Olthof S, Schreuder J, *et al*. Clonal analysis reveals multiple functional defects of aged murine hematopoietic stem cells. *J Exp Med* 2011;208:2691–703.
53 Jaiswal S, Natarajan P, Silver AJ, *et al*. Clonal hematopoiesis and risk of atherosclerotic cardiovascular disease. *N Engl J Med* 2017;377:111–21.
54 Jaiswal S, Fontanillas P, Flannick J, *et al*. Age-Related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med* 2014;371:2488–98.
55 Leins H, Mulaw M, Eiwen K, *et al*. Aged murine hematopoietic stem cells drive aging-associated immune remodeling. *Blood* 2018;132:565–76.

TRANSLATIONAL SCIENCE

NCF1-339 polymorphism is associated with altered formation of neutrophil extracellular traps, high serum interferon activity and antiphospholipid syndrome in systemic lupus erythematosus

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ABSTRACT

Objectives A single nucleotide polymorphism in the NCF1 gene (NCF1-339, rs201802880), encoding NADPH oxidase type II subunit NCF1/p47^{phox}, reducing production of reactive oxygen species (ROS) is strongly associated with the development of systemic lupus erythematosus (SLE). This study aimed at characterising NCF1-339 effects on neutrophil extracellular trap (NET) formation, type I interferon activity and antibody profile in patients with SLE.

Methods Neutrophil NET-release pathways (n=31), serum interferon (n=141) and finally antibody profiles (n=305) were investigated in SLE subjects from Lund, genotyped for NCF1-339. Then, 1087 SLE subjects from the rheumatology departments of four Swedish SLE centres, genotyped for NCF1-339, were clinically characterised to validate these findings.

Results Compared with patients with normal-ROS NCF1-339 genotypes, neutrophils from patients with SLE with low-ROS NCF1-339 genotypes displayed impaired NET formation ($p<0.01$) and increased dependence on mitochondrial ROS ($p<0.05$). Low-ROS patients also had increased frequency of high serum interferon activity (80% vs 21.4%, $p<0.05$) and positivity for anti-β2 glycoprotein I ($p<0.01$) and anticardiolipin antibodies ($p<0.05$) but were not associated with other antibodies. We confirmed an over-representation of having any antiphospholipid antibody, OR 1.40 (95% CI 1.01 to 1.95), anti-β2 glycoprotein I, OR 1.82 (95% CI 1.02 to 3.24) and the antiphospholipid syndrome (APS), OR 1.74 (95% CI 1.19 to 2.55) in all four cohorts (n=1087).

Conclusions The NCF1-339 SNP mediated decreased NADPH oxidase function, is associated with high interferon activity and impaired formation of NETs in SLE, allowing dependence on mitochondrial ROS. Unexpectedly, we revealed a striking connection between the ROS deficient NCF1-339 genotypes and the presence of phospholipid antibodies and APS.

Key messages

What is already known about this subject?
- NCF1-339 low-reactive oxygen species (ROS) genotypes are highly enriched in patients with systemic lupus erythematosus (SLE).

What does this study add?
- NCF1-339 genotype affects neutrophil extracellular trap formation, regarding quantity and possibly source of ROS (NADPH oxidase 2 vs mitochondria).
- An increased frequency of patients with SLE with NCF1-339 low-ROS genotypes have high serum interferon levels.
- NCF1-339 low-ROS genotypes are strongly associated with the secondary antiphospholipid syndrome in SLE.

How might this impact on clinical practice or future developments?
- Further characterisation of the NCF1-339 genotype is expected to generate increased insights into key pathogenic mechanisms in SLE and secondary antiphospholipid syndrome, which ultimately could lead to much needed new type of treatments.

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disease, characterised by a dysregulated balance between apoptosis and clearance of dying cells, increased type I interferon (IFN) production, autoreactive B-cells and circulating immune complexes (IC).[1] Lupus nephritis and increased incidence of cardiovascular disease cause significant morbidity and mortality in patients with SLE but the risk of cardiovascular and thrombotic disease as well as pregnancy complications are even greater in patients with secondary antiphospholipid syndrome (APS). SLE development is influenced by the genetic factors, evident by the concordance rate of 24% in monozygotic twins compared with 2% in dizygotic twins.[2]

Since the identification of NCF1 as a gene controlling reactive oxygen species (ROS) and experimental arthritis in rats,[3] accumulating evidence have established a role for ROS as an important regulator of autoimmunity. Although challenging current dogma, low rather than high ROS levels are associated with autoimmune diseases.[3–7]

The most important source of ROS is the NADPH oxidase isoform 2 (NOX2), expressed by phagocytes and antigen presenting cells.[8] NOX2 selectively produces ROS on stimulation, when the cytoplasmic subunits NCF1 ($p47^{phox}$), NCF2 ($p67^{phox}$), NCF4 ($p40^{phox}$) and small GTPase RAC1 or RAC2, (depending on cell type) are associated and translocated to the transmembrane flavocytochrome b_{558} resulting in an active enzyme complex.[9]

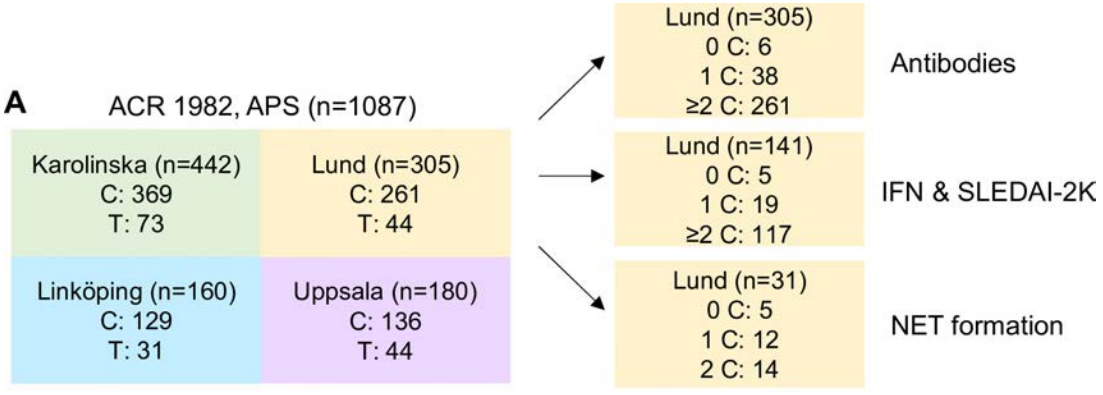
Independent reports by our group and others have associated the non-synonymous SNP NCF1-339 (rs201802880)[10] with Swedish, American and Asian SLE cases.[11 12] The NCF1 gene varies in copy number, but the most common is to have two copies of the gene, both with C at the NCF1-339 position.[10] The minor allele associated with low ROS-production has T at this position. Any genotype with less than two NCF1-339 C-alleles is predicted to have impaired ROS-production.[10 13] Low-ROS genotypes are associated with lower age at SLE onset, a type I IFN signature in patients with rheumatoid arthritis and strongly predisposes to SLE,[11 14] consistent with the concept that deficient NOX2 function promotes autoimmunity and SLE.[14]

NOX2-derived ROS are of direct importance in neutrophil oxidative burst and release of neutrophil extracellular traps (NETs).[15] Antigens associated with SLE are exposed in NETs and dysregulated NET release or impaired clearance is therefore suggested to be involved in the pathogenesis of SLE,[16] but this potential role is debated.[17–19] NOX2-derived ROS also function as regulators of the immune system,[20 21] for instance, via inhibition of IFNα production[22] or IFN-associated downstream signalling.[23] In order to investigate possible mechanisms explaining the role for ROS and the association between NCF1-339 genotype and SLE, we have studied the effects of NCF1-339 genotype on neutrophil ROS and NET formation in patients with SLE. We have also investigated potential effects on a patient with SLE type I IFN activity and antibody profile.

MATERIALS AND METHODS

Study design and patient cohorts

Four Swedish SLE patient cohorts were genotyped for NCF1-339 and included in the study (figure 1A). NCF1-339 genotype



B

NCF1 copies	NCF1-339 genotype	Genotype group
2	CC	C
3	CCC	
4	CCCC	
	CCCT	
	CCTT	
3	CCT	
	CTT	T
2	CT	
	TT	
1	T	
	C	

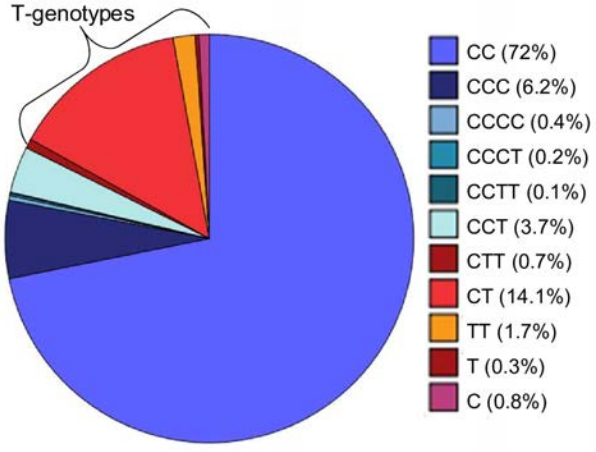


Figure 1 NCF1-339 genotype groups and study design. (A) Overview of included SLE patient cohorts, from four Swedish centres (Karolinska, Uppsala, Linköping and Lund). All patients were genotyped for NCF1-339 and evaluated by the 1982 American College of Rheumatology (ACR) classification criteria for SLE and APS manifestations. NCF1-339-dependent effects on a panel of autoantibodies were performed in the Lund cohort (n=305). Serum IFN activity and SLEDAI-2K scores were determined in a Lund subcohort (n=141). ROS and NETs were evaluated in neutrophils from 31 patients with SLE of the Lund cohort. (B) Table showing all detected NCF1-339 genotypes present among the 1087 genotyped patients and pie chart demonstrating their distribution. C-genotypes are presented in blue and T-genotypes in red and orange. APS, antiphospholipid syndrome; IFN, interferon; NET, neutrophil extracellular trap; ROS, reactive oxygen species; SLE, systemic lupus erythematosus; SLEDAI-2K, Systemic Lupus Erythematosus Disease Activity Index 2000.

influence on disease pathogenesis and phenotype were evaluated in the Lund cohort by; (1) neutrophil ROS production (n=31); (2) NET induction (n=31); (3) IFN activity (n=141); (4) SLE disease activity using Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K),[24] (n=141), originally evaluated in a previous study[25] and (5) antibodies: anti-SSA, anti-SSB, RF, anti-DNA, anti-C1q, anti-RNP, antiribosomal P protein, anti-Sm, IgG anti-β2 glycoprotein I (a_2GPI), IgG anticardiolipin (aCL) (n=305) (figure 1A). Antibodies and lupus anticoagulant (LA) were analysed at accredited clinical laboratories at Lund University hospital (ISO 17025). Genotyped patients from Linköping (n=160), Karolinska (n=442) and Uppsala (n=180) university hospitals were then included to validate findings in the Lund cohort, making a total of 1087 patients with SLE, fulfilling at least four 1982 American College of Rheumatology (ACR) classification criteria for SLE[26] (online supplementary table S1). From these, 973 were genotyped in our previous study[11] and 114 exclusively for this study, with NCF1-339 genotypes classified into C or T-genotypes. T-genotypes were further divided into '0C' and '1C' in the Lund cohort to be able to differentiate the effect of having no C-alleles (T and TT) or one C allele (CT and CTT) (figure 1B). Accordingly, C-genotypes were termed '≥2C', having 2 C or more. IgG and IgM a_2GPI, IgG and IgM aCL and LA were analysed at accredited clinical laboratories in Linköping (ISO 17025), Uppsala (ISO 15189) and Karolinska University hospitals (ISO 15189). A clinical evaluation of all 1087 patients for APS diagnosis was made based on thrombotic events and/or miscarriages in combination with repeated positive tests for IgG or IgM aCL (IgG in Lund), IgG or IgM a_2GPI (IgG in Lund) or LA, and classification according to Miyakis *et al*[27] (figure 1A).

Patient and public involvement

Patients were not invited to comment on the study design and were not consulted to develop patient relevant outcomes or interpret the results. Patients were not invited to contribute to the writing or editing of this document for readability or accuracy.

Neutrophil isolation, purification of IC and ROS induction

Details of experimental procedures regarding neutrophil isolation, purification of IC and ROS induction by phorbol-myristate-acetate (PMA), IC and ionomycin are described in online supplementary text.

NET induction and quantification

Neutrophils, 2×10^5 in 200 L RPMI 1640, were added to wells of a poly-L-lysine (0.01 %, Sigma) coated 48-well plate and incubated for 1 hour at 37°C with or without the presence of inhibitors diphenyleneiodonium (DPI) (25 µM, Sigma) and MitoTEMPO (10 µM, Sigma). NETs were induced by IC (10 µg/mL), PMA (5 and 20 nM, Sigma) and ionomycin (5 µM, Sigma) for 3 hours at 37°C. NETs were treated with micrococcal nuclease (600 mU/mL, Thermo Fisher Scientific) in nuclease buffer (10 mM Tris-HCl pH 8, 10 mM $MgCl_2$, 2 mM $CaCl_2$, 50 mM NaCl) for 30 min at 37°C and quantified by fluorometry using DNA dye Sytox Green (4 µM, Thermo Fisher Scientific) in an Infinite 200 plate reader (Tecan), excitation 485 nm/emission 535 nm and concentration determined by usage of a DNA standard (Lambda standard from Quant-iT PicoGreen kit, Thermo Fisher).

Assessment of type I IFN activity in serum

Type I IFN in serum was determined as previously described.[28 29] Briefly, WISH cells (CCL-25; American Type Culture Collection) were cultured 6 hours in the presence of SLE serum, followed by analysis of mRNA expression of six type I IFN-regulated genes (LY6E, MX1, OAS1, ISG15, IFIT1, EIFAK2) and three housekeeping genes (GAPDH, PPIB, B2M) using Quantigene Plex 2.0 assay (Panomics). Relative expression of type I IFN-regulated genes, indicating presence of active type I IFN in serum was used to calculate an IFN score. A score >2 indicates high serum IFN activity.[28]

Statistical analyses

Experimental assays on neutrophils were analysed by Kruskal-Wallis test and Mann-Whitney U test. Presence of high serum IFN activity and antibodies were analysed by Fisher's exact test. Statistical analyses of APS variables were performed by Fisher's exact test and Mantel-Haenzel meta-analysis.

RESULTS

Neutrophil ROS and NET formation is regulated by NCF1-339 genotype

First, we investigated the role of NCF1-339 genotypes on neutrophil ROS generation upon PMA, IC and ionomycin exposure in a cohort of 31 patients, each with two NCF1 alleles, but different NCF-339 genotype. They showed no genotype dependent difference in gender, age, SLICC/ACR Damage Index score, number of fulfilled ACR criteria, SLEDAI-2K, disease duration or age at diagnosis (online supplementary table S2). PMA primarily induced extracellular ROS, while IC induced intracellular and ionomycin did not induce ROS at all, as expected.[30] Neutrophils from patients with NCF1-339 0C genotype had decreased levels of extracellular and intracellular ROS on PMA stimulation, compared with the 1C and 2C genotypes (figure 2A,B). No NCF1-339 genotype dependent difference was detected by the other stimuli (figure 2A,B). Following this, we investigated if the NCF1-339 genotypes had similar effects on NET formation, again using PMA, IC and ionomycin. They are all expected to induce NET formation through different, partly overlapping pathways (online supplementary figure S1A).[31–33] The induction of NETs was confirmed by fluorescence microscopy (online supplementary figure S2). Neutrophils from patients with 0C genotype had impaired NET-releasing ability in response to low-dose PMA (5 nM), compared with neutrophils from patients with 1C and 2C genotype (figure 2C), in line with the decreased ROS production observed using PMA stimulation. Exposure to 20 nM PMA reduced the difference in amount of released NETs (figure 2D). No NCF1-339 genotype-dependent effect on NET formation on IC or ionomycin was detected (figure 2E,F). Since neutrophils from patients with SLE may exhibit enhanced NET release,[34] we evaluated both levels of spontaneous NET release and serum cell-free DNA, as a surrogate marker for circulating NETs. No genotype dependent differences were detected (online supplementary figure S3A,B). Together, these results demonstrate that the NCF1-339 genotype affects ROS and NET release specifically when PMA is used as a stimulus, suggesting a selective effect on molecular mechanisms induced by PMA, but not IC or ionomycin.

Increased dependence on mitochondrial ROS in 0C genotype NET formation

The balance between NOX2-derived and mitochondrial-derived ROS may influence NET formation and the inflammatory

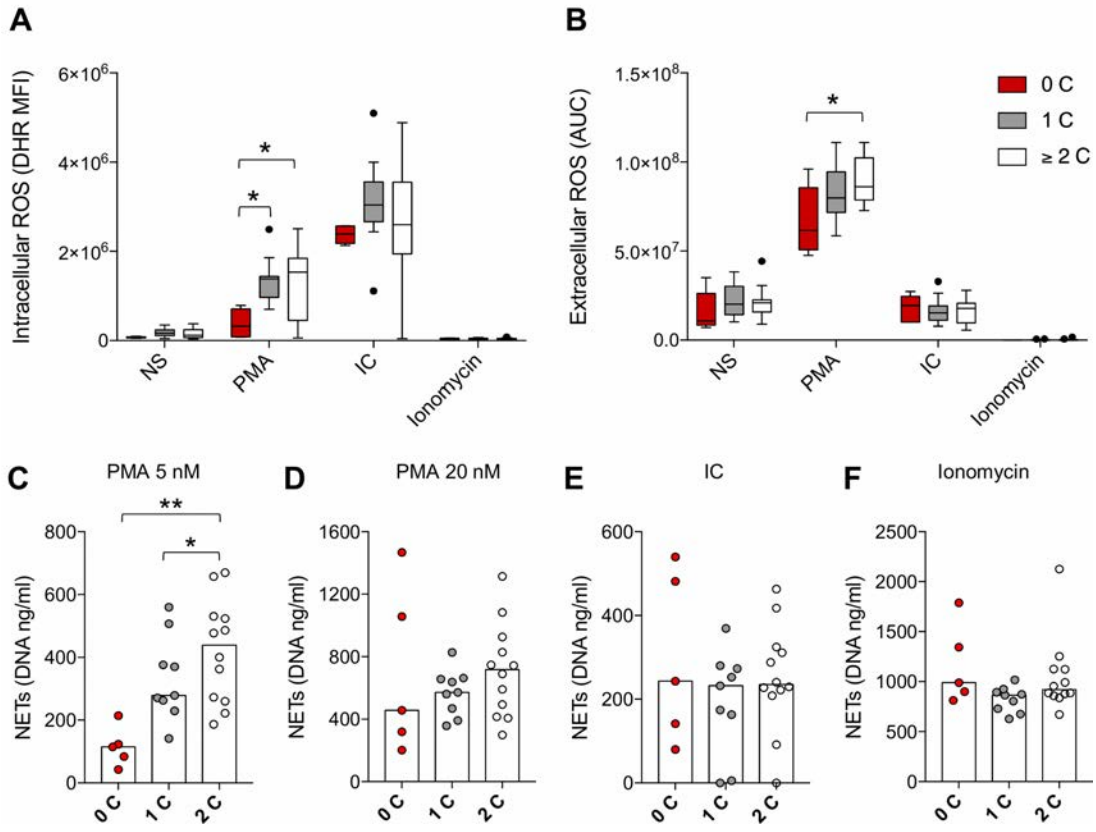


Figure 2 Oxidative burst and NET formation is dependent on NCF1-339 genotype. (A) Intracellular ROS quantified by redox sensitive DHR-123. (B) Extracellular ROS production measured by isoluminol-enhanced chemiluminescence, calculated as area under curve (AUC) from relative luminescence values. (C–F) NET formation quantified as extracellular DNA released from neutrophils treated with 5 nM (C) and 20 nM (D) PMA, IC (E) and ionomycin (F), as determined by sytox green fluorescence. 0 C, n=5; 1 C, n=12 and 2 C, n=14. Bars at median. *P<0.05, **P<0.01, Kruskal-Wallis test. IC, immune complexes; MFI, mean fluorescence intensity; NET, neutrophil extracellular trap; PMA, phorbol-myristate-acetate; ROS, reactive oxygen species.

potential of NETs.[31 35] To investigate this, neutrophils were treated with an NADPH oxidase inhibitor DPI or a scavenger of mitochondrial ROS (MitoTEMPO) prior to NET induction. DPI blocked both PMA-induced and IC-induced NETs in all genotypes but had only limited effect on ionomycin-induced NETs (figure 3A–C), consistent with PMA and IC NETs being completely or partially NOX2 dependent. In contrast, MitoTEMPO inhibited PMA-mediated NET release from neutrophils of patients with 0C genotype to a significantly larger extent than 2C genotype (figure 3D), indicating that NET induction in the 0C genotype is partially dependent on mitochondrial ROS (online supplementary figure S1B). MitoTEMPO also inhibited IC-mediated NET formation with equal effect in all genotypes, while having limited effect on ionomycin-induced NET formation. These results suggest that individuals with genetically impaired NOX2 function are partially dependent on mitochondrial ROS for release of PMA-induced NETs.

Depending on stimuli and signalling pathway, NETs might differ in DNA and protein content, where NETs released via mitochondrial signalling pathways may contain mitochondrial DNA.[36] We found no genotype dependent difference in the ratio of mitochondrial to genomic DNA in PMA-induced NETs. The NETs were also similar in levels of the granular protein myeloperoxidase (MPO) (online supplementary figure S4A,B), suggesting that NCF1-339 genotype affects quantity but not content of released NETs.

NCF1-339 0C genotype is associated with increased type I IFN activity in patients with SLE

ROS have an inhibitory effect on cytokine production and type I IFN signalling.[20] To investigate the role of NCF1-339 genotype on IFN activity in serum, we used a standardised assay to detect serum IFN levels as previously described.[28 29] Results from this analysis showed that patients with SLE with an NCF1-339 0C genotype to a larger extent had high serum IFN activity (p=0.019) compared with patients with one or more C-alleles (figure 4A). The high IFN activity was not dependent on disease activity, as there were no genotype-dependent differences in SLEDAI-2K at the time of blood collection (figure 4B). Collectively, these results argue for a genetic regulation of IFN mediated by the NCF1-339 effects on ROS, consistent with previous observations associating decreased ROS production with increased type I IFN signalling.

Patients with SLE with NCF1-339 0C genotype have higher frequency of antiphospholipid antibodies

Since IFNα may promote loss of tolerance[37 38] and NCF1-339 genotype modulated IFN activity, we hypothesised that it may

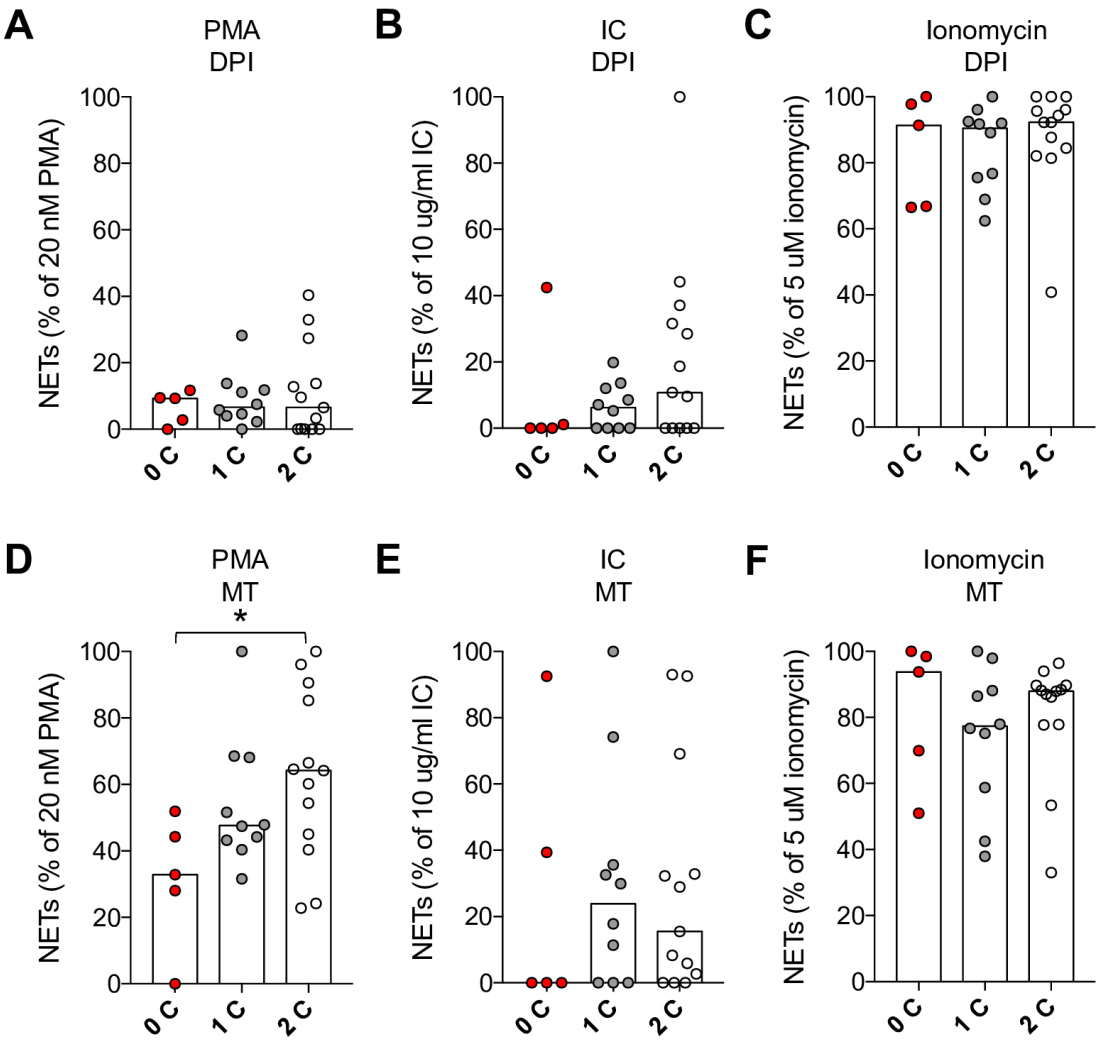


Figure 3 Dependence on mitochondrial ROS in NET formation is determined by NCF1-339 genotype. Inhibition of NET release in neutrophils treated with NADPH oxidase inhibitor DPI (upper panel) or mitochondrial scavenger MitoTEMPO (lower panel) prior to NET-induction with PMA (A, D), IC (B, E) and ionomycin (C, F). Results are calculated as amount of NETs released in presence of inhibitor as compared with amount of NETs released without inhibitor for the same stimuli. 0 C, n=5; 1 C, n=12 and 2 C, n=14. Bars at median. *P<0.05, Mann-Whitney U test. DPI, diphenyleneiodonium; IC, immune complexes; MT, MitoTEMPO; NET, neutrophil extracellular trap; PMA, phorbol-myristate-acetate; ROS, reactive oxygen species.

influence the repertoire of autoantibodies in patients with SLE. To assess this, we performed a cross-sectional analysis of conventional antibodies in serum of patients with SLE with different NCF1-339 genotypes (figure 5). Interestingly, the group of patients with NCF1-339 0C genotype were positive for aCL (50%) and anti-β_2GP1 antibodies (60%) to a significantly larger extent than patients with two or more C alleles (9.3% and 5.6%, respectively) (figure 5). No other antibody showed signs of being significantly influenced by the NCF1-339 genotype, suggesting a specific influence promoting susceptibility to develop antiphospholipid antibodies (aPL) and possibly secondary APS.

NCF1-339 0C genotype is associated with APS

To validate the association between aPL and NCF1-339 and to detect possible associations with secondary APS, we included genotyped patients from three additional Swedish University Hospitals (Linköping, Uppsala and the Karolinska institute). Investigated parameters included: clinical APS, presence of any aPL, history of any APS-related clinical event, anti-cardiolipin and anti-β_2GP1 antibodies, positive lupus anticoagluant (LA) test, arterial thrombosis, venous thrombosis or miscarriages. Results from this analysis confirmed a strong association between low-ROS NCF1-339 T-genotypes and clinical diagnosis of APS (OR 1.74 (1.19–2.55)), presence of any aPL (OR 1.40 (1.01–1.95)), presence of anti-β_2GP1 (OR 1.82 (1.02–3.24)) and positive LA (OR 1.72 (1.12–2.63)) (table 1) (online supplementary file 3). These results demonstrate that patients with SLE with NCF1-339 low-ROS genotypes have an increased prevalence of aPL and secondary APS.

DISCUSSION

The NCF1-339 missense SNP reducing NOX2 function and ROS production[10 13] is enriched in patients with SLE and associated with earlier disease onset.[11] This study adds to these findings by showing that low-ROS NCF1-339 genotypes are also associated with altered formation of NETs, high serum type I IFN activity, presence of aPL and secondary APS.

Neutrophils are important players in SLE[39] and one of the most investigated aspects is NET release that exposes autoantigens such as histones and double stranded DNA.[40 41] However, studies of the contribution of NETs to SLE are inconsistent and show opposing results.[4 17–19] It has been suggested that the effects of NETs in SLE could be dependent on variations of both protein and DNA content as well as induction pathways,[33] but we could

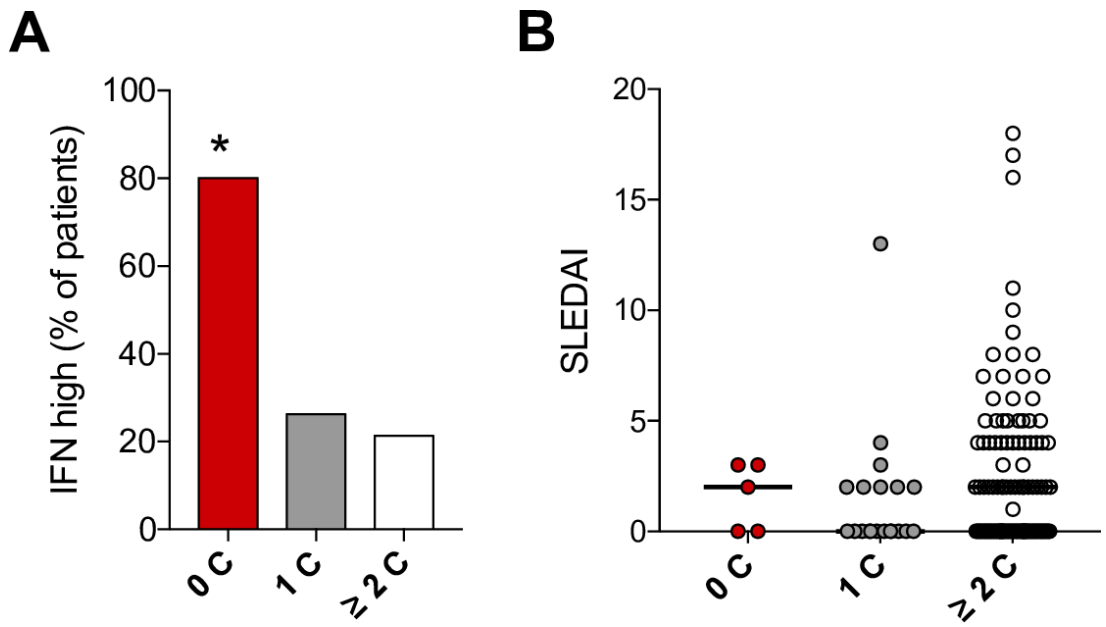


Figure 4 NCF1-339 0C genotype is associated with high serum IFN levels. (A) Frequency of patients with high serum IFN levels as determined by expression of IFN-stimulated genes in a reporter cell line. (B) Disease activity scores (SLEDAI-2K) for patients at time point of serum sampling for IFN analysis. Genotypes represented in relation to number of NCF1-339 C-alleles. 0 C, n=5; 1 C, n=19 and ≥2 C, n=117. *P<0.05 compared with expected value, Fishers exact test. IFN, interferon; SLEDAI-2K, Systemic Lupus Erythematosus Disease Activity Index 2000.

not detect any NCF1-339-dependent effects on DNA or MPO in NETs. Neither did NCF1-339 genotype seem to affect spontaneous DNA release or levels of cell-free DNA in circulation. While more studies are required to fully determine the effect of the NCF1-339 genotype on NETs, our results indicate no effects on composition but only in quantity of NETs induced by certain inductive pathways, as the NCF1-339-dependent effects were observed exclusively in response to PMA stimulation. One major difference in ROS production induced by PMA and IC is the location of the assembled NOX2 complex, where PMA stimulation leads to plasma membrane NOX2 while IC to phagosomal NOX2, suggesting that the effect of NCF1-339 is dependent on subcellular location of NOX2. NCF1 has high affinity to the plasma membrane, while another NOX2 component, NCF4, is of greater importance during phagosomal activation.[13]

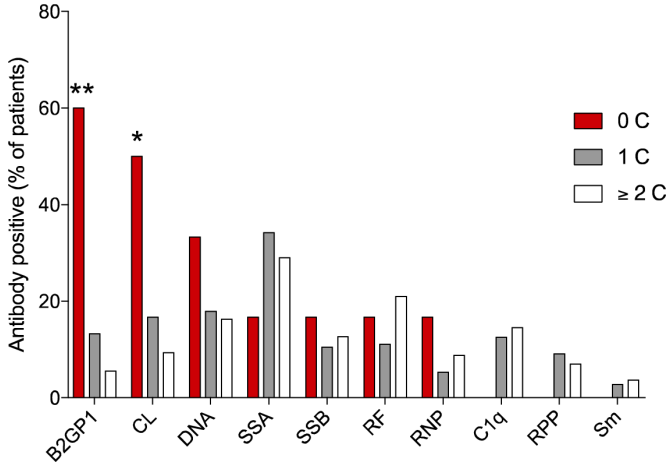


Figure 5 NCF1-339 0C genotype is associated with antiphospholipid antibodies. Frequency of patients positive for a panel of autoantibodies in a cross-sectional analysis. Genotypes represented in relation to number of NCF1-339 C-alleles. 0 C, n=6; 1 C, n=38 and ≥2 C, n=261. *P<0.05, **P<0.01 compared with expected values, Fisher's exact test.

Another clue that could help to explain the role of NCF1-339 in SLE is the potential role of ROS from mitochondrial respiration in NOX2-independent NET formation.[31] Studies have shown that NOX2 deficient low-density neutrophils can form highly interferogenic NETs, promoted by mitochondrial ROS,[36] suggesting a pathogenic role for the observed dependence on mitochondrial ROS associated with the low-ROS genotype.

NOX2 is also expressed by other immune cells, where NCF1-339 could have a modulatory role and ROS have a wide spectrum of effects on other aspects of the immune system. This is illustrated in chronic granulomatous disease (CGD), caused by genetically deficient NOX2 function. Patients with CGD are susceptible to bacterial and fungal infections but are also characterised by a variety of autoimmune manifestations, including enhanced IFN signalling and defective apoptosis.[20 23 42–45] Thus, several aberrations associated with low ROS production are shared between CGD and SLE and associated with pathogenic processes known to be important in both diseases. However, further studies are needed to determine the exact role of NCF1-339.

We have previously shown[11] that cells with 0C genotype from RA patients have an increased expression of IFN-regulated genes, but we did not see this effect in patients with SLE. In the present study, we analysed IFN response in a larger number of patients (141 instead of 23) using a reporter cell line exposed to SLE serum. The reporter cell expression is sensitive only to the IFN present in serum, and therefore, likely to more accurately reflect IFN protein activity than mRNA levels in whole blood. We therefore believe that this extended analysis gives better insight on how NCF1-339 influence IFN activity and does not contradict our previous study.

Low-ROS NCF1-339 genotype was furthermore associated with the presence of aPL (both any aPL and specific anti-β_2GP1-antibodies), LA positivity and clinical APS diagnosis. Secondary APS has its highest prevalence in patients with SLE and one or more of the major antiphospholipid antibodies are found

Table 1 APS manifestations in Swedish patients with SLE with different NCF1-339 genotypes

	Karolinska (n=442)		Linköping (n=160)		Lund (n=305)		Uppsala (n=180)		Total (n=1087)		
APS manifestation	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**	**OR**
Clinical APS	26	16	29	21	24	19	24	11	26	17	1.74 (1.19 to 2.55)
Any aPL	33	26	50	52	45	35	53	36	43	34	1.40 (1.01 to 1.95)
Any clinical event	37	31	36	28	41	45	26	26	35	34	1.14 (0.81 to 1.60)
Anti-CL	26	22	23	33	38	32	51	33	33	28	1.24 (0.80 to 1.94)
Anti-β2GP1	25	19	22	19	22	08	35	14	25	16	1.82 (1.02 to 3.24)
LA	15	13	48	31	46	23	19	14	26	17	1.72 (1.12 to 2.63)
Arterial thrombosis	14	11	16	19	21	30	16	11	16	17	0.99 (0.69 to 1.42)
Venous thrombosis	19	16	23	12	29	23	8	14	20	17	1.30 (0.86 to 1.97)
Miscarriage	13	12	7	3	8	09	13	11	11	10	1.16 (0.36 to 2.05)

Results presented as frequency of patients in each genotype group fulfilling criteria for each manifestation (%). Total results among all patients with SLE are presented both as frequency and OR with 95% CI.
Anti-CL, anticardiolipin antibodies; anti-β2GP1, anti-ß2 glycoprotein1 antibodies; aPL, antiphospholipid antibodies; APS, antiphospholipid syndrome; LA, lupus anticoagulant; SLE, systemic lupus erythematosus.

in ~20%–30% of patients with SLE.[46 47] First-degree relatives of patients with SLE and patients with primary APS share an increased risk of developing aPL[48] and APS,[49–52] suggesting a shared genetic susceptibility.

SLE autoantigens are often located on the surface of apoptotic cells and it has been proposed that their presences promote further autoimmune responses through epitope spread.[53 54] Evidence from animal models[55] and SLE[56] may also support a disease development where anti-$_2$GPI, and then SLE-associated antibodies develop in a sequential manner, followed by clinical disease, suggesting that the development of aPL may predate clinical disease and the existence of shared pathogenic mechanisms. Thus, it is tempting to speculate that a genetically determined deficient NOX2 function could modulate several pathogenetic pathways, including apoptosis and thereby the risk of developing aPL and subsequently SLE.

The association between NCF1-339 genotypes and decreased NOX2-dependent ROS and NET formation and the increased presence of aPL and serum type I IFN in patients with SLE suggests that the polymorphism at NCF1-339 regulates fundamental pathogenetic pathways in SLE, possibly mediated by ROS. This finding is further supported by observations associating NCF1-339 low-ROS genotypes with earlier disease onset and increased susceptibility to develop SLE compared with patients with normal-ROS genotypes.[11] The pathologies of APS and SLE share some striking similarities, including development of autoantibodies and impaired clearance of apoptotic cells. The evidence of a shared genetic background in SLE and APS may help further understanding of common underlaying pathogenetic mechanisms and treatment targets.

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Patient consent for publication Not required.

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Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. Data may be obtained from a third party and are not publicly available. All data relevant to the study are included in the article or uploaded as online supplementary information.

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REFERENCES

1 Bengtsson AA, Rönnblom L. Systemic lupus erythematosus: still a challenge for physicians. *J Intern Med* 2017;281:52–64.
2 Deapen D, Escalante A, Weinrib L, *et al*. A revised estimate of twin concordance in systemic lupus erythematosus. *Arthritis Rheum* 1992;35:311–8.
3 Olofsson P, Holmberg J, Tordsson J, *et al*. Positional identification of NCF1 as a gene that regulates arthritis severity in rats. *Nat Genet* 2003;33:25–32.
4 Campbell AM, Kashgarian M, Shlomchik MJ. Nadph oxidase inhibits the pathogenesis of systemic lupus erythematosus. *Sci Transl Med* 2012;4:157ra141.
5 Holmdahl R, Sareila O, Olsson LM, *et al*. Ncf1 polymorphism reveals oxidative regulation of autoimmune chronic inflammation. *Immunol Rev* 2016;269:228–47.
6 Jacob CO, Eisenstein M, Dinauer MC, *et al*. Lupus-Associated causal mutation in neutrophil cytosolic factor 2 (NCF2) brings unique insights to the structure and function of NADPH oxidase. *Proc Natl Acad Sci U S A* 2012;109:E59–67.

7 Olsson LM, Lindqvist A-K, Källberg H, *et al*. A case-control study of rheumatoid arthritis identifies an associated single nucleotide polymorphism in the NCF4 gene, supporting a role for the NADPH-oxidase complex in autoimmunity. *Arthritis Res Ther* 2007;9.
8 Nauseef WM. Assembly of the phagocyte NADPH oxidase. *Histochem Cell Biol* 2004;122:277–91.
9 DeLeo FR, Quinn MT. Assembly of the phagocyte NADPH oxidase: molecular interaction of oxidase proteins. *J Leukoc Biol* 1996;60:677–91.
10 Olsson LM, Nerstedt A, Lindqvist A-K, *et al*. Copy Number Variation of the Gene *NCF1* Is Associated with Rheumatoid Arthritis. *Antioxid Redox Signal* 2012;16:71–8.
11 Olsson LM, Johansson Åsa C, Gullstrand B, *et al*. A single nucleotide polymorphism in the *NCF1* gene leading to reduced oxidative burst is associated with systemic lupus erythematosus. *Ann Rheum Dis* 2017;76:1607–13.
12 Zhao J, Ma J, Deng Y, *et al*. A missense variant in NCF1 is associated with susceptibility to multiple autoimmune diseases. *Nat Genet* 2017;49:433–7.
13 Li XJ, Marchal CC, Stull ND, *et al*. P47Phox phox homology domain regulates plasma membrane but not phagosome neutrophil NADPH oxidase activation. *J Biol Chem* 2010;285:35169–79.
14 Zhong J, Olsson LM, Urbonaviciute V, *et al*. Association of Nox2 subunits genetic variants with autoimmune diseases. *Free Radic Biol Med* 2018;125:72–80.
15 Lambeth JD. Nox enzymes and the biology of reactive oxygen. *Nat Rev Immunol* 2004;4:181–9.
16 Hakkim A, Furnrohr BG, Amann K, *et al*. Impairment of neutrophil extracellular trap degradation is associated with lupus nephritis. *Proc Natl Acad Sci U S A* 2010;107:9813–8.
17 Lee KH, Kronbichler A, Park DD-Y, *et al*. Neutrophil extracellular traps (nets) in autoimmune diseases: a comprehensive review. *Autoimmun Rev* 2017;16:1160–73.
18 Konig MF, Andrade F. A critical reappraisal of neutrophil extracellular traps and NETosis mimics based on differential requirements for protein citrullination. *Front Immunol* 2016;7:461.
19 Kienhöfer D, Hahn J, Stoof J, *et al*. Experimental lupus is aggravated in mouse strains with impaired induction of neutrophil extracellular traps. *JCI Insight* 2017;2.
20 Thomas DC. How the phagocyte NADPH oxidase regulates innate immunity. *Free Radical Biology and Medicine* 2018;125:44–52.
21 Gelderman KA, Hultqvist M, Holmberg J, *et al*. T cell surface redox levels determine T cell reactivity and arthritis susceptibility. *Proc Natl Acad Sci U S A* 2006;103:12831–6.
22 Eloranta M-L, Lövgren T, Finke D, *et al*. Regulation of the interferon-α production induced by RNA-containing immune complexes in plasmacytoid dendritic cells. *Arthritis Rheum* 2009;60:2418–27.
23 Kelkka T, Kienhöfer D, Hoffmann M, *et al*. Reactive oxygen species deficiency induces autoimmunity with type 1 interferon signature. *Antioxid Redox Signal* 2014;21:2231–45.
24 Gladman DD, Ibañez D, Urowitz MB. Systemic lupus erythematosus disease activity index 2000. *J Rheumatol* 2002;29:288–91.
25 Tydén H, Lood C, Gullstrand B, *et al*. Endothelial dysfunction is associated with activation of the type I interferon system and platelets in patients with systemic lupus erythematosus. *RMD Open* 2017;3:e000508.
26 Tan EM, Cohen AS, Fries JF, *et al*. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis & Rheumatism* 1982;25:1271–7.
27 Miyakis S, Lockshin MD, Atsumi T, *et al*. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost* 2006;4:295–306.
28 Bengtsson AA, Sturfelt G, Lood C, *et al*. Pharmacokinetics, tolerability, and preliminary efficacy of paquinimod (ABR-215757), a new quinoline-3-carboxamide derivative: studies in lupus-prone mice and a multicenter, randomized, double-blind, placebo-controlled, repeat-dose, dose-ranging study in. *Arthritis & Rheumatism* 2012;64:1579–88.
29 Hua J, Kirou K, Lee C, *et al*. Functional assay of type I interferon in systemic lupus erythematosus plasma and association with anti–RNA binding protein autoantibodies. *Arthritis Rheum* 2006;54:1906–16.
30 Parker H, Dragunow M, Hampton MB, *et al*. Requirements for NADPH oxidase and myeloperoxidase in neutrophil extracellular trap formation differ depending on the stimulus. *J Leukoc Biol* 2012;92:841–9.
31 Douda DN, Khan MA, Grasemann H, *et al*. Sk3 channel and mitochondrial ROS mediate NADPH oxidase-independent NETosis induced by calcium influx. *Proc Natl Acad Sci U S A* 2015;112:2817–22.
32 Kaajan JP. Chronic obstructive pulmonary disease and asthma. General and medical management with special attention to exacerbations. *Pharm Weekbl Sci* 1989;11:112–7.
33 Kenny EF, Herzig A, Krüger R, *et al*. Diverse stimuli engage different neutrophil extracellular trap pathways. *eLife* 2017;6.
34 Smith CK, Kaplan MJ. The role of neutrophils in the pathogenesis of systemic lupus erythematosus. *Curr Opin Rheumatol* 2015;27:448–53.
35 Kirchner T, Möller S, Klinger M, *et al*. The impact of various reactive oxygen species on the formation of neutrophil extracellular traps. *Mediators Inflamm* 2012;2012:1–10.
36 Lood C, Blanco LP, Purmalek MM, *et al*. Neutrophil extracellular traps enriched in oxidized mitochondrial DNA are interferogenic and contribute to lupus-like disease. *Nat Med* 2016;22:146–53.
37 Liu Z, Davidson A. Ifnα inducible models of murine SLE. *Front Immunol* 2013;4:306.
38 Rönnblom L. The importance of the type I interferon system in autoimmunity. *Clin Exp Rheumatol* 2016;34:21–4.
39 Bosch X. Systemic lupus erythematosus and the neutrophil. *New England Journal of Medicine* 2011;365:758–60.
40 Villanueva E, Yalavarthi S, Berthier CC, *et al*. Netting neutrophils induce endothelial damage, infiltrate tissues, and expose immunostimulatory molecules in systemic lupus erythematosus. *J.i.* 2011;187:538–52.
41 Zharkova O, Celhar T, Cravens PD, *et al*. Pathways leading to an immunological disease: systemic lupus erythematosus. *Rheumatology* 2017;56:i55–66.
42 Barcellos-de-Souza P, Canetti C, Barja-Fidalgo C, *et al*. Leukotriene B(4) inhibits neutrophil apoptosis via NADPH oxidase activity: redox control of NF-κB pathway and mitochondrial stability. *Biochim Biophys Acta* 2012;1823:1990–7.
43 Zhang B, Hirahashi J, Cullere X, *et al*. Elucidation of molecular events leading to neutrophil apoptosis following phagocytosis: cross-talk between caspase 8, reactive oxygen species, and MAPK/ERK activation. *J Biol Chem* 2003;278:28443–54.
44 Sanford AN, Suriano AR, Herche D, *et al*. Abnormal apoptosis in chronic granulomatous disease and autoantibody production characteristic of lupus. *Rheumatology* 2006;45:178–81.
45 Cachat J, Deffert C, Hugues S, *et al*. Phagocyte NADPH oxidase and specific immunity. *Clin Sci* 2015;128:635–48.
46 Levine JS, Branch DW, Rauch J. The antiphospholipid syndrome. *N Engl J Med Overseas Ed* 2002;346:752–63.
47 Frodlund M, Vikerfors A, Grosso G, *et al*. Immunoglobulin A anti-phospholipid antibodies in Swedish cases of systemic lupus erythematosus: associations with disease phenotypes, vascular events and damage accrual. *Clin Exp Immunol* 2018;194:27–38.
48 Radic M, Pattanaik D. Cellular and molecular mechanisms of anti-phospholipid syndrome. *Front Immunol* 2018;9:969.
49 Castro-Marrero J, Balada E, Vilardell-Tarrés M, *et al*. Genetic risk factors of thrombosis in the antiphospholipid syndrome. *Br J Haematol* 2009;147:289–96.
50 Sebastiani GD, Iuliano A, Cantarini L, *et al*. Genetic aspects of the antiphospholipid syndrome: an update. *Autoimmun Rev* 2016;15:433–9.
51 Svenungsson E, Gustafsson J, Leonard D, *et al*. A STAT4 risk allele is associated with ischaemic cerebrovascular events and anti-phospholipid antibodies in systemic lupus erythematosus. *Ann Rheum Dis* 2010;69:834–40.
52 Lee YH, Choi SJ, Ji JD, *et al*. Association between the valine/leucine ^{247} polymorphism of β_2 -glycoprotein I and susceptibility to anti-phospholipid syndrome: a meta-analysis. *Lupus* 2012;21:865–71.
53 Pieterse E, van der Vlag J. Breaking immunological tolerance in systemic lupus erythematosus. *Front Immunol* 2014;5:164.
54 Andreoli L, Fredi M, Nalli C, *et al*. Antiphospholipid antibodies mediate autoimmunity against dying cells. *Autoimmunity* 2013;46:302–6.
55 Levine JS, Subang R, Setty S, *et al*. Phospholipid-Binding proteins differ in their capacity to induce autoantibodies and murine systemic lupus erythematosus. *Lupus* 2014;23:752–68.
56 Arbuckle MR, McClain MT, Rubertone MV, *et al*. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *New England Journal of Medicine* 2003;349:1526–33.

CLINICAL SCIENCE

Clinical symptoms and associated vascular imaging findings in Takayasu's arteritis compared to giant cell arteritis

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ABSTRACT

Objective To compare the presence of head, neck and upper extremity symptoms in patients with Takayasu's (TAK) and giant cell arteritis (GCA) and their association with vascular inflammation assessed by ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET) or arterial damage assessed by magnetic resonance angiography (MRA).

Methods Patients with TAK and GCA underwent clinical and imaging assessments within 24 hours, blinded to each other. Vascular inflammation was defined as arterial FDG-PET uptake greater than liver by visual assessment. Arterial damage was defined as stenosis, occlusion, or aneurysm by MRA. Clinically reported symptoms were compared with corresponding imaging findings using generalised mixed model regression. Cranial symptoms were studied in association with burden of arterial disease in the neck using ordinal regression.

Results Participants with TAK (n=56) and GCA (n=54) contributed data from 270 visits. Carotidynia was reported only in patients with TAK (21%) and was associated with vascular inflammation (p<0.01) but not damage (p=0.33) in the corresponding carotid artery. Posterior headache was reported in TAK (16%) and GCA (20%) but was only associated with corresponding vertebral artery inflammation and damage in GCA (p<0.01). Arm claudication was associated with subclavian artery damage (p<0.01) and inflammation (p=0.04) in TAK and with damage in GCA (p<0.01). Patients with an increased burden of damaged neck arteries were more likely to experience positional lightheadedness (p<0.01) or a major central nervous system event (p=0.01).

Conclusion The distribution of symptoms and association with imaging abnormalities differs in patients with TAK and GCA. These findings may help clinicians predict associated FDG-PET and MRA findings based on a specific clinical symptom.

Clinical trial registration number NCT02257866.

Key messages

What is already known about this subject?

- Angiography is useful to detect vascular damage, and ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET) is useful to detect vascular inflammation in patients with large-vessel vasculitis.

What does this study add?

- This study details the relationships between clinical features commonly reported by patients with Takayasu's arteritis and giant cell arteritis with corresponding vascular imaging findings by magnetic resonance angiography (MRA) and FDG-PET.
- Presence of specific symptoms such as carotidynia more closely align with vascular inflammation by FDG-PET and presence of other symptoms such as claudication are more tightly linked to vascular damage by MRA.
- Absence of clinical symptoms does not rule out corresponding imaging pathology in patients with large-vessel vasculitis.

How might this impact on clinical practice or future developments?

- These findings will enable clinicians to predict imaging pathology based on patient-reported symptoms and will help researchers develop and refine disease activity indices in large vessel vasculitis.

INTRODUCTION

Takayasu (TAK) and giant cell arteritis (GCA) are the two major forms of large vessel vasculitis (LVV), defined by vascular inflammation, with resultant damage of the aorta and branch arteries.[1 2] Assessment of disease activity can be challenging in LVV, as there is a wide range of vascular symptoms that could be due to ongoing vascular inflammation, vascular damage, or both. The same symptom could be attributed to active disease or damage depending in part on the chronicity of the symptom.[3]

Vascular imaging of the aorta and primary branches can be useful to assess arterial damage and inflammation in patients with LVV.[4] ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET) can demonstrate metabolic activity in the arterial wall and thus can be used as a potential surrogate marker for arterial inflammation.[5 6] In contrast, magnetic resonance angiography (MRA) is useful to assess luminal damage, including stenosis, occlusion, or aneurysm.[7]

Currently, there is no standardised reference and no reliable biomarkers to assess and measure disease activity in LVV. Although there are composite disease activity scores such as the Indian Takayasu Activity Score and Disease Extent Index-Takayasu

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Arteritis,[8 9] these tools cannot precisely predict early signs of inflammation, progression of vascular disease, or end-organ vascular damage. Further complicating matters, active vascular inflammation can be detected in patients with LVV otherwise in clinical and biochemical remission. Postmortem histological studies show the significant presence of vascular inflammation in patients considered to be in remission at the time of death.[10] Additionally, persistent disease activity can be detected by FDG-PET and MRA during periods of apparent clinical remission in many patients, indicating discordance between symptoms and imaging abnormalities in LVV.[11 12]

Involvement of the medium and large arteries in the head, neck and upper extremities is common in both TAK and GCA and is associated with frequent ocular, intra-cranial and extra-cranial manifestations including headache, lightheadedness, carotidynia, vision loss, stroke, transient ischaemic attack (TIA), syncope and upper limb claudication.[13–21] There is limited evidence comparing the presence of specific symptoms to corresponding angiographic abnormalities by both FDG-PET and MRA in LVV. Such data may inform clinicians about the relationship between specific symptoms and underlying vascular inflammation by FDG-PET or damage by MRA.

The study objectives were: (1) to compare the occurrence of common cranial and upper extremity vascular symptoms to multimodal vascular imaging assessment in LVV; (2) to explore if there are differences in the associations between vascular symptoms and imaging abnormalities in TAK versus GCA.

METHODS

Study population

Patients were included from an ongoing prospective observational cohort study at the National Institutes of Health (NIH) in Bethesda, MD. Patient visits were included in the study from September 2014 through September 2018. All patients with LVV fulfilled the American College of Rheumatology (ACR) 1990 criteria for the classification of TAK[22] or modified ACR 1990 criteria for the classification of GCA.[18 23] Patients with LVV were enrolled at various stages during the disease. Whenever possible, baseline visit imaging studies were performed during periods of clinically active disease or during remission when the patient was taking <10 mg/day of prednisone to minimise potential confounding effects of treatment.

Patient and public involvement

The authors declare no direct patient and public involvement in study design.

Clinical assessment

All patients with LVV underwent clinical assessment with categorisation of symptoms 1 day prior to imaging assessment. Symptoms present on the day of assessment were recorded, including carotidynia, posterior neck pain, frontotemporal and posterior headache, and arm claudication. Additionally, historical presence of selected symptoms at any point in the disease course were recorded, including lightheadedness, positional lightheadedness, carotidynia, vertigo, frontotemporal and posterior headache, blurred vision, vision loss, and major central nervous system (CNS) events defined as stroke, TIA, or syncope.

FDG-PET imaging assessment

PET studies were performed at each study visit within 24 hours after clinical assessment, as previously reported.[12] Patients aged <18 years underwent whole-body FDG-PET-MRI with a Siemens Biograph mMR (Siemens Medical Solutions). Patients aged ≥18 years underwent FDG-PET-CT of the torso with a Siemens Biograph mCT (Siemens Medical Solutions). A nuclear medicine physician (MAA) interpreted all PET scans included in this study blinded to clinical information and MRA data. Qualitative assessment of PET activity was performed in the carotid, vertebral and subclavian arteries. The degree of arterial uptake was visually assessed relative to liver uptake.[24 25] Vascular inflammation on PET in a specific arterial territory was defined as arterial FDG uptake greater than the FDG uptake in the liver by visual inspection.

MRA assessment

All patients underwent three-station MRA of the aorta and primary branches at each study visit within 24 hours after clinical assessment as previously described.[12] A vascular radiologist (JM) interpreted all MRAs, blinded to clinical data and PET scan assessment. Vascular damage on MRA was defined as stenosis, occlusion or aneurysm in an arterial territory of interest. Wall thickness and oedema on MRA were not included in the definition of vascular damage in this study.

Statistical analysis

Symptoms present on the day of assessment were compared with arterial involvement by imaging studies in corresponding territories. For example, left-sided carotidynia was compared with FDG uptake in the left carotid artery by PET and to left carotid damage by MRA. Performance characteristics of clinical symptoms were detailed in association with corresponding imaging findings. In the context of this study, high sensitivity means that the absence of symptoms is likely to correspond to normal imaging studies (low false negatives) and high specificity means that the presence of symptoms is likely to correspond to abnormal imaging studies (low false positives). Generalised linear mixed model regression analysis adjusting for repeated study visits, daily prednisone dose, and use of additional immunosuppressant medication was used to examine the relationship between symptoms and imaging modalities in TAK and GCA. P values <0.05 were considered statistically significant.

Historical presence of symptoms at any point in the disease course was compared with the burden of arterial disease in the four major neck arteries (carotid and vertebral arteries). Ordinal logistic regression was performed to determine the association between each neurovascular symptom and the number of affected neck arteries (range 0–4 arteries).

RESULTS

Demographic characteristics of the study population

A total of 110 patients with LVV were recruited into the study. There were 56 patients with TAK and 54 patients with GCA. A total of 105 FDG-PET and 102 MRA were performed in TAK, and 134 FDG-PET and 129 MRA were performed in GCA. Among patients with GCA, 56% had a positive temporal artery biopsy and 76% had involvement of the large arteries by FDG-PET or MRA. Baseline demographic information of the study population is shown in table 1. Seventy nine out of 110 (72%) patients were studied during clinically active disease, and 70/110 (64%) patients were taking prednisone <10 mg/day at the baseline visit.

Frequency of clinical symptoms in patients with TAK and GCA

The occurrence of symptoms in patients with TAK and GCA is compared in table 2. No significant differences in the frequency

Table 1 Demographic characteristics of the study population

Diagnosis	TAK	GCA
Number of subjects	56	54
Number of visits	123	147
Number of visits per patient		
1	26 (46%)	22 (40 %)
2–3	23 (41%)	16 (30 %)
>3	7 (13%)	16 (30 %)
Age, years (mean±SD)	33.2±12.1	69.9±8.9
Female (%)	45 (80.4%)	44 (78.6%)
Disease duration, years (mean±SD)	10.6±9.8	2.9±2.4
Prednisone (%)	33 (59%)	44 (81%)
Daily prednisone dose (mg, mean±SD)	7.4±12.2	9.0±14.1
Other medications		
Methotrexate	27 (48%)	27 (50%)
Tocilizumab	8 (14%)	21 (39%)
Infliximab	17 (30%)	1 (2%)
Azathioprine	9 (16%)	3 (6%)
Other	20 (36%)	6 (11%)

GCA, giant cell arteritis;TAK, Takayasu's arteritis.

of symptoms were observed between patients with TAK and GCA for six of the 11 symptoms of interest. There was significantly more carotidynia (21 vs 0%, p<0.01), lightheadedness (30 vs 9%, p<0.01), positional lightheadedness (29 vs 5%, p<0.01), major CNS events (25 vs 9%, p=0.04) and arm claudication (52 vs 28%, p=0.01) in patients with TAK compared with GCA. The most common symptom in patients with TAK was arm claudication (52%) and in patients with GCA was blurred vision (37%).

Prevalence of cephalic and upper extremity arterial involvement in TAK and GCA

The prevalence of arterial disease detected by FDG-PET or by MRA was compared between patients with TAK and GCA (table 3). In general, there was more vascular inflammation in patients with GCA and more vascular damage in patients with TAK. In the carotid arteries, there was more PET activity in patients with GCA compared with TAK (52 vs 31%, p=0.05) and more arterial damage in patient with TAK compared with GCA (69 vs 33%, p<0.01). In the vertebral arteries, there was more PET activity in patients with GCA compared with TAK (19 vs 2%, p=0.05), but vertebral artery damage was not significantly different in patients with TAK compared with GCA (31 vs 17%, p=0.17). In the subclavian arteries, there was more PET activity in patients with GCA compared with TAK (50 vs 28%, p=0.03), but subclavian artery damage was not significantly different in patients with TAK compared with GCA (63 vs 52%, p=0.32).

Table 2 Frequency of clinical symptoms in patients with TAK and GCA

Symptom	TAK (n=56)	GCA (n=54)	P value
Carotidynia	12 (21%)	0 (0%)	<0.01
Lightheadedness	17 (30%)	5 (9%)	<0.01
Positional lightheadedness	16 (29%)	3 (5%)	<0.01
Posterior neck pain	4 (7%)	10 (18%)	0.09
Frontotemporal headache	14 (25%)	17 (31%)	0.53
Posterior headache	9 (16%)	11 (20%)	0.63
Vertigo	3 (5%)	5 (9%)	0.48
Blurred vision	18 (32%)	20 (37%)	0.69
Vision loss	6 (11%)	4 (7%)	0.74
Major CNS event	14 (25%)	5 (9%)	0.04
Arm claudication	29 (52%)	15 (28%)	0.01

GCA, giant cell arteritis; major CNS event, central nervous system event defined as stroke, transient ischaemic attack, or syncope; TAK, Takayasu's arteritis.

Table 3 Frequency of cephalic and upper extremity arterial disease detected by FDG-PET or MRA in patients with TAK and GCA

Artery	Imaging study	TAK	GCA	P value
Carotid	FDG-PET	17/54 (31%)	28/54 (52%)	0.05
	MRA	35/51 (69%)	17/52 (33%)	<0.01
Vertebral	FDG-PET	1/54 (2%)	10/54 (19%)	<0.01
	MRA	16/52 (31%)	9/52 (17%)	0.17
Subclavian	FDG-PET	15/54 (28%)	27/54 (50%)	0.03
	MRA	32/51 (63%)	27/52 (52%)	0.32

FDG-PET, ^{18}F-fluorodeoxyglucose positron emission tomography; GCA, giant cell arteritis; MRA, magnetic resonance angiography; TAK, Takayasu's arteritis.

Comparison of specific vascular symptoms to imaging studies in TAK and GCA

Carotidynia and posterior neck pain

Carotidynia on the day of evaluation was reported during 12 out of 123 study visits (8%) in patients with TAK. Carotidynia was not reported by any patient with GCA. Carotidynia was more strongly associated with inflammation of the carotid artery by FDG-PET (p<0.01) than carotid artery damage by MRA (p=0.33) in patients with TAK (table 4, figure 1A,B). Sensitivity was low for the association of carotidynia with FDG-PET or MRA abnormalities (27% and 11%, respectively) indicating that an absence of carotidynia could still be associated with imaging abnormalities in the carotid artery, particularly on MRA compared with FDG-PET. Specificity was high for both FDG-PET and MRA (96% and 95%, respectively) indicating that the presence of carotidynia was strongly associated with corresponding carotid artery abnormalities on both FDG-PET and MRA.

Posterior neck pain was more commonly reported by patients with GCA than TAK (10 (18%) vs 4 (7%), p=0.09). Posterior neck pain contributed to 6% of the total visits in patients with GCA and significantly associated with inflammation of the vertebral artery by FDG-PET in patients with GCA (p<0.01) but not TAK (p=1.00) (table 4). Posterior neck pain was not significantly associated with vertebral damage by MRA in either patients with GCA or TAK (p=0.78 and p=0.51, respectively). Sensitivity was low (range 0%–12%) for the association of posterior neck pain with FDG-PET or MRA abnormalities in patients with TAK and GCA. Specificity was excellent (range 98%–100%) for the association between posterior neck pain and both FDG-PET and MRA in patients with TAK and GCA, indicating the presence of posterior neck pain was strongly associated with corresponding vertebral artery abnormalities on both FDG-PET and MRA.

Posterior and frontotemporal headache

Posterior headache was reported in 5% of the total visits of patients with GCA and was significantly associated with both vertebral artery damage (p<0.01) and PET activity (p<0.01) in patients with GCA (figure 1C). No association of posterior headache with vertebral PET activity (p=1.00) or damage (p=0.79) was observed in patients with TAK (table 4). Sensitivity was low (range 0%–12%) and specificity was high (98%) for the association between posterior headache and imaging abnormalities by FDG-PET or MRA in patients with TAK and GCA.

Table 4 Comparison of specific vascular symptoms to imaging studies in patients with TAK and GCA

Symptom	Artery	LVV	Image	TN	TP	FN	FP	P value	Sensitivity	Specificity
Carotidynia	Carotid	TAK	PET	166	10	27	7	<0.01	27% (14–44)%	96% (92–98)%
			MRA	90	12	95	5	0.33	11% (6–19)%	95% (88–98)%
		GCA	PET	No patients with GCA had carotidynia						
			MRA							
Posterior neck pain	Vertebral	TAK	PET	208	0	1	3	1.00	0% (0–97)%	98% (96–100)%
			MRA	159	0	42	3	0.78	0% (0–8)%	98% (95–100)%
		GCA	PET	244	3	22	1	<0.01	12% (3–31)%	100% (98–100)%
			MRA	229	0	25	4	0.51	0% (0–14)%	98% (96–100)%
Posterior headache	Vertebral	TAK	PET	206	0	1	4	1.00	0% (0–97)%	98% (95–99)%
			MRA	159	1	40	3	0.79	2% (0–13)%	98% (95–100)%
		GCA	PET	238	3	21	4	<0.01	12% (3–32)%	98% (96–99)%
			MRA	229	3	22	4	<0.01	12% (2–31)%	98% (96–99)%
Frontotemporal headache	Carotid	TAK	PET	165	4	33	8	0.33	11% (3–25)%	95% (91–98)%
			MRA	92	7	99	3	0.74	7% (3–13)%	97% (91–99)%
		GCA	PET	149	7	63	18	0.20	10% (4–19)%	89% (83–93)%
			MRA	163	11	73	13	0.21	13% (7–22)%	93% (88–96)%
Arm claudication	Subclavian	TAK	PET	142	8	19	42	0.04	29% (14–50)%	77% (70%–83%)
			MRA	89	42	60	9	<0.01	41% (31–51)%	91% (83–96)%
		GCA	PET	129	25	81	32	0.57	23% (16–33)%	80% (73–86)%
			MRA	126	49	75	10	<0.01	40% (31–49)%	93% (87–96)%

P values were derived by mixed model regression adjusting for repeated measures, daily prednisone dose, and use of additional immunosuppressant medication (yes/no).
FN, false negative; FP, false positive; GCA, giant cell arteritis; LVV, large vessel vasculitis; MRA, magnetic resonance angiography; PET, positron emission tomography; TAK, Takayasu's arteritis; TN, true negative; TP, true positive.

Frontotemporal headache was present in seven out of the 123 total visits (5.7%) of patients with TAK and 15 out of the 147 total visits (10.2%) of patients with GCA. Frontotemporal headache was not associated with either carotid PET activity or damage in patients with TAK or GCA (table 4). Sensitivity was low (range 7%–13%) and specificity was high (range 89%–97%) for the association between frontotemporal headache and FDG-PET or MRA findings in patients with TAK and GCA.

Arm claudication

Arm claudication at the time of clinical assessment was a highly prevalent symptom, present in 37% and 23% of the total visits in patients with TAK and GCA, respectively. Arm claudication was more strongly associated with damage to the subclavian arteries by MRA ($p<0.01$) than inflammation by FDG-PET ($p=0.04$) in patients with TAK (table 4). Similarly, in patients with GCA, arm claudication was significantly associated with damage to the subclavian arteries by MRA ($p<0.01$) but was not associated with PET activity ($p=0.57$). Sensitivity was moderate (range 23%–40%) and specificity was high (range 77%–93%) for the association between arm claudication and abnormalities by FDG-PET or MRA in patients with TAK and GCA. Presence of arm claudication had higher sensitivity in association with MRA findings compared with PET findings in both diseases.

Association of cephalic and neck symptoms with burden of arterial neck disease in patients with LVV

The association between specific clinical symptoms and the number of affected neck arteries was assessed. Stronger associations were observed between presence of clinical symptoms and burden of arterial disease by MRA rather than by FDG-PET (figure 2A,B). Patients with LVV who had increased number of damaged neck arteries detected by MRA were significantly more likely to experience lightheadedness (OR=2.61, $p=0.04$), positional lightheadedness (OR=3.51, $p<0.01$), or a major CNS event (OR=3.23, $p=0.01$) at some point in their disease course. Patients with LVV who had increased number of inflamed neck arteries detected by FDG-PET were only significantly more likely to experience posterior headache (OR=2.84, $p=0.03$) at some point in the course of their disease.

DISCUSSION

Distinct associations between clinical symptoms and corresponding imaging pathology by FDG-PET and MRA were observed in a

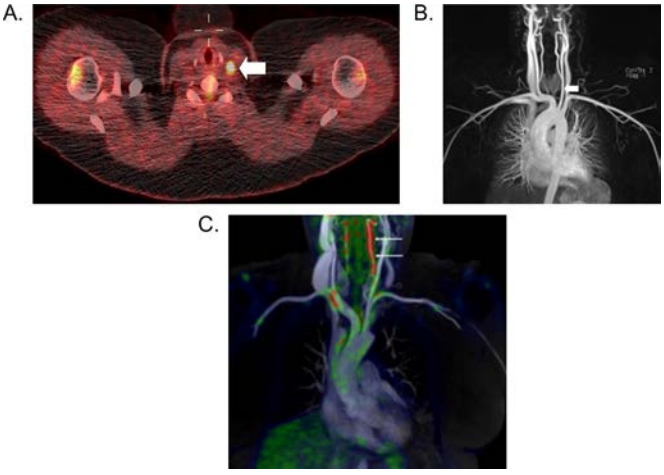

Figure 1 Clinical images from patients with large-vessel vasculitis. A patient with Takayasu's arteritis who complained of left sided carotidynia had corresponding vascular inflammation by FDG-PET (A) without vascular damage by magnetic resonance angiography (B). Fused FDG-PET and angiography images from a patient with giant cell arteritis who complained of a left sided posterior headache demonstrate increased FDG uptake (red) and throughout a stenotic left vertebral artery (white arrows) (C). FDG-PET, ^{18}F-fluorodeoxyglucose positron emission tomography.

prospective, longitudinal observational cohort of patients with LVV. Certain vascular symptoms on the day of clinical and imaging assessment were more closely aligned with abnormal FDG-PET activity, angiographic damage, both, or neither. Some of these associations differed significantly between patients with TAK and GCA. Increased burden of neck arterial disease was associated with an increased likelihood of major CNS events. Clinical symptoms were not sensitive markers of underlying vascular pathology but were specific when present. Vascular imaging should be considered in the management of these patients since reliance on the presence of clinical symptoms may not be sensitive to detect vascular pathology within an acceptable window to prevent or minimise damage.

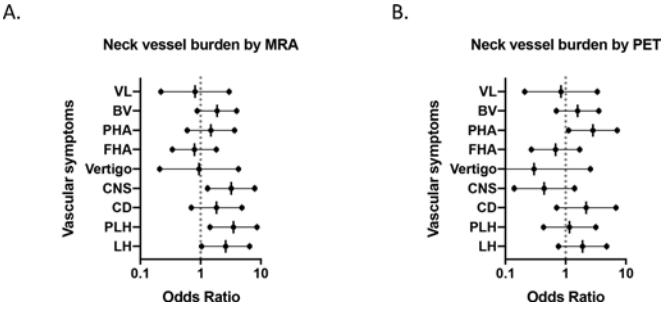

Figure 2 OR plots comparing the presence of cranial symptoms at any point during the disease course to the cumulative burden of vascular damage by MRA (A) or vascular inflammation by FDG-PET (B) in the four major neck arteries. Associations of each symptom with the burden of neck vessel disease in the carotid and vertebral arteries are displayed. ORs >1 indicate that a specific symptom is associated with increased odds of vascular disease affecting increasingly more of the neck arteries. BV, blurred vision; CD, carotidynia; CNS, central nervous system event defined as stroke, TIA or syncope; FHA, frontotemporal headache; LH, lightheadedness; MRA, magnetic resonance angiography; PHA, posterior headache; PLH, positional lightheadedness; FDG-PET, ^{18}F-fluorodeoxyglucose positron emission tomography; VL, vision loss.

FDG-PET and MRA can be used to assess different aspects of vascular pathology, and imaging findings on these modalities correlate differently to specific symptoms. Symptoms of vascular pain, for example, carotidynia, were significantly associated with FDG-PET abnormalities rather than angiographic damage and can therefore be considered more likely to reflect vascular inflammation. In contrast, arm claudication was only weakly associated with PET activity in patients with TAK but was strongly associated with angiographic damage in both patients with TAK and GCA. Symptoms of carotidynia could therefore be considered a stronger indicator of corresponding active vascular inflammation as compared with symptoms of limb claudication, which more strongly reflected vascular damage.

There were similarities and differences in the clinical and vascular imaging associations observed in patients with TAK compared with GCA. Symptoms related to carotid artery involvement aligned to carotid artery imaging findings only in patients with TAK, while symptoms related to vertebral artery involvement aligned to corresponding vertebral artery imaging findings only in patients with GCA. Posterior neck pain and posterior headache was associated with vertebral artery imaging abnormalities in patients with GCA. While posterior headaches in the occipital region are uncommon in patients with GCA,[26 27] this study emphasises that presence of a posterior headache should alert the clinician to the likelihood of associated vascular inflammation and damage in the corresponding vertebral artery.

In contrast to posterior headache, frontotemporal headache was not associated with disease activity or damage of the carotid artery in TAK nor GCA. While frontotemporal headaches frequently occur in patients with TAK,[2 28] and are a cardinal feature of GCA,[26] headaches in this region may reflect inflammation in smaller branches of cranial arteries, rather than the corresponding larger arteries of the neck. Additionally, most patients in this study were studied several years into the course of disease, when frontotemporal headaches may be less specific for vasculitis compared with a potentially stronger association at the time of diagnosis.

Presence of clinical symptoms aligned with corresponding imaging abnormalities by both FDG-PET and MRA. High specificity was observed between clinical symptoms and corresponding vascular imaging findings. In the context of this study, high specificity indicated that when a patient reported a specific head and neck symptom, there was often a corresponding vascular imaging abnormality. Thus, new symptoms of head, neck and upper limbs should prompt consideration of vascular imaging in order to confirm new inflammation or damage in corresponding arterial territories.

However, these results also suggest that the absence of clinical symptoms does not necessarily rule out underlying imaging pathology. Low sensitivity was observed between clinical symptoms and both FDG-PET and MRA, indicating that imaging abnormalities were frequently detected in the absence of corresponding symptoms. In the context of FDG-PET, these findings align with several prior studies that demonstrated that subclinical vascular inflammation is relatively common in patients with LVV.[29–33] In the context of angiography, presence of vascular damage in the absence of accompanying clinical symptoms underscores the importance of angiography for categorising disease extent. Progression of vascular damage is often insidious and accompanied by compensatory collateral circulation,[11 34 35] which may explain how patients with LVV can sometimes develop profound vascular damage in the absence of ischaemic symptoms.

The current study has some limitations. First, most patients were studied during later phases of disease and not necessarily at

time of diagnosis. Many patients were on treatment for vasculitis which may have weakened the associations between symptoms and imaging findings, in particular the FDG-PET results. Although statistical analysis adjusted for differences in glucocorticoid use, residual confounding from treatment effect is possible as only daily rather than cumulative glucocorticoid exposure was considered. More vascular inflammation was observed in patients with GCA compared with TAK which could be due to biological differences, shorter disease duration in the patients with GCA, or differences in concomitant atherosclerosis. A large, international study also reported more FDG-PET activity in GCA and more angiographic damage in TAK in data collected at the time of diagnosis.[36] Symptoms were defined as present or absent on the day of assessment, but the duration of those symptoms was not considered. New or worsening claudication may have a different relationship to imaging findings compared with persistent claudication.

The study has several important strengths. MRA and FDG-PET were performed on the same day within 24 hours of clinical assessment. Imaging assessments were performed by central readers independent of clinical assessment. This study does not dictate how angiography and FDG-PET should be used in a clinical setting. Rather, these findings may help clinicians predict imaging pathology in specific vascular territories based on patient-reported symptoms and may inform which type of imaging modality would be the most useful to obtain in certain clinical scenarios, recognising that additional sequences to detect wall morphology may augment the ability of MR-based assessments to detect vascular inflammation in addition to luminal damage.[12] Additionally, these findings may facilitate the development and refinement of disease activity indices in LVV for use in future clinical research studies as clinical features that are more strongly associated with vascular inflammation than damage may be preferentially considered.

TAK and GCA are complex diseases that pose clinical management challenges, particularly at later stages of disease when accurate assessment of disease activity can be difficult. Findings from this study support the concept that clinical assessment should be integrated with imaging assessment in order to facilitate clinical care and research in these conditions.

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REFERENCES

1 Jennette JC, Falk RJ, Bacon PA, *et al*. 2012 revised international chapel Hill consensus conference Nomenclature of vasculitides. *Arthritis Rheum* 2013;65:1–11.
2 Maksimowicz-McKinnon K, Clark TM, Hoffman GS. Takayasu arteritis and giant cell arteritis: a spectrum within the same disease? *Medicine* 2009;88:221–6.
3 Langford CA, Cuthbertson D, Ytterberg SR, *et al*. A randomized, double-blind trial of abatacept (CTLA-4Ig) for the treatment of Takayasu arteritis. *Arthritis Rheumatol* 2017;69:846–53.
4 Cimmino MA, Camellino D. Large vessel vasculitis: which imaging method? *Swiss Med Wkly* 2017;147:w14405.
5 Danve A, O'Dell J, O'Dell J. The role of 18F fluorodeoxyglucose positron emission tomography scanning in the diagnosis and management of systemic vasculitis. *Int J Rheum Dis* 2015;18:714–24.
6 Grayson PC, Alehashemi S, Bagheri AA, *et al*. [18] F-Fluorodeoxyglucose-positron emission tomography as an imaging biomarker in a prospective, longitudinal cohort of patients with large vessel vasculitis. *Arthritis Rheumatol* 2018;70:439–49.
7 Koster MJ, Matteson EL, Warrington KJ. Large-vessel giant cell arteritis: diagnosis, monitoring and management. *Rheumatology* 2018;57:ii32–42.
8 Misra R, Danda D, Rajappa SM, *et al*. Development and initial validation of the Indian Takayasu clinical activity score (ITAS2010). *Rheumatology* 2013;52:1795–801.
9 Aydin SZ, Yilmaz N, Akar S, *et al*. Assessment of disease activity and progression in Takayasu's arteritis with Disease Extent Index-Takayasu. *Rheumatology* 2010;49:1889–93.
10 Östberg G. Morphological changes in the large arteries in polymyalgia arteritica. *Acta Med Scand* 1972;191:135–59.
11 Kerr GS*et al*. Takayasu arteritis. *Ann Intern Med* 1994;120:919–29.
12 Quinn KA, Ahlman MA, Malayeri AA, *et al*. Comparison of magnetic resonance angiography and [18]F-fluorodeoxyglucose positron emission tomography in large-vessel vasculitis. *Ann Rheum Dis* 2018;77:1165–71.
13 Bond KM, Nasr D, Lehman V, *et al*. Intracranial and extracranial neurovascular manifestations of Takayasu arteritis. *AJNR Am J Neuroradiol* 2017;38:766–72.
14 Bolaman Z, Yavasoglu I, Kadikoylu G, *et al*. Takayasu arteritis with intracranial involvement mimicking epilepsy: case report and review of the literature. *Intern Med* 2011;50:1345–8.
15 Duarte MM, Geraldes R, Sousa R, *et al*. Stroke and transient ischemic attack in Takayasu's arteritis: a systematic review and meta-analysis. *J Stroke Cerebrovasc Dis* 2016;25:781–91.
16 Kim HJ, Suh DC, Kim JK, *et al*. Correlation of neurological manifestations of Takayasu's arteritis with cerebral angiographic findings. *Clin Imaging* 2005;29:79–85.
17 Soriano A, Muratore F, Pipitone N, *et al*. Visual loss and other cranial ischaemic complications in giant cell arteritis. *Nat Rev Rheumatol* 2017;13:476–84.
18 Grayson PC, Maksimowicz-McKinnon K, Clark TM, *et al*. Distribution of arterial lesions in Takayasu's arteritis and giant cell arteritis. *Ann Rheum Dis* 2012;71:1329–34.
19 Schmidt WA, Seipelt E, Krause A, *et al*. Carotidynia in Takayasu arteritis. *J Rheumatol* 2007;34:231–2.
20 Cannon CR. Carotidynia: an unusual pain in the neck. *Otolaryngol Head Neck Surg* 1994;110:387–90.
21 De Hertogh W, Vaes P, Versijpt J. Diagnostic work-up of an elderly patient with unilateral head and neck pain. A case report. *Man Ther* 2013;18:598–601.
22 Arend WP, Michel BA, Bloch DA, *et al*. The American College of rheumatology 1990 criteria for the classification of Takayasu arteritis. *Arthritis Rheum* 1990;33:1129–34.
23 Hunder GG, Bloch DA, Michel BA, *et al*. The American College of rheumatology 1990 criteria for the classification of giant cell arteritis. *Arthritis Rheum* 1990;33:1122–8.
24 Walter MA, Melzer RA, Schindler C, *et al*. The value of [18F]FDG-PET in the diagnosis of large-vessel vasculitis and the assessment of activity and extent of disease. *Eur J Nucl Med Mol Imaging* 2005;32:674–81.
25 Meller J, Strutz F, Siefker U, *et al*. Early diagnosis and follow-up of aortitis with [18F] FDG PET and MRI. *Eur J Nucl Med Mol Imaging* 2003;30:730–6.
26 Rana AQ, Saeed U, Khan OA, *et al*. Giant cell arteritis or tension-type headache?: a differential diagnostic dilemma. *J Neurosci Rural Pract* 2014;05:409–11.
27 Weyand CM, Goronzy JJ. Medium- and large-vessel vasculitis. *N Engl J Med* 2003;349:160–9.
28 Li-xin Z, Jun N, Shan G, *et al*. Neurological manifestations of Takayasu arteritis. *Chin Med Sci J* 2011;26:227–30.
29 Newman KA, Ahlman MA, Hughes M, *et al*. Diagnosis of giant cell arteritis in an asymptomatic patient. *Arthritis Rheumatol* 2016;68:1135.
30 Folco EJ, Sheikine Y, Rocha VZ, *et al*. Hypoxia but not inflammation augments glucose uptake in human macrophages: implications for imaging atherosclerosis with 18fluorine-labeled 2-deoxy-D-glucose positron emission tomography. *J Am Coll Cardiol* 2011;58:603–14.
31 Rosenbaum D, Millon A, Fayad ZA. Molecular imaging in atherosclerosis: FDG PET. *Curr Atheroscler Rep* 2012;14:429–37.
32 Blockmans D, Ceuninck Lde, Vanderschueren S, *et al*. Repetitive18F-fluorodeoxyglucose positron emission tomography in giant cell arteritis: a prospective study of 35 patients. *Arthritis Rheum* 2006;55:131–7.
33 Clifford A, Burrell S, Hanly JG. Positron emission tomography/computed tomography for the diagnosis and assessment of giant cell arteritis: when to consider it and why: table 1. *J Rheumatol* 2012;39:1909–11.
34 Subramanyan R, Joy J, Balakrishnan KG. Natural history of aortoarteritis (Takayasu's disease). *Circulation* 1989;80:429–37.
35 Kermani TA, Sreih AG, Cuthbertson D, *et al*. Evaluation of damage in giant cell arteritis. *Rheumatology* 2018;57:322–8.
36 Gribbons KB, Ponte C, Carette S, *et al*. Patterns of arterial disease in Takayasu's arteritis and giant cell arteritis. *Arthritis Care Res* 2019. doi:10.1002/acr.24055. [Epub ahead of print: 23 Aug 2019].

TRANSLATIONAL SCIENCE

Germline genetic patterns underlying familial rheumatoid arthritis, systemic lupus erythematosus and primary Sjögren's syndrome highlight T cell-initiated autoimmunity

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ABSTRACT

Objectives Familial aggregation of primary Sjögren's syndrome (pSS), systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and co-aggregation of these autoimmune diseases (ADs) (also called familial autoimmunity) is well recognised. However, the genetic predisposition variants that explain this clustering remains poorly defined.

Methods We used whole-exome sequencing on 31 families (9 pSS, 11 SLE, 6 RA and 5 mixed autoimmunity), followed by heterozygous filtering and cosegregation analysis of a family-focused approach to document rare variants predicted to be pathogenic by *in silico* analysis. Potential importance in immune-related processes, gene ontology, pathway enrichment and overlap analyses were performed to prioritise gene sets.

Results A range from 1 to 50 rare possible pathogenic variants, including 39 variants in immune-related genes across SLE, RA and pSS families, were identified. Among this gene set, regulation of T cell activation ($p=4.06\times10^{-7}$) and T cell receptor (TCR) signalling pathway ($p=1.73\times10^{-6}$) were particularly concentrated, including *PTPRC* (*CD45*), *LCK*, *LAT–SLP76* complex genes (*THEMIS*, *LAT*, *ITK*, *TEC*, *TESPA1*, *PLCL1)*, *DGKD*, *PRKD1*, *PAK2* and *NFAT5*, shared across 14 SLE, RA and pSS families. TCR-interactive genes *P2RX7*, *LAG3*, *PTPN3* and *LAX1* were also detected. Overlap analysis demonstrated that the antiviral immunity gene *DUS2* variant cosegregated with SLE, RA and pSS phenotypes in an extended family, that variants in the TCR-pathway genes *CD45*, *LCK* and *PRKD1* occurred independently in three mixed autoimmunity families, and that variants in *CD36* and *VWA8* occurred in both RA-pSS and SLE-pSS families.

Conclusions Our preliminary results define common genetic characteristics linked to familial pSS, SLE and RA and highlight rare genetic variations in TCR signalling pathway genes which might provide innovative molecular targets for therapeutic interventions for those three ADs.

Key messages

What is already known about this subject?

► It is well recognised that rheumatoid arthritis (RA), primary Sjögren's syndrome (pSS) and systemic lupus erythematosus (SLE) cluster in families, indicating the presence of shared variants underlying the genetic predisposition to familial RA, pSS and SLE. Genome-wide association studies have identified common variants (minor allelic frequency >1%) shared among RA, pSS and SLE.

What does this study add?

► This study comprehensively characterised the genetic profiles across RA, pSS and SLE families, and further investigated the genetic background for their familial clustering. Our data identified certain rare and potential pathogenic variants concentrated in the T cell receptor (TCR) signalling pathway.

How might this impact on clinical practice or future developments?

► The TCR signalling pathway genes found in this study may provide novel molecular targets for therapeutic interventions for RA, pSS and SLE, which remain a challenge to manage clinically.

INTRODUCTION

Rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and primary Sjögren's syndrome (pSS) are three closely related autoimmune diseases (ADs), which share multiple disease aspects, including epidemiological characteristics, clinical manifestations and serological profiles. Indeed, SLE, RA and pSS co-occurring at the same or at different times in the same patient support the concept that these disorders share common molecular ties.[1] Furthermore, co-aggregation of RA, SLE and pSS within family, also known as familial autoimmunity, indicates that common germline variants may predispose to these ADs.[2] Previously, several associated genes shared among RA, SLE and pSS were identified by whole genome-wide association studies, including HLA, IRF5, STAT4 and TNFAIP3.[3 4] Recently, whole-exome sequencing also has been used to uncover rare variants for SLE and RA.[5 6] Nevertheless, rare variants for familial AD still remain an unsettled question.

Personalised therapies based on individual genetic susceptibility may increase the probability of improved efficacy.[7] Harnessing the plasticity of $CD4^+$ T cells could provide improved therapeutic efficacy for patients with AD.[8] $CD4^+$ T cells, including Th1, Th2 and Th17 cells, were identified in target organ infiltrates and peripheral blood in early ADs. It was also shown that $CD4^+$ T cells play a major role in AD pathogenesis, amplifying inflammation by secretion of pro-inflammatory cytokines plus helping B cells to generate autoantibodies.[9] However, the contribution of genetic factors to inappropriate T cell homing of Th1, Th2 and Th17 cells in AD still unknown.

Herein, we describe rare and deleterious variants in genes involved in TCR signalling pathway genes underlying predisposition to familial ADs.

PATIENTS AND METHODS

Family ascertainment

Subjects met research classification criteria using the 2002 American-European Consensus Group for pSS, the 2012 Systemic Lupus Collaborating Clinics for SLE and the 2010 ACR/EULAR for RA. Familial RA, pSS, SLE and autoimmunity were defined as patients with at least one first-degree relative with confirmed RA, pSS or SLE. Voluntary, written, fully informed consent was obtained from all participants.

For more details about experimental and bioinformatic methods, see the online supplementary methods and figure S1.

RESULTS

Genetic spectrum and gene enrichment for T cell-mediated immunity

Our filtering pipeline showed that pathogenic variants cosegregated with phenotypes on a per-family basis. We did not identify any significant linkage peak and clustering of variants located in genomic 'hotspots'. Depending on family size, between 1 and 50 variants were shared by all affected individuals within each family (see online supplementary figure S2 and table S2), including 39 immune-related genes (table 1 and figure 1A). Previously implicated autoimmune-associated genes with new rare variants were identified, such as CFHR4, RPP21, IL12A

Table 1 List of candidate genes prioritised by immune-related function and enrichment in T cell receptor signalling pathway

Families	Number of genes	Immune-related genes	T cell differentiation and development genes	TCR proximal signalling genes	TCR interactive genes
pSS					
pSS-001	22	*CRHR2, ITK*	*ITK*	*ITK*	
pSS-002	30	*CD36, TRIM16*	*CD36, TRIM16*		
pSS-003	6	*PLCL1, VWA8*	*PLCL1*	*PLCL1*	
pSS-004	1	*DUS2*			
pSS-005	1	*CERK*	*CERK*		*CERK (TCR)*
pSS-006	26	*ADAMTS5, TRIM16*	*TRIM16*		
pSS-007	7	*VWA8*			
pSS-027	8	*VGLL3*			
pSS-031	20	*PTPRC (CD45)*	*PTPRC (CD45)*	*PTPRC (CD45)*	
RA					
RA-008	14	*NFAT5*	*NFAT5*	*NFAT5*	
RA-009	21	*CD36, THEMIS,CFHR5*	*CD36, THEMIS*	*THEMIS*	
RA-010	22	*PTPN3*			*PTPN3*
RA-011	48	*CR2, TESPA1*		*TESPA1*	
RA-028	23	*IRAK3, PTPRC (CD45)*	*PTPRC (CD45)*	*PTPRC (CD45)*	
RA-029	30	*LAX1, IL31RA*	*LAX1*		*LAX1*
SLE					
SLE-012	14	*P2RX7*	*P2RX7*		*P2RX7* (ATP and calcium)
SLE-013	28	*LAT*	*LAT*	*LAT*	
SLE-014	22	*DGKD, IL12A, SEMA4D*	*DGKD, IL12A, SEMA4D*	*DGKD*	*SEMA4D (CD3)*
SLE-015	47	*CD276*			
SLE-016	20	*PAK2*		*PAK2*	
SLE-017	20	*CFHR4*			
SLE-018	17	*RPP21*			
SLE-019	43	*LAG3*			*LAG3 (TCR)*
SLE-020	35	*RIPK1*			
SLE-021	22	*TEC*	*TEC*	*TEC*	
SLE-030	33	*VWA8, IL1RL2*			
Autoimmunity					
Autoimmunity-022	44	*PRKD1, IRAK4*	*PRKD1*	*PRKD1*	IRAK4
Autoimmunity-023	7	*ASL*	*ASL*		
Autoimmunity-024	50	*DPP4, LCK, RIPK1*	*DPP4, LCK*	*LCK*	
Autoimmunity-025	35				
Autoimmunity-026	25	*ARL14,PTPRC (CD45)*	*PTPRC (CD45)*	*PTPRC (CD45)*	

Number of genes: total number of candidate genes with cosegregated with phenotype, rare and potential pathogenic variants by whole-exome sequencing and filtering.
TCR-proximal signalling genes: the core elements of the TCR signalling pathway, which downstream of TCR are responsible for the cascade of events leading to T cell activation.
TCR-interactive genes: crosstalk between TCR singling network and other immune pathways.
pSS, primary Sjögren's syndrome; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; TCR, T cell receptor.

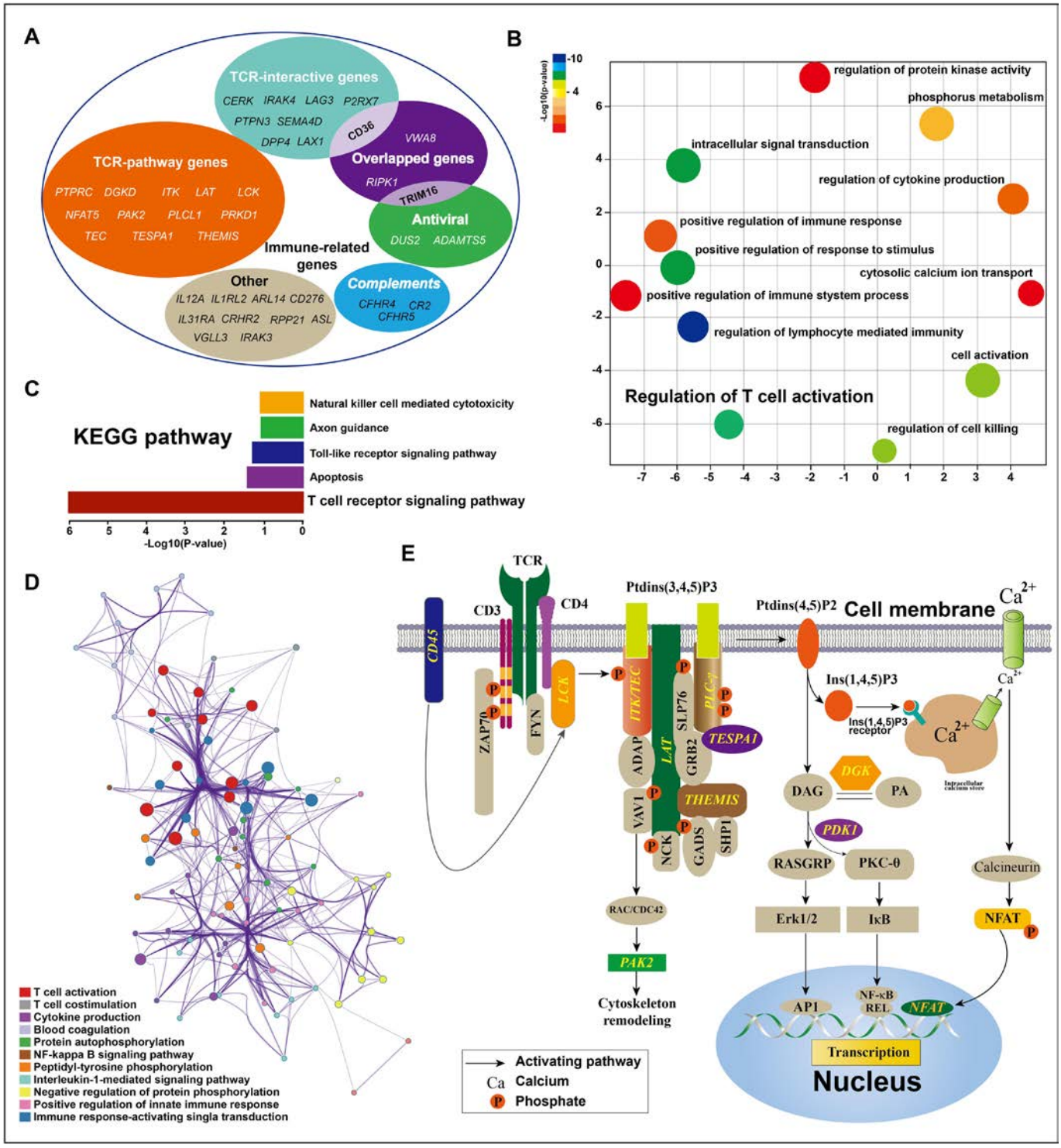


Figure 1 Results of whole-exome sequencing revealed T cell-mediated autoimmune. (A) Functional category of immune-related gene set identified by whole-exome sequencing. (B) Gene ontology analysis suggested T cell activation was a functional category by REVIGO scatterplot. (C) KEGG pathway enrichment indicated T cell receptor (TCR) pathway was significantly highlighted. (D) Metascape network of the enriched gene ontology terms showed the T cell activation was the central node. (E) Genetic variants in TCR pathway genes. Genes with variants were highlighted in yellow colour and italic type.

and ARL14.[10] To further dissect the shared genetic spectrum across families, we assessed whether there were enriched gene ontology terms within our immune-related gene set. Notably, regulation of T cell activation was highlighted ($p=4.06\times10^{-7}$, figure 1B, D), indicating that T cells probably contribute to the genetic basis of AD via different genes and/or pathways.

Sharing TCR-pathway genes across RA, SLE and PSS families

The immune-related genes were further classified according to their potential roles in signalling pathways. T cell receptor (TCR) signalling pathways ($p=1.73\times10^{-6}$) were aggregated (figure 1C), including PTPRC (CD45), *ITK*, *TEC*, *LAT*, *LCK*, PAK2 and *NFAT5*. To identify more TCR-pathway genes which were not

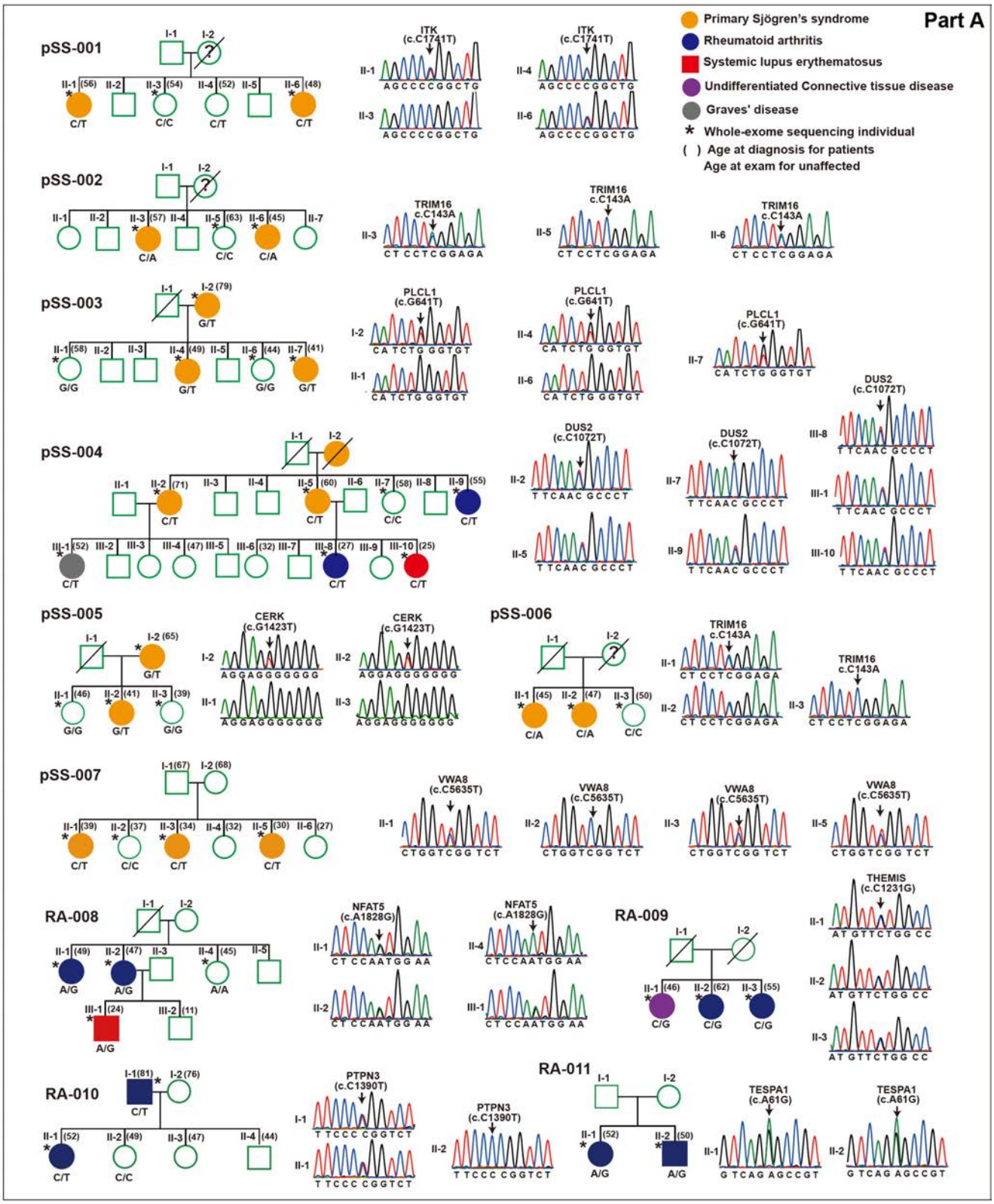
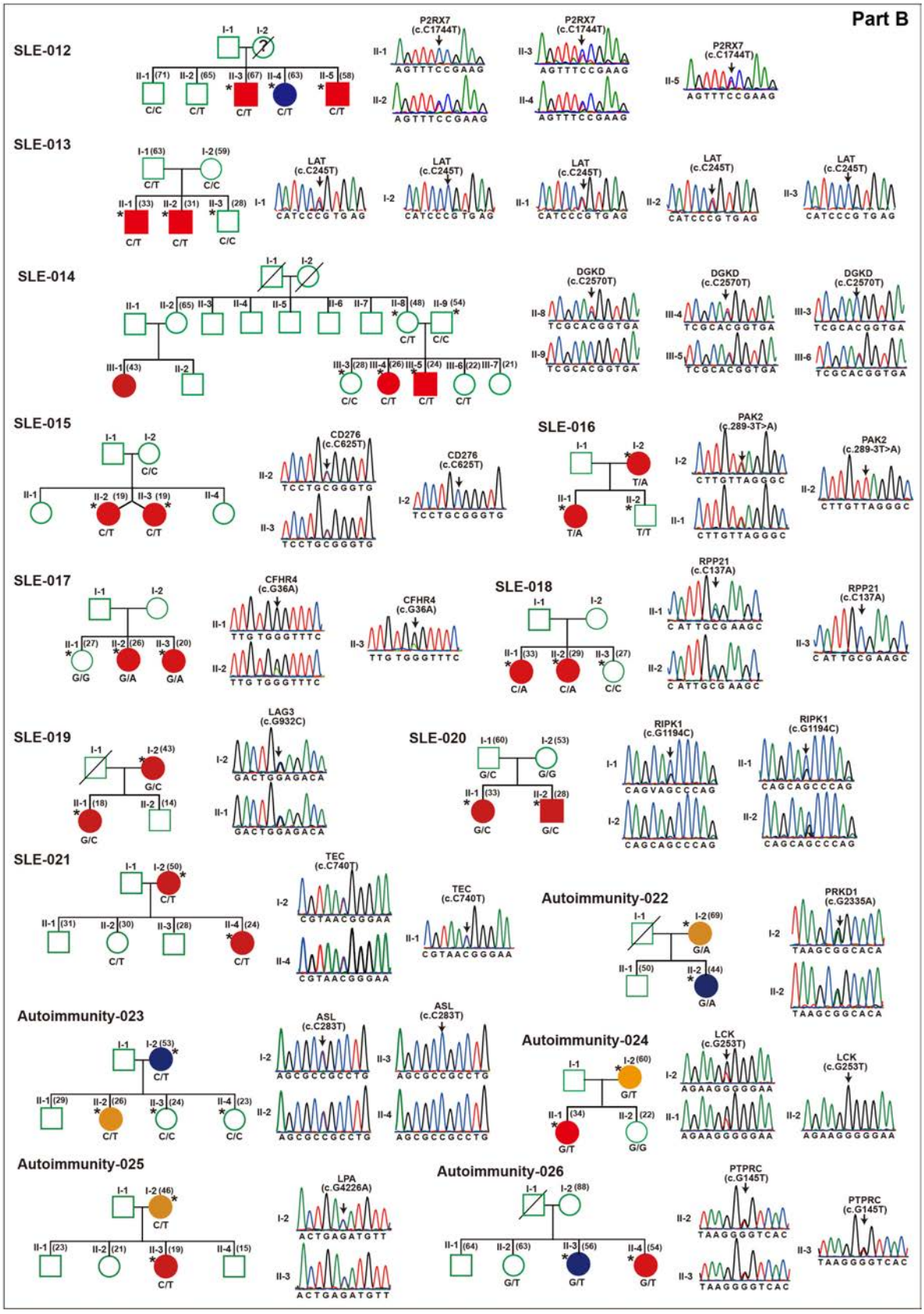


Figure 2 *(Continued)*



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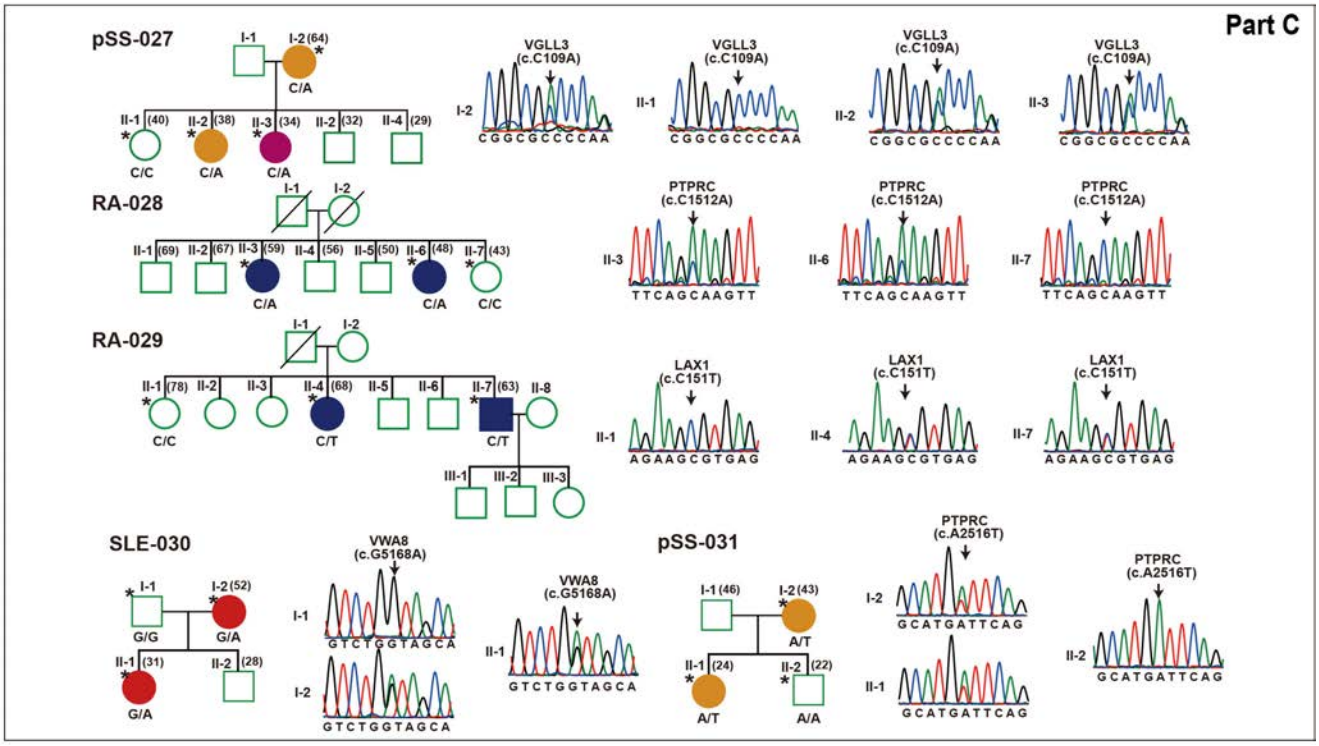

Figure 2 Rare variants segregating with phenotypes within each family. Pedigree structures of 31 families with genetic variants listed below each family member are shown. Males are indicated with squares, females with circles, slashes indicate deceased members and clinical diagnoses are shown by different colour. All subjects with DNA available are indicated by genotype, individuals with whole-exome sequencing are indicated by single asterisks and extended subjects for validation are indicated only by genotype. Sanger sequencing chromatograms are shown near the pedigrees.

included in the current KEGG database, we manually filtered immune-related gene sets further and found additional TCR-proximal genes for *THEMIS*, *PLCL1*, *PRKD1*, *DGKD* and *TESPA1* (figure 1E). Notably, six TCR-pathway genes (*ITK, LAT, THEMIS, PLCL1, TEC* and *TESPA1*) were found within the LAT–SLP76 complex.[11] The positions and conservation in different species of these variants and their corresponding gene expression were shown. Furthermore, the T cell proliferation and cytokine production were significantly increased with mutant TCR-pathway genes (see online supplementary figures S3–S6).

TCR interactive genes across RA, SLE and PSS families

We also identified TCR-interactive genes which are cross-linked to the TCR-pathway, including *P2RX7, CERK, LAG3, SEMA4D, IRAK4, LAX1, CD36* and *DPP4 (CD26)*. *LAG3* and PTPN3 inhibit the activation of the TCR pathway[12 13] and SEMA4D–CD72 interactions were necessary for TCR-induced T cell proliferation; in contrast, CD36, CD26 and IRAK4 influenced TCR signalling via lipid rafts.[14 15] Also, P2RX7 signalling affected TCR signalling at the αβ/γδ lineage bifurcation checkpoint.[16] The detailed connection of these genes and the TCR-pathway are shown in table 1.

Overlapping genes across RA, SLE and PSS families

Almost all of the identified variants are present only once in our families, indicating private genetic profiles and polygenic characteristics. Five immune-related genes with fully segregating variants in more than one family revealed a common pattern of genetic variants across pSS, RA and SLE (figure 1C and table 1), including *CD36*, PTPRC, *RIPK1*, *VWA8* and *TRIM16*.

CD36 was embedded in RA and pSS families and *VWA8* was shared in two extended pSS families and SLE family; while variants in TCR-pathway gene CD45 occurred in pSS, RA and mixed autoimmunity families. Recurrent stop-gain variants (p.S48X) in *TRIM16* were replicated in pSS families and reflect the same *TRIM* family as *TRIM21* (Ro52/SSA), which had been demonstrated to promote antiviral immunity.

We also detected a *DUS2* variant (p.R358C) which cosegregated with SLE, RA and pSS phenotypes (figure 2) in an extended family; DUS2 and interferon-induced protein kinase are involved in the regulation of innate antiviral immunity.[17] This provided direct evidence for sharing genes in these disorders. The presence of the *TRIM16* and *DUS2* variants supported the hypothesis that antiviral immunity may contribute to the development of familial AD.[18]

DISCUSSION

It is well known that RA, SLE and pSS cluster in families. However, not all of the genetic background that explains this clustering has been discovered and it remains unclear whether specific genetic variants and pathways are shared in familial SLE, RA and pSS. Our study describes genetic profiles across SLE, RA and pSS in Chinese families.

In fact, symptoms of SLE, RA and pSS at an early stage are similar, although tissue damage may intervene or features of one may dominate over another. We identified a single variant in the *DUS2* gene responsible for part of SLE, RA and pSS phenotypes. CD45, LCK and PRKD1 in mixed autoimmunity families further supported the presence of some common genetic variants underlying aspects of SLE, RA and pSS.

Consistent with previous studies showing polygenic variants in SLE families,[19] we identified 39 immune-related genes (the majority being T cell variants but not B variants), which supported the hypothesis that T cell-initiated immunity drives AD parthenogenesis. Our results show that genetic variants in T cells, but not in B cells, supply a genetic basis for AD in some familial forms of pSS, SLE and RA. We speculate that common variants of T cell activation differentiate and acquire distinct functions in subtypes which contribute to AD phenotypes and serological profiles via B cells.

TCR-pathway genes accounted for up to 48.7% of the immune-related genes in our AD families, suggesting SLE, RA and pSS could be influenced by multiple rare variants acting on a common pathway. A dynamic role for Th-cell activation is characterised by cytokine secretion, including Th1, Th2 and Th17, critical contributors to pSS, SLE and RA pathogenesis.[20 21] In this study, variants we found in *CD45*, LCK, LAT, TEC and *NFAT5* activate the coagulation cascades and the TCR signalling pathway and they might explain the triggering of naive $CD4^+$ T cell differentiation in pSS, RA and SLE. Most of them were TCR proximal rheostats serving the LAT–SLP76 complex. We propose primarily variants linking genetic susceptibility and naive $CD4^+$ T cell differentiation, amplified small initial difference in the efficacy of triggering in TCR signalling results the profound effects underlying AD pathogenesis. Furthermore, diverse AD presented in mixed autoimmunity families, strongly indicated genetic variants in TCR-pathway genes contributed to phenotypic heterogeneity.

Our results present variants in the TCR interactive genes leading to T cell activation, resulting in T cell differentiation, survival and effectors functions also contributed AD phenotypes. TCR-pathway and its interactive genes may not represent the full spectrum of AD susceptibility. The different genetic variants were underling not only AD susceptibility but also with specific AD phenotypes.

A total of five shared gene patterns added further data to help understand the root and specific genetic aetiologies of ADs. *CD36* is a known susceptibility gene for malaria,[22] and regulates malaria-induced pro-inflammatory cytokine responses and activation of T cells.[23] P2XR7 drives the fine-tuning between Th1 and Tfh cell differentiation to protect against malaria.[24] The antimalarial agent chloroquine has an established role and is routinely used in the treatment of RA, SLE and pSS. However, the common mechanism of action of this medication in malaria and ADs remains unclear. Therefore, we suspected that *CD36* and *P2XR7* might be novel immune links between malaria and ADs.

Currently, B cell depletion interrupts T cell/B cell interaction,[25] and may lead to worsening of the AD.[26] Characterisation of the TCR gene might also open new avenues to design specific and more effective therapies. Our study provides potential therapeutic targets, such as inhibitors AX-024 for the LAT–SLP76 complex,[25] ITK inhibitor ibrutinib[27] and TEC inhibitor PRN694.[28] Overlapped immune-related genes also could be severed as therapeutic targets, such as GSK2982772 for RIP1K-I40 for CD26.[29]

In conclusion, the families presented here identified common genetic patterns across RA, SLE and pSS, and provide data on the genetic background for SLE, pSS and RA which may help understand their pathogeneses. They also illustrate an important concept that could explain some of the heterogeneity in SLE, pSS and RA families. Further studies are warranted to evaluate the consequences of TCR-pathway variant and the mechanism by which it contributes to the development of familial ADs.

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REFERENCES

1 Anaya J-M. The autoimmune tautology. *Arthritis Res Ther* 2010;12:147.
2 Kuo C-F, Grainge MJ, Valdes AM, *et al*. Familial risk of Sjögren's syndrome and co-aggregation of autoimmune diseases in affected families: a nationwide population study. *Arthritis Rheumatol* 2015;67:1904–12.
3 Márquez A, Vidal-Bralo L, Rodríguez-Rodríguez L, *et al*. A combined large-scale meta-analysis identifies *COG6* as a novel shared risk *locus* for rheumatoid arthritis and systemic lupus erythematosus. *Ann Rheum Dis* 2017;76:286–94.
4 Zhao J, Ma J, Deng Y, *et al*. A missense variant in NCF1 is associated with susceptibility to multiple autoimmune diseases. *Nat Genet* 2017;49:433–7.
5 Mitsunaga S, Hosomichi K, Okudaira Y, *et al*. Exome sequencing identifies novel rheumatoid arthritis-susceptible variants in the BTNL2. *J Hum Genet* 2013;58:210–5.
6 Ellyard JI, Jerjen R, Martin JL, *et al*. Identification of a pathogenic variant in TREX1 in early-onset cerebral systemic lupus erythematosus by whole-exome sequencing. *Arthritis Rheumatol* 2014;66:3382–6.
7 Bluestone JA, Bour-Jordan H, Cheng M, *et al*. T cells in the control of organ-specific autoimmunity. *J Clin Invest* 2015;125:2250–60.
8 DuPage M, Bluestone JA. Harnessing the plasticity of CD4+ T cells to treat immune-mediated disease. *Nat Rev Immunol* 2016;16:149–63.
9 Moulton VR, Tsokos GC. T cell signaling abnormalities contribute to aberrant immune cell function and autoimmunity. *J Clin Invest* 2015;125:2220–7.
10 Acosta-Herrera M, Kerick M, González-Serna D, *et al*. Genome-wide meta-analysis reveals shared new *loci* in systemic seropositive rheumatic diseases. *Ann Rheum Dis* 2019;78:311–9.
11 Wange RL. LAT, the linker for activation of T cells: a bridge between T cell-specific and general signaling pathways. *Sci Signal* 2000;2000:re1.
12 Maruhashi T, Okazaki I-mi, Sugiura D, *et al*. LAG-3 inhibits the activation of CD4+ T cells that recognize stable pMHCII through its conformation-dependent recognition of pMHCII. *Nat Immunol* 2018;19:1415–26.
13 Stanford SM, Rapini N, Bottini N. Regulation of TCR signalling by tyrosine phosphatases: from immune homeostasis to autoimmunity. *Immunology* 2012;137:1–19.
14 Ishii T, Ohnuma K, Murakami A, *et al*. CD26-mediated signaling for T cell activation occurs in lipid rafts through its association with CD45RO. *Proc Natl Acad Sci U S A* 2001;98:12138–43.

15 Suzuki N*et al*. A critical role for the innate immune signaling molecule IRAK-4 in T cell activation. *Science* 2006;311:1927–32.
16 Frascoli M, Marcandalli J, Schenk U, *et al*. Purinergic P2X7 receptor drives T cell lineage choice and shapes peripheral γδ cells. *J Immunol* 2012;189:174–80.
17 Mittelstadt M, Frump A, Khuu T, *et al*. Interaction of human tRNA-dihydrouridine synthase-2 with interferon-induced protein kinase PKR. *Nucleic Acids Res* 2008;36:998–1008.
18 Münz C, Lünemann JD, Getts MT, *et al*. Antiviral immune responses: triggers of or triggered by autoimmunity? *Nat Rev Immunol* 2009;9:246–58.
19 Schmiedel BJ, Singh D, Madrigal A, *et al*. Impact of genetic polymorphisms on human immune cell gene expression. *Cell* 2018;175:e1716:1701–15.
20 Suárez-Fueyo A, Bradley SJ, Tsokos GC. T cells in systemic lupus erythematosus. *Curr Opin Immunol* 2016;43:32–8.
21 Karabiyik A, Peck AB, Nguyen CQ. The important role of T cells and receptor expression in Sjögren's syndrome. *Scand J Immunol* 2013;78:157–66.
22 Zhou Y, Zhou B, Pache L, *et al*. Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. *Nat Commun* 2019;10:1523.
23 Thylur RP, Wu X, Gowda NM, *et al*. Cd36 receptor regulates malaria-induced immune responses primarily at early blood stage infection contributing to parasitemia control and resistance to mortality. *J Biol Chem* 2017;292:9394–408.
24 Salles Érika Machado de, Menezes MNde, Siqueira R, *et al*. P2X7 receptor drives Th1 cell differentiation and controls the follicular helper T cell population to protect against Plasmodium chabaudi malaria. *PLoS Pathog* 2017;13:e1006595.
25 Petersone L, Edner NM, Ovcinnikovs V, *et al*. T cell/B cell collaboration and autoimmunity: an intimate relationship. *Front Immunol* 2018;9:1941.
26 Musette P, Bouaziz JD. B cell modulation strategies in autoimmune diseases: new concepts. *Front Immunol* 2018;9:622.
27 Sagiv-Barfi I, Kohrt HEK, Czerwinski DK, *et al*. Therapeutic antitumor immunity by checkpoint blockade is enhanced by ibrutinib, an inhibitor of both Btk and Itk. *Proc Natl Acad Sci U S A* 2015;112:E966–72.
28 Cho H-S, Shin HM, Haberstock-Debic H, *et al*. A small molecule inhibitor of Itk and RLK impairs Th1 differentiation and prevents colitis disease progression. *J Immunol*2015;195:4822–31.
29 Harris PA, Berger SB, Jeong JU, *et al*. Discovery of a first-in-class receptor interacting protein 1 (RIP1) kinase specific clinical candidate (GSK2982772) for the treatment of inflammatory diseases. *J Med Chem* 2017;60:1247–61.

CLINICAL SCIENCE

Open-label randomised pragmatic trial (CONTACT) comparing naproxen and low-dose colchicine for the treatment of gout flares in primary care

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ABSTRACT

Objectives To compare the effectiveness and safety of naproxen and low-dose colchicine for treating gout flares in primary care.

Methods This was a multicentre open-label randomised trial. Adults with a gout flare recruited from 100 general practices were randomised equally to naproxen 750 mg immediately then 250 mg every 8 hours for 7 days or low-dose colchicine 500 mcg three times per day for 4 days. The primary outcome was change in worst pain intensity in the last 24 hours (0–10 Numeric Rating Scale) from baseline measured daily over the first 7 days: mean change from baseline was compared between groups over days 1–7 by intention to treat.

Results Between 29 January 2014 and 31 December 2015, we recruited 399 participants (naproxen n=200, colchicine n=199), of whom 349 (87.5%) completed primary outcome data at day 7. There was no significant between-group difference in average pain-change scores over days 1–7 (colchicine vs naproxen: mean difference −0.18; 95% CI −0.53 to 0.17; p=0.32). During days 1–7, diarrhoea (45.9% vs 20.0%; OR 3.31; 2.01 to 5.44) and headache (20.5% vs 10.7%; 1.92; 1.03 to 3.55) were more common in the colchicine group than the naproxen group but constipation was less common (4.8% vs 19.3%; 0.24; 0.11 to 0.54).

Conclusion We found no difference in pain intensity over 7 days between people with a gout flare randomised to either naproxen or low-dose colchicine. Naproxen caused fewer side effects supporting naproxen as first-line treatment for gout flares in primary care in the absence of contraindications.

Trial registration number ISRCTN (69836939), clinicaltrials.gov (NCT01994226), EudraCT (2013-001354-95).

Key messages

What is already known about this subject?

- Non-steroidal anti-inflammatory drugs (NSAIDs) are effective treatments for gout flare, but side effects are frequent.
- Lower doses of colchicine are as effective as and better tolerated than high doses but have never been compared directly with an NSAID.

What does this study add?

- There was no difference between the effect of naproxen and low-dose colchicine on pain from gout flare.
- Naproxen was associated with fewer side effects, lower use of other analgesics and was cost-effective.

How might this impact on clinical practice or future developments?

- In the absence of contraindications, naproxen should be used ahead of low-dose colchicine in primary care on the grounds of effectiveness, safety and cost.

INTRODUCTION

Gout affects 2.5% of adults in the UK and 3.8% in the USA.[1 2] It causes sudden flares of excruciating joint pain and swelling, which are treated with non-steroidal anti-inflammatory drugs (NSAIDs), low-dose colchicine or corticosteroids.[3–5]

Numerous randomised trials demonstrate that NSAIDs treat gout flares effectively.[6 7] However, side effects are frequent and can be life-threatening. NSAIDs are commonly used in all age groups: three-quarters of NSAID prescriptions for gout flares in the UK in 2001–2004 were for diclofenac or indomethacin,[8] two of the most toxic NSAIDs.[9] Naproxen is associated with lower vascular risk than other NSAIDs and is as effective as oral prednisolone for gout flares.[9 10]

High-dose colchicine is effective but commonly causes gastrointestinal side effects.[6 8 11–13] Lower doses are as effective but better tolerated.[14] The recommended 'low-dose' regimen in the UK is 500 mcg two to four times per day,[3 15] however, the effectiveness and tolerability of this dose have never been evaluated. A direct comparison of an NSAID and low-dose colchicine is needed to inform choice for patients and practitioners.

The Colchicine Or Naproxen Treatment for ACute gouT (CONTACT) trial aimed to compare the clinical effectiveness of naproxen and low-dose colchicine at reducing pain from gout flares in primary care, their side-effect profiles and cost-effectiveness.

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METHODS

Study design

This was a randomised, multicentre, open-label, pragmatic clinical trial. The trial protocol is available at https://www.keele.ac.uk/pchs/research/inflammatoryconditions/contact/

Participants

We recruited participants from 100 general practices across England. Registered patients who had consulted for gout in the preceding 2 years were mailed trial information before trial commencement and then 3 monthly inviting them to consult their general practitioner (GP) about the trial if they experienced a gout flare. Patients experiencing their first-ever flare were provided with trial information when they consulted.

Eligibility was assessed by the GP during a routine consultation. Participants were aged 18 years and over, consulting for a current gout flare, and had capacity and willingness to give consent and complete trial documentation. A clinical diagnosis of gout was made by the GP without joint aspiration, blood tests, imaging or diagnostic criteria. Exclusion criteria were unstable medical conditions (eg, ischaemic heart disease, impaired liver function); known stage 4/5 chronic kidney disease (estimated glomerular filtration rate/creatinine clearance <30 mL/min); recent surgery or gastrointestinal bleed; history of gastric ulcer; current anticoagulant use; allergy to aspirin or NSAID; previous inability to tolerate naproxen or low-dose colchicine; other contraindication to either study drug described in the Summary of Product Characteristics; prescription of naproxen or colchicine in the previous 24 hours; pregnancy or lactation; potentially vulnerable patients; and participation in the CONTACT trial during a previous gout flare or involvement in another clinical trial in the last 90 days or other research within the last 30 days. Written informed consent was obtained prior to participation.

Randomisation, masking and interventions

Participants were randomly allocated 1:1 using simple randomisation to either:

1. Single initial dose of oral naproxen 750 mg (three 250 mg tablets) followed by 250 mg (one tablet) every 8 hours for up to 7 days. Co-prescription of a proton-pump inhibitor was at the GP's discretion.
2. Oral colchicine 500 mcg (one tablet) every 8 hours for 4 days. Participants prescribed a statin were advised to omit the statin during colchicine treatment.

Randomisation was undertaken by the healthcare professional using web-access to a secure remote allocation system or, if this could not be accessed, a telephone randomisation service. Clinicians did not know which treatment a participant would receive prior to randomisation ensuring allocation concealment.

The GP prescribed the allocated medication. Participants and treating clinicians were aware of treatment allocation. Participants received a drug-specific advice leaflet that included advice about non-pharmacological treatment (rest, application of ice) and were offered reimbursement for prescription charges.

Data collection

Baseline data were collected by self-complete questionnaire prior to randomisation. Outcome measures were collected by self-complete daily diary (days 1–7) and a questionnaire at week 4. On study entry, participants chose between paper (postal) or web-based (e-mail invitation) follow-up. Reminders were sent during week 1 (postcard or daily e-mail reminders). If diary data were not received by day 10, a blinded research nurse telephoned participants to capture key outcome data. Non-responders to the 4-week questionnaire were sent postal/e-mail reminders at 2 weeks and 4 weeks after initial mailing. Non-responders to the second reminder were telephoned by the research nurse and, if not successfully contacted, mailed a brief questionnaire.

Participants provided consent for review of their medical records over the 4-week study period to capture serious adverse events including hospitalisations and deaths.

Outcomes

On days 0–7 and at week 4, participants rated the intensity of the worst pain experienced in the last 24 hours using a validated 0–10 Numeric Rating Scale (NRS).[16] The primary outcome was change in pain intensity from baseline measured over the first 7 days. Secondary outcomes were time-to-treatment effect; complete pain resolution (reporting 0 or 1 on NRS); self-reported side effects (nausea, vomiting, headache, skin rash, dyspepsia, abdominal pain, constipation and diarrhoea); patient global assessment of treatment response (completely better/much better/somewhat better/about the same/somewhat worse/much worse); use of corticosteroids, paracetamol, NSAIDs or opiates for gout pain; treatment adherence; relapse/recurrent gout flare; quality of life (EQ-5D-5L)[17]; attendance at GP, emergency department or primary care out-of-hours service; and absence from work/education. Worst pain intensity in the last 24 hours, side effects, medication use for gout pain and treatment adherence were assessed daily during days 1–7 and at week 4. EQ-5D-5L and patient global assessment of treatment response were assessed at day 7 and week 4. Relapse/recurrent gout flare, re-attendance and work absence were assessed at week 4.

Sample size

We aimed to assess the superiority of naproxen or colchicine (two-tailed hypothesis testing). A sample size of 200 participants per arm was required to detect a small standardised effect size (ES) of 0.3, allowing for the repeated measures structure (assumed autocorrelation 0.6), 20% loss to follow-up, 1:1 allocation ratio, 90% power and two-sided type 1 error of 0.05.[18]

Statistical analysis

The main analysis was by intention-to-treat (ITT) evaluating participants as per allocation assignment. Mean change in worst pain intensity in the last 24 hours from baseline to each follow-up time point was calculated for each group. Analysis of the primary outcome was by linear mixed model with autoregressive covariance for repeated measures.[19] Between-group mean differences for each day (and at week four) were derived from the group×time interaction within the model. Standardised between-group mean differences for pain were expressed as the estimated mean differences relative to the baseline SD of pain scores (ES).[18] Analyses of primary and secondary outcomes were performed before and after adjustment for baseline pain score, age and gender.

The proportion of participants reporting complete pain resolution and time-to-first resolution of pain was compared between groups through χ^2 and Mann-Whitney U tests, respectively. Stepped per-protocol evaluations of between-group difference in the primary outcome were undertaken by excluding: (1) protocol violators related to treatment and eligibility; (2) those who did not take their designated treatment at any point and (3) those who did not take the full treatment course (naproxen <7 days, colchicine <4 days).

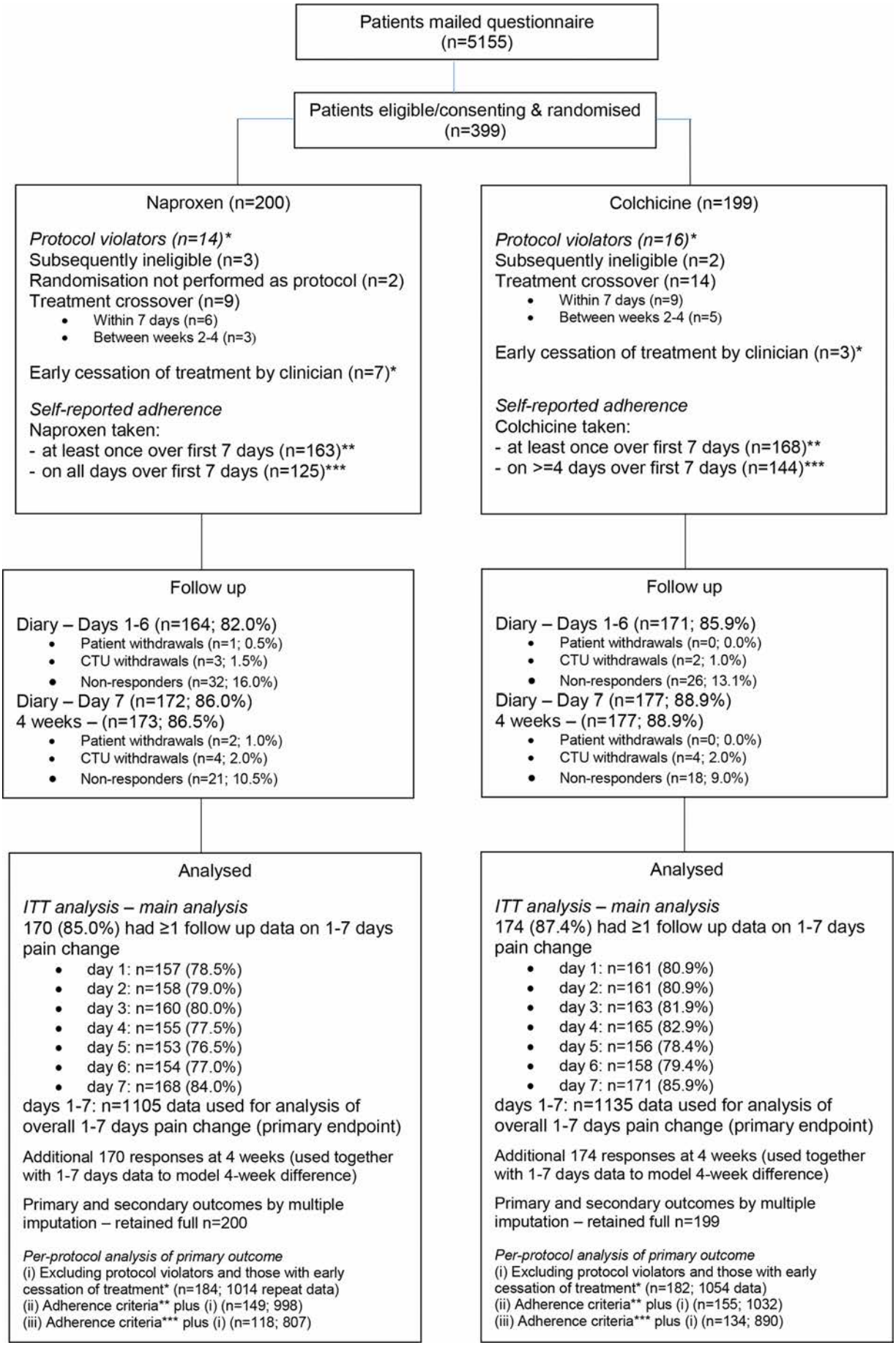


Figure 1 Participant flow. CTU, Clinical Trials Unit. ITT, intention-to-treat.

Binary or ordinal logistic models were used to estimate ORs for between-group comparisons of secondary outcomes: patient global assessment of treatment response; relapse/recurrent gout flare; re-attendance; time off work because of gout; use of other medications for gout pain; and side effects, based on complete data and multiple imputation (MI) using chained equations based on 50 imputed data sets including treatment and sociodemographic variables as predictors. Separate MI evaluations were undertaken to maintain reasonable cases-to-variables ratio >5[20]: imputed variables comprised (i) primary/secondary health variables (excluding side effects) across baseline and follow-up and health utilisation at week 4 and (ii) key health variables (ie, pain, global response, EQ-5D-5L) plus days 1–7 and week 4 medication and side-effect variables.[21] Number needed to treatment harm was estimated for side effects as the reciprocal of the absolute risk difference.[22]

In a sensitivity analysis, between-group differences in the primary outcome based on more inclusive baseline covariates including adjustment for first episode, age at first flare, location of gout, EQ-5D-5L, index of deprivation (fixed factors) and GP practice (random factor) were examined via MI evaluation (imputation data set (i) above).

Analysis was performed when all participants had completed follow-up; no interim analysis was performed. Primary and secondary outcomes (except per-protocol and health economic evaluations) were analysed blind to treatment allocation. The primary endpoint analysis was independently analysed by two statisticians. All analyses were carried out using SPSS V.21.0 and STATA V.14.0.

Health economics

An incremental cost-utility analysis from a National Health Service (NHS)/personal social services perspective was undertaken. Unit costs (2015/2016 prices) from standard UK sources were applied to resource use data. EQ-5D-5L index scores were generated using the UK value set to calculate QALYs over the 4-week follow-up period.[23]

Resource use, costs and EQ-5D-5L scores were summarised using descriptive statistics. Missing EQ-5D-5L scores and costs were imputed using MI. QALYs were calculated for each participant using EQ-5D-5L responses. A regression approach controlled for imbalances in baseline EQ-5D-5L scores between treatment arms. Mean costs were estimated by treatment arm and the difference in mean costs (95% CI) calculated using non-parametric bootstrapping.[24]

Incremental cost-effectiveness ratios were estimated by dividing the mean cost difference between arms by the difference in mean QALYs. Five thousand pairs of mean cost and QALY differences were estimated by non-parametric bootstrapping and presented on a cost-effectiveness plane. Cost-effectiveness acceptability curves were plotted to determine the probability that naproxen was cost-effective.[25]

The human capital approach was used to estimate productivity costs from employment status and days off work due to health. The average wage for each respondent was identified using UK Standard Occupational Classification coding and annual earnings data.[26]

Patient and public involvement

This trial was developed with research users with gout who provided feedback on the proposed recruitment and consent processes and choice of trial outcomes. Two patient representatives sat on the independent trial steering committee, playing a full part in monitoring trial progress and conduct, and provided advice on the design of questionnaires and Participant Information Leaflets.

Table 1 Baseline characteristics

Key characteristics	Categories	Naproxen	Colchicine
Age: mean (SD)	–	58.7 (14.4)	60.0 (13.4)
Male, n (%)		173 (86.5)	174 (87.4)
Pain NRS (0–10), mean (SD)	–	7.1 (2.1)	6.9 (2.2)
	Missing data	7	5
First instance of gout, n (%)	–	35 (17.9)	51 (26.2)
	Missing data	4	4
Age when diagnosed, mean (SD)		52.1 (15.2)	53.4 (14.6)
	Missing data	6	7
Body part affected, n (%)	First MTPJ	142 (72.4)	135 (69.2)
	Other foot joints	58 (29.6)	48 (24.6)
	Other lower limb	46 (23.5)	47 (24.1)
	Upper limb	23 (11.7)	31 (15.9)
	Missing data	4	4
Number of body parts affected, n (%)	1	139 (70.9)	145 (74.3)
	2	34 (17.3)	27 (13.8)
	3	13 (6.6)	9 (4.6)
	4	6 (3.1)	13 (6.7)
	≥5	4 (2.0)	1 (0.5)
	Missing data	4	4
EQ-5D-5L, mean (SD)	–	0.665 (0.210)	0.666 (0.225)
	Missing data	8	6

MTPJ, metatarsophalangeal joint; NRS, Numeric Rating Scale.

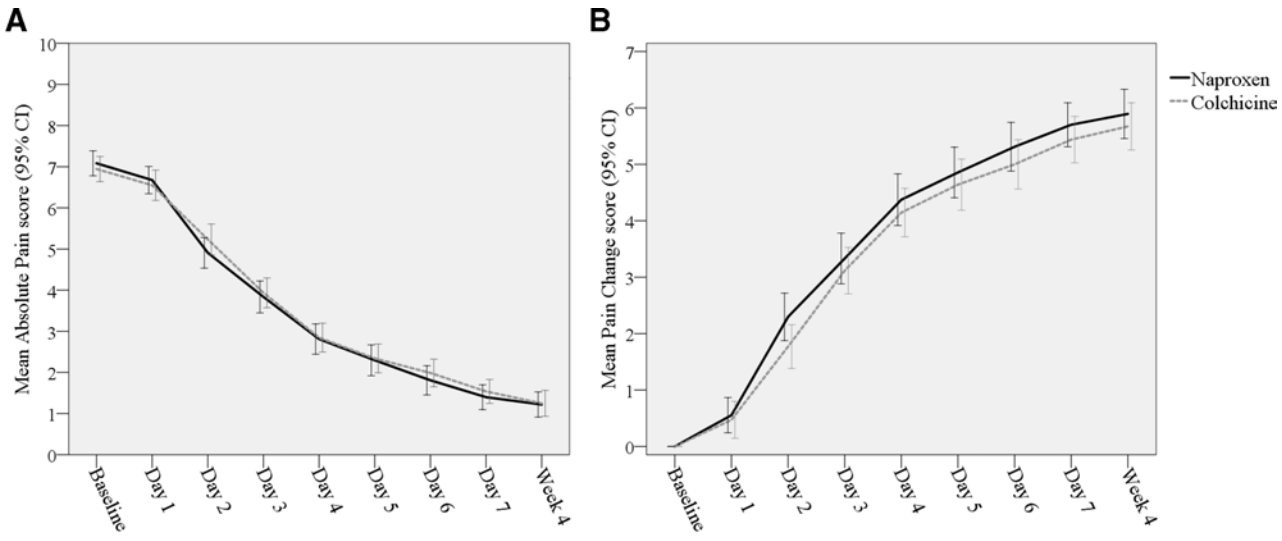
RESULTS

Between 29 January 2014 and 31 December 2015, 5155 patients were mailed. Three-hundred and ninety-nine participants were randomised: 200 to receive naproxen and 199 to receive colchicine (figure 1). Groups were similar at baseline although more people allocated to colchicine reported experiencing their first-ever gout flare (table 1, online supplementary table 1). Primary outcome data were collected for 86.0% in the naproxen group at day 7 and 86.5% at 4 weeks and 88.9% in the colchicine group at both day 7 and 4 weeks (figure 1).

There were 30 protocol violations (8% of participants) relating to treatment or eligibility (naproxen n=14, colchicine n=16). Of those returning diary data, 99% (163/164) reported taking the allocated treatment at least once and 75% (125) taking it on each day of the course in the naproxen group compared with 98% (168/171) and 85% (144), respectively, for colchicine.

Within-group improvements in the primary outcome were seen in both groups over days 1–7 (figure 2). There was no significant between-group difference in mean change in worst pain intensity over days 1–7 (colchicine vs naproxen: adjusted mean difference −0.18; 95% CI −0.53 to 0.17; p=0.32; ES 0.09). Unadjusted estimates and MI evaluation with extended covariate adjustment were similar. There was a small between-group difference favouring naproxen on day 2 only.

Per-protocol analysis (1) showed comparable between-group mean differences to the ITT evaluation (online supplementary



	Naproxen		Colchicine		Unadjusted*		Adjusted**		Sensitivity***	
	Absolute mean (SD) n	Change mean (SD) n	Absolute mean (SD) n	Change mean (SD) n	Mean difference (95% CI)	P value	Mean difference (95% CI)	P value	Mean difference (95% CI)	P value
Day 1	6.7 (2.1) 160	0.6 (2.0) 157	6.5 (2.4) 164	0.5 (2.1) 161	-0.07 (-0.62 to 0.47)	0.79	-0.06 (-0.51 to 0.39)	0.79	-0.05 (-0.45 to 0.35)	0.80
Day 2	4.9 (2.4) 161	2.3 (2.7) 158	5.2 (2.4) 164	1.8 (2.5) 161	-0.50 (-1.04 to 0.05)	0.075	-0.45 (-0.90 to -0.01)	0.047	-0.47 (-0.95 to -0.01)	0.049
Day 3	3.8 (2.5) 163	3.3 (2.9) 160	3.9 (2.4) 166	3.1 (2.7) 163	-0.22 (-0.76 to 0.33)	0.43	-0.16 (-0.61 to 0.28)	0.48	-0.21 (-0.69 to 0.28)	0.40
Day 4	2.8 (2.3) 158	4.4 (2.9) 155	2.8 (2.3) 168	4.1 (2.8) 165	-0.18 (-0.73 to 0.36)	0.51	-0.12 (-0.56 to 0.33)	0.61	-0.16 (-0.62 to 0.30)	0.49
Day 5	2.3 (2.4) 156	4.9 (2.8) 153	2.3 (2.2) 159	4.6 (2.9) 156	-0.19 (-0.74 to 0.35)	0.48	-0.13 (-0.57 to 0.32)	0.58	-0.18 (-0.64 to 0.28)	0.44
Day 6	1.8 (2.2) 157	5.3 (2.7) 154	2.0 (2.1) 160	5.0 (2.8) 158	-0.20 (-0.74 to 0.34)	0.47	-0.13 (-0.57 to 0.31)	0.57	-0.17 (-0.61 to 0.26)	0.44
Day 7#	1.4 (2.0) 171	5.7 (2.6) 168	1.5 (2.0) 173	5.4 (2.7) 171	-0.30 (-0.83 to 0.24)	0.28	-0.21 (-0.65 to 0.22)	0.34	-0.28 (-0.68 to 0.13)	0.18
Overall (Days 1-7)	3.4 (2.9) 1126	3.8 (3.2) 1105	3.5 (2.8) 1154	3.5 (3.1) 1135	-0.24 (-0.70 to 0.23)	0.31	-0.18† (-0.53 to 0.17)	0.32†	-0.22 (-0.57 to 0.13)	0.22
Week 4	1.2 (2.0) 173	5.9 (2.9) 170	1.2 (2.1) 177	5.7 (2.8) 174	-0.22 (-0.76 to 0.32)	0.43	-0.09 (-0.53 to 0.35)	0.68	-0.19 (-0.63 to 0.25)	0.40

*/** Between-group difference in mean change scores (colchicine – naproxen) by linear mixed model * unadjusted for baseline covariates ** adjusted for age, gender and baseline pain score (autoregressive (1st order) residual covariance structure was assumed for the model of daily differences).

*** By MI (multiple imputation via chained equations) adjusted for baseline pain, age and gender plus previous gout, age at first episode, single or multisite location, EQ5D (health dimensions), GP-Practice index of multiple deprivation [fixed effects] and GP-Practice site [random effect].

Summary is inclusive of minimum data collection (for scores at day 7).

† Primary endpoint.

Standardized mean difference (effect size) based on adjusted** evaluation i.e. absolute mean difference relative to overall baseline SD of 2.1: 0.03 (day 1); 0.21 (day 2); 0.08 (day 3) 0.06 (days 4 through 6); 0.10 (day 7); 0.09 (overall); 0.04 (week 4).

Figure 2 Comparison of pain scores (primary outcome measure) at follow-up (intention-to-treat analysis).

table 2). Per-protocol analyses (2) and (3) showed similar between-group differences to the ITT analysis overall and on days 1–6 but found small significant differences favouring naproxen at week 4.

There were no between-group differences in complete pain resolution or patient global assessment of treatment response at any time-point (table 2, online supplementary table 3). At week 4, there were no between-group differences in proportions reporting a relapse/recurrent gout flare; consulting a GP, practice nurse or emergency department; or time off work.

More participants in the colchicine group used paracetamol or codeine for gout during days 1–7 than in the naproxen group (table 3). At week 4, ibuprofen use was more common in the colchicine group on complete case analysis but not in the MI data set.

There were three serious adverse events, none related to trial interventions, and no deaths. Two participants who received naproxen were hospitalised: one for non-cardiac chest pain and one for hospital-acquired pneumonia following a transcatheter aortic valve implantation. One participant who received colchicine was hospitalised with osteomyelitis. During days 1–7, self-reported diarrhoea and headache were more common with colchicine than naproxen, whereas constipation was less common with colchicine (table 4). Diarrhoea peaked on day 4 in the colchicine group and constipation on day 3 in the naproxen group (online supplementary table 4). Both reduced considerably during weeks 2–4.

Naproxen was slightly less costly and more effective than colchicine (online supplementary table 5). At a willingness-to-pay threshold of £20 000 per QALY, naproxen had an 80%

Table 2 Comparison of secondary outcome measures at day 7 and week 4 follow-up

	Naproxen	Colchicine	OR (95% CI) (p value)*	OR (95% CI) (p value)†
Complete pain resolution, n (%)				
7 days	115 (67.3)	116 (67.1)	0.96 (0.60 to 1.54) (p=0.87)	0.95 (0.60 to 1.48) (p=0.81)
4 weeks	130 (75.1)	130 (73.4)	0.83 (0.51 to 1.36) (p=0.46)	0.90 (0.56 to 1.44) (p=0.66)
Days to complete pain resolution, median (IQR)	5 (day 4, week 4)	6 (day 4, week 4)	–	–
Patient assessment of global treatment response (completely/much better), n (%)				
7 days	114 (71.3)	110 (72.4)	1.11 (0.67 to 1.84) (p=0.69)	1.03 (0.62 to 1.70) (p=0.91)
4 weeks	140 (80.9)	143 (80.8)	0.88 (0.51 to 1.52) (p=0.64)	0.96 (0.56 to 1.64)(p=0.87)
Recurrence/relapse of gout flare during 4-week follow-up, n (%)	40 (30.1)	54 (35.1)	1.28 (0.78 to 2.13) (p=0.33)	1.24 (0.77 to 1.99) (p=0.37)
Consultation/re-attendance for gout during 4-week follow-up, n (%)				
Health professional‡	30 (22.6)	41 (26.6)	1.43 (0.82 to 2.51) (p=0.213)	1.39 (0.82 to 2.34) (p=0.22)
GP	26 (19.4)	39 (25.3)	1.69 (0.93 to 3.05) (p=0.083)	1.56 (0.89 to 2.72) (p=0.12)
Number of times				
1	14 (58.3)	27 (69.2)	–	–
2	8 (33.3)	10 (25.6)		
3	2 (8.3)	2 (5.1)		
Practice nurse	7 (5.3)	10 (6.6)	1.31 (0.47 to 3.64) (p=0.61)	1.23 (0.45 to 3.32) (p=0.69)
Number of times				
1	5 (71.4)	9 (90.0)	–	–
2	1 (14.3)	1 (10.0)		
3	1 (14.3)	0 (0.0)		
Emergency GP	6 (4.5)	6 (3.9)	0.84 (0.26 to 2.68) (p=0.77)	0.87 (0.30 to 2.57) (p=0.81)
Emergency department	1 (0.8)	1 (0.7)	–	1.23 (0.07 to 21.5) (p=0.89)
Taken time off work because of gout during 4-week follow-up, n (%)	11 (8.6)	8 (5.3)	0.61 (0.22 to 1.64) (p=0.33)	0.76 (0.31 to 1.91) (p=0.57)
Days, median (IQR)	4 (2, 12)	3 (3, 17)	–	–

OR for colchicine relative to naproxen.
*Analysis of complete case data (adjusted for baseline pain, age and gender).
†Analysis through multiple imputation via chained equations with logistic (binary/ordinal) regression model (adjusted for age, sex and baseline pain) based on full ITT on 50 imputations.
‡Health professional: GP, practice nurse, emergency GP and/or accident and emergency.
GP, general practitioner; ITT, intention-to-treat.

chance of being cost-effective compared with colchicine (online supplementary figure).

DISCUSSION

We found substantial within-group improvements in pain intensity in both groups but no statistically significant difference between naproxen and low-dose colchicine over the first 7 days. Naproxen appeared to provide faster pain relief, which could be explained by the 750 mg loading dose although the between-group difference at day 2 was small and possibly spurious. Side effects, particularly diarrhoea, and analgesic use were more frequent with colchicine. There were no major harms with naproxen. Naproxen was slightly more cost-effective than colchicine. These findings suggest that naproxen should be considered ahead of low-dose colchicine to treat gout flares in primary care in the absence of contraindications.

This is the first head-to-head comparison of naproxen and colchicine for gout flares and the first randomised trial of colchicine at this dose. In an equivalence trial comparing naproxen and prednisolone for gout flare,[10] mean pain reduction (0–100 mm visual analogue scale) was 46 mm with naproxen by day 4 similar to the 4.1 mean reduction in our trial. A reduction of 2 points on a 0–10 pain NRS has been shown to be clinically significant in chronic pain.[27] Only 70% of participants were completely/much better by day 7 and 80% by week 4, consistent with clinical observations that flares often persist beyond 1 week and one-third of participants reporting a recurrent flare by week 4. There have been two placebo-controlled trials of colchicine for gout flare, one used a traditional high-dose regime[13] whereas the AGREE trial included both high-dose and low-dose arms.[14] Lower doses are recommended to lessen gastrointestinal side effects while maintaining effectiveness.[3–5] We used the UK recommended dose of colchicine, which is intermediate to the regime used by Ahern *et al* and the AGREE trial.[13–15] Forty-two per cent of participants reported diarrhoea in week 1 compared with

Table 3 Use of medication for relief of gout pain over the first week (diary days 1–7) and between weeks 2 and 4 (week 4 follow-up)

	Days 1–7				Weeks 2–4			
	Naproxen	Colchicine	OR (95% CI) (p value)		Naproxen	Colchicine	OR (95% CI) (p value)	
	N (%)*	N (%)*	Complete case*	Imputed†	N (%)*	N (%)*	Complete case*	Imputed†
Paracetamol	20 (13.4)	34 (23.6)	2.09 (1.11 to 3.93) (p=0.022)	1.91 (1.05 to 3.51) (p=0.035)	10 (7.5)	11 (7.1)	1.12 (0.45 to 2.82) (p=0.81)	0.98 (0.40 to 2.37) (p=0.96)
Ibuprofen	16 (10.7)	20 (13.9)	1.54 (0.72 to 3.29) (p=0.27)	1.58 (0.80 to 3.12) (p=0.19)	12 (9.0)	27 (17.5)	2.34 (1.11 to 4.94) (p=0.026)	1.93 (0.90 to 4.14) (p=0.089)
Diclofenac	2 (1.3)	4 (2.8)	–	–	4 (3.0)	6 (3.9)	–	–
Indomethacin	1 (0.7)	0 (0.0)	–	–	2 (1.5)	5 (3.2)	–	–
Tramadol	1 (0.7)	0 (0.0)	–	–	1 (0.7)	2 (1.3)	–	–
Codeine	7 (4.7)	21 (14.6)	3.62 (1.47 to 8.93) (p=0.005)	3.20 (1.35 to 7.57) (p=0.008)	12 (9.0)	8 (5.2)	0.60 (0.22 to 1.65) (p=0.32)	0.59 (0.23 to 1.50) (p=0.27)
Prednisolone	3 (2.0)	2 (1.4)	–	–	2 (1.5)	1 (0.6)	–	–
Any analgesic or non-naproxen NSAID‡	37 (24.8)	61 (42.4)	2.23 (1.35 to 3.66) (p=0.001)	1.89 (1.24 to 2.88) (p=0.003)	37 (27.6)	52 (33.8)	1.34 (0.81 to 2.21) (p=0.26)	0.95 (0.63 to 1.43) (p=0.81)

OR for colchicine relative to naproxen (adjusted for age, gender and baseline pain score). *Analysis of complete case data (days 1–7: n=288; five cases excluded due to missing baseline pain scores; week 4: n=283; five cases excluded due to missing baseline pain scores). †Analysis of imputed data (n=399). n/a: analysis not applicable (as it is an evaluation of compliance with allocated treatment). –, ORs not estimated due to small frequency counts.
*Complete response to medication questions: diary days 1–7—149 in naproxen group and 144 in colchicine group; week 4—134 in naproxen group and 154 in colchicine group.
†Imputed data set: 200 in naproxen group; 199 in colchicine group (full ITT analysis).
‡Paracetamol or codeine or tramadol or ibuprofen or diclofenac or indomethacin.
ITT, intention-to-treat; NSAID, non-steroidal anti-inflammatory drug.

100% with the regime of Ahern *et al* and 77% and 23% in the AGREE trial high-dose and low-dose regimes, respectively. Eighteen per cent in the naproxen group reported diarrhoea, similar to 14% in the placebo group in the AGREE trial. It was unexpected that headache differed between the groups, but it is plausible that naproxen may have a protective effect to treat or prevent headaches. Colchicine is considered to be more effective if given in the first 12–36 hours of a flare.[4 5 14] Two-thirds of our participants initiated medication over 24 hours after symptom-onset providing 'real-world' evidence that low-dose colchicine is effective even when treatment is delayed due to patient or service-related factors.

Strengths of this trial include its primary care setting and pragmatic design. Although this should ensure generalisability to most patients with gout who are managed in the community, we did not assess existing comorbidities, use of urate-lowering therapy or prior flare rates to verify this. Gout diagnosis was made clinically rather than using validated criteria or additional investigations risking misclassification, although clinical diagnosis of gout in UK primary care has a positive predictive value of 90%.[28] Further limitations include the open-label design without blinded outcome assessment or placebo tablets, and collection of solely self-reported outcomes without assessing the effect of NSAIDs on objective measures such as blood pressure or renal function. More participants in the naproxen group had experienced gout in the past and hence probably taken trial medications previously, possibly influencing perception of treatment effect, although participating clinical staff were trained to maintain equipoise. Since having recurrent flares increases the likelihood of a correct diagnosis,[29] misclassification could have been greater in the colchicine group. Hence, it is possible that the naproxen group could have been advantaged, if previous treatment experiences influenced outcome reporting or alternative diagnoses such as osteoarthritis or palindromic rheumatism respond better to NSAID than colchicine. Finally, recruitment fell one short of the target of 400 participants. However, follow-up was better than anticipated and exceeded the required number of participants at the primary end-point.

We chose the dose of naproxen specified for gout flares in its marketing authorisation,[30] although two times per day dosing is not uncommon in clinical practice. A previous randomised trial demonstrated equivalence of naproxen 500 mg two times per day to prednisolone for gout flares.[10] Colchicine treatment was limited to 4 days, consistent with UK guidance, which advises a maximum total dose of 6 mg per course.[15] In contrast, the AGREE trial low-dose arm comprised a total dose of 1.8 mg over 2 hours,[14] although the American College of Rheumatology gout guideline recommended that this can be followed by 600 mcg one time or two times per day until flare resolution.[4] While the longer treatment duration could have biased towards naproxen, colchicine was effective within the treatment period and there were no statistically significant between-group differences between days 3 and 7.

NSAIDs and colchicine are not the only drugs used to treat gout flares. The American College of Physicians recommends corticosteroids as first-line treatment, whereas other guidelines advise being guided by comorbidities, contraindications, previous response and the pattern of joint involvement.[3–5 31] While randomised trials have compared NSAIDs and prednisolone,[10] future research should compare the effectiveness and safety of colchicine and corticosteroids, particularly in patients with contraindications to NSAIDs. We found little difference in pain reduction between naproxen and low-dose colchicine, but naproxen was associated with fewer side effects, less analgesic use and slightly lower costs, suggesting that, in the absence of contraindications, naproxen should be used ahead of low-dose colchicine to treat gout flares in primary care.

Table 4 Self-reported side effects over the first week and between weeks 2 and 4 (week 4 follow-up)

	Days 1–7					Weeks 2–4				
	Naproxen	Colchicine	OR (95% CI) {p value}			Naproxen	Colchicine	OR (95% CI) {p value}		
	N (%)†	N (%)†	Complete case†	Imputed‡	NNTH (95%CI)*	N (%)†	N (%)†	Complete case†	Imputed‡	NNTH (95%CI)*
Nausea and/or vomiting	21 (14.0)	30 (20.5)	1.82 (0.96 to 3.46) {p=0.066}	1.28 (0.71 to 2.30) {p=0.42}	31^{1} ($8\text{-}\infty^{1}$ to $27\text{-}\infty^{2}$)	7 (5.2)	5 (3.2)	0.51 (0.14 to 1.83) {p=0.30}	0.59 (0.19 to 1.90) {p=0.38}	48^{2} ($24\text{-}\infty^{2}$ to $24\text{-}\infty^{1}$)
Dyspepsia	20 (13.3)	20 (13.7)	0.89 (0.48 to 1.90) {p=0.95}	1.09 (0.58 to 2.04) {p=0.79}	98^{1} ($9\text{-}\infty^{1}$ to $19\text{-}\infty^{2}$)	13 (9.7)	8 (5.2)	0.44 (0.17 to 1.15) {p=0.094}	0.59 (0.24 to 1.45) {p=0.25}	27^{2} ($14\text{-}\infty^{2}$ to $26\text{-}\infty^{1}$)
Abdominal pain	16 (10.7)	16 (11.0)	1.07 (0.51 to 2.25) {p=0.86}	0.83 (0.40 to 1.71) {p=0.61}	53^{2} ($16\text{-}\infty^{2}$ to $16\text{-}\infty^{1}$)	4 (3.0)	8 (5.2)	1.57 (0.44 to 5.53) {p=0.49}	1.32 (0.43 to 4.09) {p=0.63}	108^{1} ($12\text{-}\infty^{1}$ to $59\text{-}\infty^{2}$)
Headache	16 (10.7)	30 (20.5)	2.38 (1.21 to 4.68) {p=0.012}	1.92 (1.03 to 3.55) {p=0.039}	12^{1} (5^{1} to 350^{1})	4 (3.0)	4 (2.6)	0.92 (0.22 to 3.86) {p=0.91}	0.80 (0.21 to 3.10) {p=0.75}	171^{2} ($42\text{-}\infty^{2}$ to $17\text{-}\infty^{1}$)
Constipation	29 (19.3)	7 (4.8)	0.20 (0.08 to 0.48) {p<0.001}	0.24 (0.11 to 0.54) {p<0.001}	7^{2} (6^{2} to 13^{2})	9 (6.7)	6 (3.9)	0.49 (0.16 to 1.54) {p=0.22}	0.57 (0.21 to 1.55) {p=0.27}	36^{2} ($19\text{-}\infty^{2}$ to $31\text{-}\infty^{1}$)
Diarrhoea	30 (20.0)	67 (45.9)	3.54 (2.10 to 5.99) {p<0.001}	3.31 (2.01 to 5.44) {p<0.001}	4^{1} (3^{1} to 7^{1})	5 (3.7)	10 (6.5)	1.75 (0.58 to 5.26) {p=0.32}	1.59 (0.54 to 4.66) {p=0.40}	49^{1} ($9\text{-}\infty^{1}$ to $60\text{-}\infty^{2}$)
Skin rash	3 (2.0)	3 (2.1)	1.13 (0.22 to 5.83) {p=0.88}	1.06 (0.21 to 5.39) {p=0.95}	851^{1} ($13\text{-}\infty^{1}$ to $64\text{-}\infty^{2}$)	3 (2.2)	3 (1.9)	0.98 (0.19 to 5.09) {p=0.98}	0.97 (0.19 to 5.03) {p=0.97}	1548^{2} ($56\text{-}\infty^{2}$ to $13\text{-}\infty^{1}$)
Any side effect(s)§	91 (60.7)	101 (69.2)	1.49¶ (0.92 to 2.43) {p=0.11}¶	1.60 (1.03 to 2.49) {p=0.038}	10^{1} (5^{1} to 142^{1})	37 (27.6)	28 (18.2)	0.58 (0.33 to 1.03) {p=0.064}	0.71 (0.41 to 1.23) {p=0.23}	16^{2} ($7\text{-}\infty^{2}$ to $23\text{-}\infty^{1}$)

OR for colchicine relative to naproxen (adjusted for age, gender and baseline pain score). †Analysis of complete data (days 1–7: n=291; five cases excluded due to missing baseline pain scores; week 4: n=283; five cases excluded due to missing baseline pain scores). ‡ Analysis of imputed data (n=399).

*Number needed to treatment harm (NNTH): [1]for colchicine over naproxen; [2]for naproxen over colchicine (based on imputed OR estimates and observed rates for the naproxen group).

†Complete response to side-effect questions: diary days 1–7 naproxen=150, colchicine=146; week 4 naproxen n=134, colchicine=154.

‡Imputed data set: 200 in naproxen group; 199 in colchicine group (full ITT analysis).[1]

§Includes the side effects listed and 'other' (nominated free-text) side effects.

ITT, intention-to-treat.

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REFERENCES

1 Kuo C-F, Grainge MJ, Mallen C, *et al*. Rising burden of gout in the UK but continuing suboptimal management: a nationwide population study. *Ann Rheum Dis* 2015;74:661–7.
2 Juraschek SP, Miller ER, Gelber AC. Body mass index, obesity, and prevalent gout in the United States in 1988-1994 and 2007-2010. *Arthritis Care Res* 2013;65:127–32.
3 Hui M, Carr A, Cameron S, *et al*. The British Society for rheumatology guideline for the management of gout. *Rheumatology* 2017;56:1056–9.
4 Khanna D, Fitzgerald JD, Khanna PP, *et al*. 2012 American College of rheumatology guidelines for management of gout. Part 1: systematic nonpharmacologic and pharmacologic therapeutic approaches to hyperuricemia. *Arthritis Care Res* 2012;64:1431–46.
5 Richette P, Doherty M, Pascual E, *et al*. 2016 updated EULAR evidence-based recommendations for the management of gout. *Ann Rheum Dis* 2017;76:29–42.
6 Sutaria S, Katbamna R, Underwood M. Effectiveness of interventions for the treatment of acute and prevention of recurrent gout—a systematic review. *Rheumatology* 2006;45:1422–31.
7 van Durme CMPG, Wechalekar MD, Buchbinder R, *et al*. Non-Steroidal anti-inflammatory drugs for acute gout. *Cochrane Database Syst Rev* 2014:CD010120.
8 Roddy E, Mallen CD, Hider SL, *et al*. Prescription and comorbidity screening following consultation for acute gout in primary care. *Rheumatology* 2010;49:105–11.
9 Bhala N, Emberson J, Merhi A, *et al*. Vascular and upper gastrointestinal effects of non-steroidal anti-inflammatory drugs: meta-analyses of individual participant data from randomised trials. *Lancet* 2013;382:769–79.
10 Janssens HJEM, Janssen M, van de Lisdonk EH, *et al*. Use of oral prednisolone or naproxen for the treatment of gout arthritis: a double-blind, randomised equivalence trial. *Lancet* 2008;371:1854–60.
11 Schlesinger N, Schumacher R, Catton M, *et al*. Colchicine for acute gout. *Cochrane Database Syst Rev* 2006:CD006190.
12 van Echteld I, Wechalekar MD, Schlesinger N, *et al*. Colchicine for acute gout. *Cochrane Database Syst Rev* 2014:CD006190.
13 Ahern MJ, Reid C, Gordon TP, *et al*. Does colchicine work? the results of the first controlled study in acute gout. *Aust N Z J Med* 1987;17:301–4.
14 Terkeltaub RA, Furst DE, Bennett K, *et al*. High versus low dosing of oral colchicine for early acute gout flare: twenty-four-hour outcome of the first multicenter, randomized, double-blind, placebo-controlled, parallel-group, dose-comparison colchicine study. *Arthritis Rheum* 2010;62:1060–8.
15 Joint Formulary Committee. *British National formulary*. 75th ed. London: BMJ Group and Pharmaceutical Press, 2018.
16 Dworkin RH, Turk DC, Farrar JT, *et al*. Core outcome measures for chronic pain clinical trials: IMMPACT recommendations. *Pain* 2005;113:9–19.
17 Herdman M, Gudex C, Lloyd A, *et al*. Development and preliminary testing of the new five-level version of EQ-5D (EQ-5D-5L). *Qual Life Res* 2011;20:1727–36.
18 Cohen J. *Statistical power analysis for the behavioral sciences*. 2nd edn. Hillsdale: NJ, Lawrence Earlbaum Associates, 1988.
19 Diggle P, Heagerty LK, *et al*. *Analysis of longitudinal analysis*. 2nd edn. Oxford: Oxford University Press, 2002.
20 Hardt J, Herke M, Leonhart R. Auxiliary variables in multiple imputation in regression with missing X: a warning against including too many in small sample research. *BMC Med Res Methodol* 2012;12:184.
21 White IR, Royston P, Wood AM. Multiple imputation using chained equations: issues and guidance for practice. *Stat Med* 2011;30:377–99.
22 Altman DG. Confidence intervals for the number needed to treat. *BMJ* 1998;317:1309–12.
23 Devlin NJ, Shah KK, Feng Y, *et al*. Valuing health-related quality of life: an EQ-5D-5L value set for England. *Health Econ* 2018;27:7–22.
24 Briggs AH, Gray AM. Handling uncertainty in economic evaluations of healthcare interventions. *BMJ* 1999;319:635–8.
25 van Hout BA, Al MJ, Gordon GS, *et al*. Costs, effects and C/E-ratios alongside a clinical trial. *Health Econ* 1994;3:309–19.
26 Standard occupational classification (SOC), 2000. Available: http://www.ons.gov.uk/ons/guide-method/classifications/archived-standard-classifications/standard-occupational-classification-2000/about-soc-2000/index.html
27 Farrar JT, Young JP, LaMoreaux L, *et al*. Clinical importance of changes in chronic pain intensity measured on an 11-point numerical pain rating scale. *Pain* 2001;94:149–58.
28 Meier CR, Jick H. Omeprazole, other antiulcer drugs and newly diagnosed gout. *Br J Clin Pharmacol* 1997;44:175–8.
29 Janssens HJEM, Fransen J, van de Lisdonk EH, *et al*. A diagnostic rule for acute gouty arthritis in primary care without joint fluid analysis. *Arch Intern Med* 2010;170:1120–6.
30 Accord Healthcare Ltd. Summary of product characteristics. naproxen 250mg tablets. Available: https://www.medicines.org.uk/emc/medicine/25766 [Accessed 13 Feb 2017].
31 Qaseem A, Harris RP, Forciea MA, *et al*. Management of acute and recurrent gout: a clinical practice guideline from the American College of physicians. *Ann Intern Med* 2017;166:58–68.

EPIDEMIOLOGICAL SCIENCE

Comparative risk of serious infections among real-world users of biologics for psoriasis or psoriatic arthritis

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ABSTRACT

Objective To examine whether initiation of interleukin (IL)-17, IL-12/23 or tumour necrosis factor (TNF) inhibitor is associated with an increased risk of serious infection among real-world psoriasis (PsO) or psoriatic arthritis (PsA) patients.

Methods We assembled a retrospective cohort of commercially insured adults in the USA diagnosed with PsO or PsA between 2015 and 2018. Exposure was dispensation for IL-17 (ixekizumab or secukinumab), IL-12/23 (ustekinumab) or TNF (adalimumab, certolizumab pegol, etanercept, golimumab and infliximab). The outcome was infection requiring hospitalisation after biologic initiation. Incidence rates (IRs) per 100 person-years were computed, and hazard ratios (HRs) with 95% confidence intervals (CIs) were estimated using Cox proportional hazards regression models, adjusted for inverse probability of treatment-weighted propensity scores.

Results A total of 11 560 new treatment episodes were included. Overall, 190 serious infections (2% of treatment episodes) were identified in 9264 person-years of follow-up. Class-specific IRs were similar among IL-17 and TNF, yet significantly lower for IL-12/23. After adjustment for propensity scores, there was no increased risk with IL-17 compared with either TNF (HR=0.89, 95% CI 0.48 to 1.66) or IL-12/23 (HR=1.12, 95% CI 0.62 to 2.03). By contrast, IL-23/23 were associated with a lower risk of infections than TNF (HR=0.59, 95% CI 0.39 to 0.90).

Conclusions Relative to TNF and IL-17, IL-12/23 inhibitors were associated with a reduced risk of serious infection in biologic-naïve patients with PsO or PsA. In biologic-experienced individuals, there was no difference in infection risk across TNF, IL-17 or IL-12/23 inhibitors.

Key messages

What is already known about this subject?

► In randomised controlled trials, and some observational cohort studies, biologic therapies such as interleukin (IL) and tumour necrosis factor (TNF) inhibitors are associated with an increased risk of serious infection.

What does this study add?

► Risks of serious infections were similar among new users of IL-17 and TNF inhibitors, while persons newly treated with an IL-12/23 were less likely to be hospitalised with a serious infection than those newly treated with a TNF. Risks of serious infections were similar among the biologic experienced users who initiated a new class of biologic.

How might this impact on clinical practice or future developments?

► While many factors guide treatment choices for psoriasis and psoriatic arthritis, our results, derived from real-world cohorts, may be useful in guiding clinicians and patients regarding the selection of biologic treatments for these conditions.

INTRODUCTION

Tumour necrosis factor (TNF) inhibitors have transformed the care of many rheumatologic and autoimmune conditions, including psoriasis (PsO) and psoriatic arthritis (PsA). In the past 10 years, additional biologic options approved by the US Food and Drug Administration (FDA) include the interleukin-12/23 (IL-12/23) inhibitor ustekinumab as well as the human interleukin-IL-17A (IL-17) antagonists secukinumab and ixekizumab.

Despite efficacy for the management of moderate-to-severe PsO and PsA, biologics' immunosuppressive properties also contribute to an increased risk of serious infections in placebo-controlled randomised controlled trials (RCTs).[1–4] Head-to-head RCTs between biologic agents with adequate power to inform comparative safety questions have been limited.[3 5 6]

It is important to understand whether these findings from RCTs persist in real-world practice, where patients are more heterogeneous and drug utilisation is far less controlled.[7] Evidence from observational studies between biologic and non-biologic drugs have yielded inconsistent findings: some have shown an increased risk,[8 9] while others have not found a difference.[10–14] To our knowledge, no published studies have yet quantified the comparative real-world risk of serious infections among IL-17, IL-12/23 and TNF inhibitors.

We examined the absolute and relative comparative risk of serious infections in patients initiating IL-17, IL-12/23 and TNF inhibitors, among commercially insured adults in the USA diagnosed with PsO or PsA between 2015 and 2018.

METHODS

Data source

We conducted a retrospective cohort analysis using the OptumLabs Data Warehouse.[15] The OptumLabs data consist of administrative claims for over 100 million individuals in all 50 states, of all ages, ethnic and racial groups. Claims include limited patient sociodemographic characteristics as well as inpatient, outpatient and pharmacy dispensation claims. Analysis of secondary, deidentified data is considered exempt by the Johns Hopkins Institutional Review Board.

Patient and public involvement

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

Study population

First, we identified a cohort of all prescription dispensation or medical infusion procedure claims for any of the biologics of interest between 1 January 2015 and 1 May 2018. We were not able to study broadulamb (IL-17) nor guselkumab (IL-12/23), as they were FDA approved towards the end of the study period. We then included only those with at least one diagnosis code prior to the index date for PsO (International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) code 696.1 or ICD-10-CM code L40.9) or PsA (ICD-9-CM code 696.0; ICD-10-CM codes L40.50, L40.51, L40.52, L40.53, L40.54, L40.59) from a dermatologist or rheumatologist visit. Prior work suggests a sensitivity of 77%–91% and positive predictive value of 67%–89% for this approach.[16]

We defined the index date as the date of the first dispensing of any IL-17, IL-12/23 or TNF inhibitor of interest, requiring individuals to have at least 6 months of continuous enrolment with full medical and pharmacy data before the index date to establish new user status.[17] Since these biologics were only approved for use in adults, we required patients to be at least 18 years old at the index date. We excluded individuals with overlapping claims for multiple biologics, due to our inability to ascertain which biologic was truly used given the contraindication of simultaneous use. We also excluded persons who had a diagnosis of rheumatoid arthritis, Crohn's disease, ulcerative colitis, osteoarthritis, HIV, cancer, chronic lymphocytic leukaemia and non-Hodgkin's lymphoma at any point during 24 months prior to the index date, given the potential impact of these comorbid conditions on the incidence of serious infection.[18] We further excluded persons who had a serious infection (using our outcome definition, below) in the 60 days prior to index date.

Exposures

We defined three mutually exclusive exposures (IL-17: ixekizumab and secukinumab; IL-12/23: ustekinumab; TNF: adalimumab, certolizumab pegol, etanercept, golimumab and infliximab) based on pharmacologic drug class (online supplementary table S1). We defined treatment episodes as the initiation of a new biologic agent without any claim for that specific treatment at any time previously, requiring a minimum of 6 months of medical and pharmacy coverage.[18 19] We allowed for a grace period of 90 days for non-overlapping prescriptions to define periods of continuous treatment.

Each person could contribute more than one treatment episode. We defined biologic-naïve as no claims of PsO-related or PsA-related biologics prior to the index date based on all available data. Biologic-experienced was defined as having at least one other biologic, but not the index biologic, before the index date of the current treatment episode.

Self-administered biologics were identified from pharmacy claims using National Drug Codes. Prescription fill date and days of supply were used to calculate duration of treatment. Biologics that require infusions under supervision of a physician were identified through Healthcare Common Procedure Coding System procedure codes from medical claims. As these medical claims lack information on days of supply, we assigned the duration of treatment based on a typical dosage regimen. For infliximab, days of continuous drug exposure were based on a loading schedule of 0, 2, 6, 14, and then every 8 weeks. For ustekinumab, first administration was assumed to be 4-week supply, and any subsequent refill was assumed to be 12-week supply.[20]

Outcome

Our primary outcome was serious infection, defined as hospitalisation with the listing of infection in the inpatient claims diagnosis codes, including the primary and all non-primary positions (list available from authors on request). Subjects were followed from biologic initiation until their first hospitalisation with serious infection, or were censored if they developed a competing comorbidity that would have been exclusionary at baseline (such as Crohn's disease), discontinued biologic therapy (defined as switching to another biologic, or a treatment gap of at least 90 days),[21] lost continuous enrolment, died or 31 December 2018, whichever came first.

Covariates

We measured covariates using a 6-month lookback period from the index date. Patient covariates included demographics (age, sex, calendar year) and socioeconomic characteristics (race, education, household income level). We conducted multiple imputation by simple random sampling for missing data including race, income level and education (less than 5%).[22] Other pharmacologic covariates included prior use of phototherapy, and non-biologic PsO and PsA drugs (online supplementary table S2). We also included Charlson Comorbidity Index scores using ICD-9 or ICD-10 codes from claims,[23] selected comorbidities and health services utilisation (number of prior hospitalisations, emergency room, outpatient and physician specialist visits). For the number of prior biologic agents, we used all available lookback data to ascertain prior drug experience.

Propensity scores

We used inverse probability weighting for the average treatment effect of the treated weighting,[24] with a propensity score to adjust for differences in baseline demographic and clinical characteristics between groups which may confound their drug treatment exposure. Propensity scores were calculated based on the probability of being exposed to either IL-17, IL-12/23 or TNF using multivariable logistic regression models.[18 25] Weights were trimmed at 0.1 and 10 to minimise the influence of outliers.[26] We assessed the balance of covariates after weighting by standardised mean differences (SMD). Propensity scores were recalculated for each of the stratified analyses.

Statistical analysis

We estimated the incidence rate (IR) of serious infections per 100 person-years for each drug class, with 95% confidence intervals (CIs) calculated using Poisson models. Kaplan-Meier curves were constructed to describe time from drug initiation to serious infection. We used weighted Cox proportional hazard

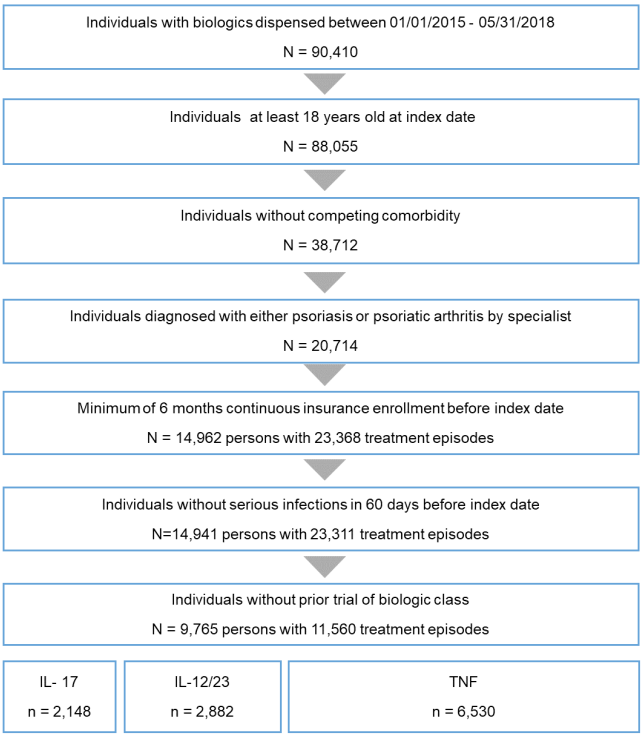

Figure 1 Patient selection process. IL,interleukin; TNF, tumour necrosis factor.

models to estimate hazard ratios (HRs) and corresponding 95% CI for risk of serious infections, adjusted for imbalanced covariates with SMD >10% after weighting.[27] We calculated corrected standard errors clustered on an individual level, to account for a patient contributing more than one treatment episode. The proportional hazards assumption was verified by Schoenfeld residuals and complementary log-log plots. For each analysis, we compared IL-17, IL-12/23 and TNF inhibitor groups to each other (ie, three pairwise comparisons).

We performed subgroup analyses stratifying by PsO or PsA, as well as by history with other biologics (naïve vs experienced). Diagnosis groups were not exclusive, as patients with PsO can also have PsA, and vice versa; stratified regression models were adjusted for the presence of the alternative disease type.

We conducted two sensitivity analyses. First, we restricted our outcome definition to the primary diagnosis code on inpatient admission diagnoses (rather than any position). We also narrowed the length of the grace period to 60 days to produce reduced estimates of treatment duration. All analyses were done in R V.3.5.3.[28]

RESULTS

We identified a total of 11 560 treatment episodes from 9305 adults: 19% IL-17, 25% IL-12/23% and 56% in the TNF groups, respectively (figure 1). Overall, the population mean age was 46 years and 53% male (table 1).

Among the treatment episodes, 6044 (52%) were for persons with a recorded diagnosis of PsO only, 1872 (16%) with PsA only, and 3648 (32%) both. Using all available data, the proportion of patients with any historical exposure to previous biologics differed greatly by cohort (85% among IL-17, 53% IL-12/23% and 34% in TNF inhibitor groups, respectively), and may reflect utilisation management strategies by pharmacy benefit managers. Conventional synthetic disease-modifying antirheumatic drugs (DMARDs) were most commonly used in the TNF inhibitors group, and 35% were dispensed oral steroids during the 6-month lookback period. Additional cohort characteristics are presented in online supplementary table S3, as well as stratified for the PsO (online supplementary table S4) and PsA (online supplementary table S5) subgroups. Compared with the PsO cohort, the PsA cohort had a higher mean Charlson score, and a larger proportion of patients using other medications (disease-modifying antirheumatic drugs, steroids and non-steroidal anti-inflammatory drugs) during the lookback period. A larger proportion of biologic-naïve, compared with biologic experienced persons, used methotrexate. Steroids treatment was similar between the two subgroups.

Incidence rate of serious infections

Overall, 190 serious infections (2% of treatment episodes) occurred after initiation of study biologics. The most commonly diagnosed serious infections on hospitalisation were sepsis and pneumonia (table 2).

Class-specific IRs were similar among IL-17 and TNF, and significantly lower for IL-12/23 (table 3).

While statistically significant, the absolute burden of serious infection was one or two serious infections per 100 person-years. There were no significant differences in incidence after stratification for disease type (PsO vs PsA).

Cumulative incidence of serious infections

Kaplan-Meier curves were constructed to show the time from biologic drug initiation to serious infection, by drug class (figure 2). The total follow-up time was 9264 person-years, with median follow-up time of 0.6 years (IQR 0.2–1.1 years) per treatment episode. The cumulative incidence of infection was lowest for IL-12/23 over the entire follow-up period, followed by IL-17 then TNF inhibitors.

Adjusted risk of serious infections

After propensity score weighting and adjustment for imbalanced baseline covariates, there was no increased risk of serious infections with IL-17 compared with either IL-12/23 (HR=1.12, 95% CI 0.62 to 2.03) or to TNF (HR=0.89, 95% CI 0.48 to 1.66) (table 4).

However, we observed a 41% lower risk of serious infection in the IL-12/23 inhibitor users as compared with TNF (HR=0.59, 95% CI 0.39 to 0.90). This finding remained significant in subgroup analyses of both the PsO cohort and the biologic-naïve cohort. Additionally, within the biologic-naïve subgroup, there was a significantly increased risk of infections with IL-17 versus IL-12/23 (HR=3.34, 95% CI 1.10 to 10.12). Similar results were found in sensitivity analyses restricting to the primary admission diagnosis (online supplementary table S6), and narrowing the permissible treatment gap to 60 days (online supplementary table S7). The common imbalanced variables after weighting were cohort entry year and baseline DMARD use.

DISCUSSION

Risk of serious infections were similar among new users of IL-17 and TNF inhibitors, while persons treated with an IL-12/23 were less likely to be hospitalised with a serious infection than TNF. Risks of serious infections were not significantly different between biologics among biologic-experienced patients.

Our findings are important as IL inhibitors are relatively new products, and the comparative safety of each of the IL-17, IL-12/23 and TNF inhibitors has not yet been closely examined

Table 1 Characteristics of PsO or PsA patients at the time of index date, overall and by drug class

	All (n=11 560)	IL-17 (n=2148)	IL-12/23 (n=2882)	TNF (n=6530)
Age	46 (12)	48 (11)	46 (12)	46 (12)
Male	6107 (53%)	1141 (53%)	1569 (54%)	3397 (52%)
Household income				
<$40 000	1372 (12%)	277 (13%)	333 (12%)	762 (12%)
$40 000–$74 999	2818 (24%)	477 (22%)	678 (24%)	1663 (26%)
$75 000–$124 999	3694 (32%)	682 (32%)	922 (32%)	2090 (32%)
$125 000–$199 999	2137 (19%)	415 (19%)	529 (18%)	1193 (18%)
>$200 000	1539 (13%)	297 (14%)	420 (15%)	822 (13%)
Diagnosis				
PsO only	6043 (52%)	1204 (56%)	1994 (69%)	2846 (44%)
PsA only	1869 (16%)	245 (11%)	239 (8%)	1388 (21%)
PsO and PsA	3648 (32%)	699 (33%)	650 (23%)	2299 (35%)
Charlson Comorbidity Index score				
0	8248 (71%)	1429 (67%)	2158 (75%)	4661 (71%)
1	2192 (19%)	477 (22%)	468 (16%)	1247 (19%)
2 or more	1120 (10%)	242 (11%)	256 (9%)	622 (10%)
Number of previous biologics				
0	5995 (52%)	332 (16%)	1344 (47%)	4319 (66%)
1	3614 (31%)	817 (38%)	1121 (39%)	1676 (26%)
2 or more	1951 (17%)	999 (46%)	417 (14%)	535 (8%)
DMARDs in past 6 months	3115 (27%)	519 (24%)	619 (22%)	1977 (30%)
Methotrexate	2032 (18%)	251 (12%)	345 (12%)	1436 (22%)
Sulfasalazine	249 (2%)	22 (1%)	28 (1%)	199 (3%)
Apremilast	796 (7%)	228 (11%)	240 (8%)	328 (5%)
Other	369 (3%)	77 (4%)	81 (3%)	211 (3%)
Oral steroids in past 6 months, %	3989 (35%)	732 (34%)	891 (31%)	2366 (36%)
NSAIDs prescribed in past 6 months	1594 (14%)	278 (13%)	262 (9%)	1054 (16%)
Phototherapy in past 6 months	261 (2%)	39 (2%)	93 (3%)	129 (2%)

Continuous variables are presented as mean (SD), and categorical variables are presented as counts (percentages).
DMARD, disease-modifying antirheumatic drugs; IL, interleukin; NSAID, non-steroidal anti-inflammatory drug; PsA, psoriatic arthritis; PsO, psoriasis.

in a real-world cohort. Prior investigations of the safety of IL-17 inhibitors have generally been placebo-controlled or have not reported the risks for serious infection separately from overall adverse events.[3 29–31] Our results build on the recent work of Kalb *et al*[8] and Dommasch *et al*,[14] which found reduced risk of infection with ustekinumab compared with non-biologic treatments. However, in contrast to that reports, we used more recent data, included two IL-17 inhibitors, and focused on TNF products, rather than methotrexate, as our referent group. Our findings also extend a recent report comparing the rate of serious infections of IL-17 to IL-12/23 and some, but not all, TNF inhibitors.[32]

Interestingly, we found that IL-12/23 inhibitors were associated with a significant reduction in risk of serious infection compared with TNF inhibitors, which held in subgroup analyses of PsO but not PsA and in biologic-naïve but not biologic-experienced patients. While we cannot exclude the possibility of residual confounding or the play of chance, PsA patients appear to have been at higher risk for infection due to other factors (older, female, more comorbidities and physician office visits, greater utilisation of DMARDs and glucocorticoids). Similarly, biologic-experienced patients are at higher risk compared with biologic naïve patients. For example, the rate in biologic naïve ustekinumab-treated patients (0.9/100py) was approximately half the rate in the biologic-experienced ustekinumab-treated patients (1.7/100py). Thus, in higher risk patients who have multiple infection-related risk factors, it may be somewhat more difficult to detect the specific contribution of biologic exposure compared with other infection-related risk factors. Additionally, the proportion of biologic-naïve episodes was larger in the PsO cohort. Nevertheless, we note that the effect estimate even in the PsA patients for IL-12/23 exposure was numerically lower (HR=0.74, 95% CI 0.40 to 1.36) and compatible with the protective association observed in the PsO patients (HR=0.59, 95% CI 0.38 to 0.92).

Among the biologic-naïve cohort, we observed a lower risk of serious infections in IL-12/23 than both IL-17 and TNF. However, given the small sample size of biologic-naïve persons using IL-17 (8 infections, 332 treatment episodes), further studies are needed to confirm these results.

Table 2 Frequency counts of ICD codes for serious infection

Code	Description	Frequency
A419	Sepsis unspecified organism	38
J189	Pneumonia unspecified organism	30
N390	UTI site not specified	20
L03116	Cellulitis of left lower limb	13
L0390	Cellulitis unspecified	12
	Other types of serious infection*	77

*Clinical description of cells with 10 or fewer observations suppressed per data license.

Table 3 Incidence of serious infections among biologic users with PsO or PsA, overall and by drug class

	All biologic classes	IL-17	IL-12/23	TNF
Total cohort				
Number of treatment episodes	11 560	2148	2882	6530
Total person-years of follow-up	9264	1528	2461	5275
Incident serious infections, n (%)	190 (2)	32 (1)	32 (1)	126 (2)
Incidence rate (95% CI), per 100 person-years	2.1 (1.8 to 2.4)	2.1 (1.5 to 2.9)	1.3 (0.9 to 1.8)	2.4 (2.0 to 2.8)
PsO				
Number of treatment episodes	9691	1903	2644	5144
Total person-years of follow-up	8010	1406	2311	4293
Incident serious infections, n (%)	156 (2)	26 (1)	29 (1)	101 (2)
Incidence rate (95% CI), per 100 person-years	2.0 (1.7 to 2.3)	1.9 (1.2 to2.7)	1.3 (0.9 to 1.8)	2.4 (1.9 to 2.8)
PsA				
Number of treatment episodes	5517	944	888	3685
Total person-years of follow-up	4159	605	647	2907
Incident serious infections, n (%)	105 (2)	14 (1)	13 (1)	78 (2)
Incidence rate (95% CI), per 100 person-years	2.5 (2.1 to 3.1)	2.3 (1.3 to 3.7)	2.0 (1.1 to 3.3)	2.7 (2.1 to 3.3)
Biologic-naïve				
Number of treatment episodes	5995	332	1344	4319
Total person-years of follow-up	5019	237	1217	3565
Incident serious infections, n (%)	*	*	11 (1)	80 (2)
Incidence rate (95% CI), per 100 person-years	2.0 (1.6 to 2.4)	3.4 (1.6 to 6.3)	0.9 (0.5 to 1.6)	2.2 (1.8 to 2.8)
Biologic experienced				
Number of treatment episodes	5565	1816	1538	2211
Total person-years of follow-up	4.246	1292	1244	1710
Incident serious infections, n (%)	91 (2)	24 (1)	21 (1)	46 (2)
Incidence rate (95% CI), per 100 person-years	2.1 (1.7 to 2.6)	1.9 (1.2 to 2.7)	1.7 (1.1 to 2.5)	2.7 (2.0 to 3.5)

*Clinical description of cells with 10 or fewer observations suppressed per data license.
IL, interleukin; TNF, tumour necrosis factor.

Our analysis has limitations. First, the ICD codes used to define serious infections have not been fully validated in PsO and PsA patients. However, the ICD codes were derived from a combination of validation studies of patients with inflammatory arthritis (e.g., rheumatoid arthritis) and clinical expertise. Moreover, our absolute infection rates of approximately 1–2

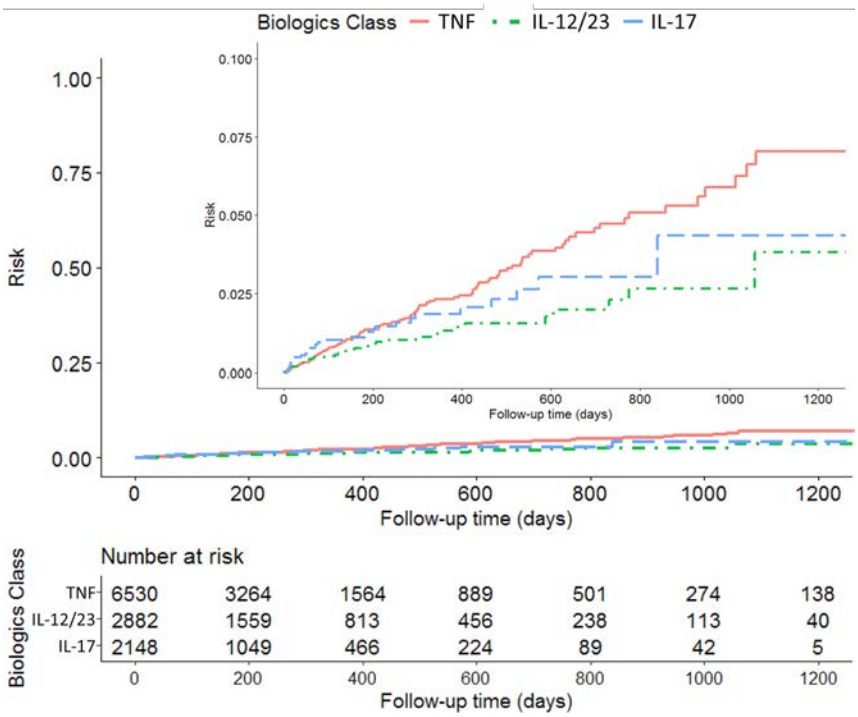

Figure 2 Kaplan-Meier curve showing the cumulative incidence of serious infection over time, by biologic class. IL,interleukin; TNF, tumour necrosis factor.

Table 4 HRs (with 95% CIs) of risk of first serious infection among persons with psoriasis or psoriatic arthritis, by drug class

	Unadjusted	Adjusted for propensity score and imbalanced covariates
Total cohort		
IL-17 vs TNF	0.86 (0.58 to 1.27)	0.89 (0.48 to 1.66)
IL-12/23 vs TNF	0.55 (0.37 to 0.80)	0.59 (0.39 to 0.90)
IL-17 vs IL-12/23	1.53 (0.94 to 2.51)	1.12 (0.62 to 2.03)
Psoriasis		
IL-17 vs TNF	0.76 (0.50 to 1.18)	0.73 (0.36 to 1.45)
IL-12/23 vs TNF	0.53 (0.35 to 0.81)	0.59 (0.38 to 0.92)
IL-17 vs IL-12/23	1.42 (0.83 to 2.41)	1.01 (0.53 to 1.92)
Psoriatic arthritis		
IL-17 vs TNF	0.83 (0.47 to 1.47)	0.67 (0.25 to 1.73)
IL-12/23 vs TNF	0.74 (0.41 to 1.34)	0.74 (0.40 to 1.36)
IL-17 vs IL-12/23	1.10 (0.52 to 2.35)	1.23 (0.50 to 3.01)
Biologic-naïve		
IL-17 vs TNF	1.45 (0.70 to 3.00)	2.02 (0.94 to 4.33)
IL-12/23 vs TNF	0.41 (0.22 to 0.76)	0.46 (0.23 to 0.89)
IL-17 vs IL-12/23	3.63 (1.44 to 9.12)	3.34 (1.10 to 10.12)
Biologic experienced		
IL-17 vs TNF	0.68 (0.42 to 1.12)	0.72 (0.40 to 1.32)
IL-12/23 vs TNF	0.62 (0.37 to 1.04)	0.72 (0.42 to 1.26)
IL-17 vs IL-12/23	1.05 (0.59 to 1.90)	0.92 (0.49 to 1.74)

IL, interleukin; PsA, psoriatic arthritis; PsO, psoriasis; TNF, tumour necrosis factor.

per 100 patient-years are consistent with PsO and PsA trials and registries.[3 9] Second, our study had a relatively short duration of follow-up, with a median of 6 months, and thus comparative risks between drugs should be interpreted accordingly. However, most evidence suggests that the risks of serious infections are greatest during the first months of treatment.[33] Third, our data are limited to persons with commercial insurance and may not represent the Medicare, Medicaid and uninsured populations or experiences outside of the US healthcare system. Finally, as with any observational analysis, we acknowledge the potential for unmeasured or residual confounding related to confounders that were not available in these data.

While many factors inform the choice among TNF, IL-17 and IL-12/23 inhibitors, our results suggest that the risks of serious infections associated with specific biologics may differ between PsO and PsA patients, and between biologic-naïve and biologic-experienced patients. Given the relatively small magnitude of absolute effect (difference less than 1 per 100 person-years) yet strong relative reduction in risk, this potentially clinically relevant signal for reduced infections among the IL-12/23 inhibitors warrants further investigation and surveillance efforts.

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Competing interests GCA is past Chair of FDA's Peripheral and Central Nervous System Advisory Committee, has served as a paid advisor to IQVIA, and is a consultant and holds equity in Monument Analytics, a healthcare consultancy whose clients include the life sciences industry as well as plaintiffs in opioid litigation; and is a member of OptumRx's National P&T Committee. This arrangement has been reviewed and approved by Johns Hopkins University in accordance with its conflict of interest policies.

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REFERENCES

1 Leonardi CL, Kimball AB, Papp KA, *et al*. Efficacy and safety of ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with psoriasis: 76-week results from a randomised, double-blind, placebo-controlled trial (Phoenix 1). *The Lancet* 2008;371:1665–74.
2 Papp KA, Tyring S, Lahfa M, *et al*. A global phase III randomized controlled trial of etanercept in psoriasis: safety, efficacy, and effect of dose reduction. *Br J Dermatol* 2005;152:1304–12.
3 Langley RG, Elewski BE, Lebwohl M, *et al*. Secukinumab in plaque psoriasis--results of two phase 3 trials. *N Engl J Med* 2014;371:326–38.
4 Griffiths CEM, Reich K, Lebwohl M, *et al*. Comparison of ixekizumab with etanercept or placebo in moderate-to-severe psoriasis (UNCOVER-2 and UNCOVER-3): results from two phase 3 randomised trials. *The Lancet* 2015;386:541–51.
5 Blauvelt A, Reich K, Tsai T-F, *et al*. Secukinumab is superior to ustekinumab in clearing skin of subjects with moderate-to-severe plaque psoriasis up to 1 year: Results from the CLEAR study. *J Am Acad Dermatol* 2017;76:60–9.
6 Griffiths CEM, Strober BE, van de Kerkhof P, *et al*. Comparison of ustekinumab and etanercept for moderate-to-severe psoriasis. *N Engl J Med* 2010;362:118–28.
7 Yiu ZZN, Mason KJ, Barker JNWN, *et al*. A standardization approach to compare treatment safety and effectiveness outcomes between clinical trials and real-world populations in psoriasis 2019.
8 Kalb RE, Fiorentino DF, Lebwohl MG, *et al*. Risk of serious infection with biologic and systemic treatment of psoriasis. *JAMA Dermatol* 2015;151.
9 Dobry AS, Quesenberry CP, Ray GT, *et al*. Serious infections among a large cohort of subjects with systemically treated psoriasis. *J Am Acad Dermatol* 2017;77:838–44.
10 Garcia-Doval I, Cohen AD, Cazzaniga S, *et al*. Risk of serious infections, cutaneous bacterial infections, and granulomatous infections in patients with psoriasis treated with anti-tumor necrosis factor agents versus classic therapies: prospective meta-analysis of Psonet registries. *J Am Acad Dermatol* 2017;76:299–308.
11 Yiu ZZN, Smith CH, Ashcroft DM, *et al*. Risk of Serious Infection in Patients with Psoriasis Receiving Biologic Therapies: A Prospective Cohort Study from the British Association of Dermatologists Biologic Interventions Register (BADBIR). *J Invest Dermatol* 2018;138:534–41.
12 Reich K, Mrowietz U, Radtke MA, *et al*. Drug safety of systemic treatments for psoriasis: results from the German psoriasis registry PsoBest. *Arch Dermatol Res* 2015;307:875–83.
13 Carneiro C, Bloom R, Ibler E, *et al*. Rate of serious infection in patients who are prescribed systemic biologic or nonbiologic agents for psoriasis: a large, single center, retrospective, observational cohort study. *Dermatol Ther* 2017;30:e12529.
14 Dommasch ED, Kim SC, Lee MP, *et al*. Risk of serious infection in patients receiving systemic medications for the treatment of psoriasis. *JAMA Dermatology* 2019.
15 Wallace PJ, Shah ND, Dennen T, *et al*. Optum Labs: building a novel node in the learning health care system. *Health Aff* 2014;33:1187–94.
16 Asgari MM, Wu JJ, Gelfand JM, *et al*. Validity of diagnostic codes and prevalence of psoriasis and psoriatic arthritis in a managed care population, 1996-2009. *Pharmacoepidemiol Drug Saf* 2013;22:842–9.
17 Ray WA. Evaluating medication effects outside of clinical trials: new-user designs. *Am J Epidemiol* 2003;158:915–20.
18 Herrinton LJ, Curtis JR, Chen L, *et al*. Study design for a comprehensive assessment of biologic safety using multiple healthcare data systems. *Pharmacoepidemiol Drug Saf* 2011;20:1199–209.

19 Yun H, Xie F, Delzell E, *et al*. Comparative risk of hospitalized infection associated with biologic agents in rheumatoid arthritis patients enrolled in Medicare. *Arthritis Rheumatol* 2016;68:56–66.
20 Doshi JA, Takeshita J, Pinto L, *et al*. Biologic therapy adherence, discontinuation, switching, and restarting among patients with psoriasis in the US Medicare population. *J Am Acad Dermatol* 2016;74:1057–65.
21 Grijalva CG, Chen L, Delzell E, *et al*. Initiation of tumor necrosis factor-α antagonists and the risk of hospitalization for infection in patients with autoimmune diseases. *JAMA* 2011;306.
22 Buuren Svan, Groothuis-Oudshoorn K. mice : Multivariate Imputation by Chained Equations in *R*. *J Stat Softw* 2011;45:1–67.
23 Deyo RA, Cherkin DC, Ciol MA. Adapting a clinical comorbidity index for use with ICD-9-CM administrative databases. *J Clin Epidemiol* 1992;45:613–9.
24 Austin PC. An introduction to propensity score methods for reducing the effects of confounding in observational studies. *Multivariate Behav Res* 2011;46:399–424.
25 Walsh JA, Adejoro O, Chastek B, *et al*. Treatment patterns among patients with psoriatic arthritis treated with a biologic in the United States: descriptive analyses from an administrative claims database. *J Manag Care Spec Pharm* 2018:1–11.
26 Harder VS, Stuart EA, Anthony JC. Propensity score techniques and the assessment of measured covariate balance to test causal associations in psychological research. *Psychol Methods* 2010;15:234–49.
27 Nguyen T-L, Collins GS, Spence J, *et al*. Double-adjustment in propensity score matching analysis: choosing a threshold for considering residual imbalance. *BMC Med Res Methodol* 2017;17.
28 R Core Team. R: a language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria, 2018. Available: https://www.R-project.org/
29 Yiu ZZN, Exton LS, Jabbar-Lopez Z, *et al*. Risk of serious infections in patients with psoriasis on biologic therapies: a systematic review and meta-analysis. *J Invest Dermatol* 2016;136:1584–91.
30 Thaçi D, Blauvelt A, Reich K, *et al*. Secukinumab is superior to ustekinumab in clearing skin of subjects with moderate to severe plaque psoriasis: clear, a randomized controlled trial. *J Am Acad Dermatol* 2015;73:400–9.
31 Egeberg A, Ottosen MB, Gniadecki R, *et al*. Safety, efficacy and drug survival of biologics and biosimilars for moderate-to-severe plaque psoriasis. *Br J Dermatol* 2018;178:509–19.
32 Ibler E, Rangel SM, Huynh TN, *et al*. The rate of infection in patients with psoriasis who are exposed to secukinumab compared to the rate of infection for other biologic agents: a retrospective cohort analysis from a large U.S. patient population. *J Amer Acad Derm* 2017;76.
33 Singh JA. Infections with biologics in rheumatoid arthritis and related conditions: a scoping review of serious or hospitalized infections in observational studies. *Curr Rheumatol Rep* 2016;18.

EPIDEMIOLOGICAL SCIENCE

Tuberculosis in biologic users for rheumatic diseases: results from the South African Biologics Registry (SABIO)

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ABSTRACT

Objectives To evaluate the rate of tuberculosis (TB) in biologic users for rheumatic diseases in South Africa, the effectiveness of our latent TB infection (LTBI) programme, risk factors and outcome.

Methods TB cases were collected from the South African Biologics Registry (SABIO), rheumatologists and pharmaceutical companies. Demographics, LTBI screening and treatment, biological and disease modifying antirheumatic drug (DMARD) therapies, TB diagnosis and outcomes were recorded.

Results 96 TB cases were collected from 1999 to June 2017: rheumatoid arthritis 55, ankylosing spondylitis 27, psoriatic arthritis 4, and juvenile inflammatory arthritis 10. The TB rate was 1240/100 000 person years for biologic users (n=96) versus the biologic naive cohort of 0/100 000 years with an incidence rate difference of 0.0124 (p<0.0001). 60/96 had pulmonary and 36/96 had extra-pulmonary TB. Reactivation TB occurred in 45/96 cases. TB occurred in all biologics licenced in South Africa, the majority in monoclonal inhibitors (1683/100 000 person years) compared with etanercept (861/100 000 person years) and non-tumour necrosis factor (TNF) inhibitors (681/100 000 person years). The incidence rate ratio for monoclonal inhibitors compared with etanercept was 1.96 (p=0.005) and 2.47 (p=0.002) compared with non-TNF inhibitors with no significant difference between non-TNF inhibitors and etanercept (p=0.336). From those (12.9%) who screened LTBI positive, 14 developed TB, while the majority (77) screened LTBI negative. Black race, male sex, younger age and residence in the Western Cape were statistical risk factors. Two drug resistant TB cases and six deaths occurred.

Conclusion Reactivation and new onset TB is a significant risk for all biologics users in SA. Screening for LTBI is an imperative preventative strategy.

Key messages

What is already known about this subject?
- There is an increased risk of tuberculosis (TB) in patients using TNF inhibitors.
- Monoclonal TNF inhibitors are consistently reported to have higher TB infection rates than etanercept, a soluble TNF receptor.
- Screening and treatment of latent TB infection (LTBI) reduces the risk.

What does this study add?
- All biologics increase the risk of TB, especially monoclonal inhibitors, but importantly also non-TNF inhibitors with a risk not statistically different to etanercept.
- In South Africa, the TB incidence rate among biologic users is around ten times higher than in European countries.
- Black race, male sex, younger age and residence in the Western Cape were statistical risk factors for TB.
- Reactivation TB occurred in around 50% of cases.

How might this impact on clinical practice or future developments?
- Risk stratification, screening and treatment for LTBI are important mitigating strategies in preventing TB infection.

BACKGROUND

TB is a leading cause of morbidity and mortality in the world, accounting for about 10.4 million new cases and 1.4 million deaths annually.[1] More than two thirds of the global TB burden is reported in Africa and Asia, and six countries account for 60% of new cases: India, Indonesia, China, Nigeria, Pakistan and South Africa. Latent TB infection (LTBI) is defined as a state of persistent immune response to Mycobacterium tuberculosis antigens detected either by the tuberculin skin test (TST) or by interferon gamma release assay (IGRA) without evidence of clinical tuberculosis.[2] LTBI subjects have an increased risk of progression to TB, augmented by immune impairment such as HIV coinfection, therapies with TNF inhibitors and other immune regulators used for inflammatory diseases, transplantation and diseases such as type 2 diabetes.[3–10] The use of biologics in our country is an obvious concern.

The incidence rate of TB in anti-TNF users has been reported from registries in Europe and strategies for prevention of reactivation TB have been proposed.[11] Monoclonal TNF inhibitors are consistently reported to have higher TB infection rates than etanercept, a soluble TNF receptor; however, non-TNF inhibitors have not been evaluated.[11–15] Screening for LTBI was not mandatory in some countries and rheumatic disease other than rheumatoid arthritis (RA) were not always considered.[12 13] The South African Biologics Registry (SABIO) was started in 2008 and upgraded in 2013, with special

emphasis on evaluating screening for LTBI, patient demographics and drug-related risk factors for acquiring TB.[16 17]

Most patients accessing biological treatment in SA are from the private sector funded by medical aids, with the minority from state institutions, clinical trials or self-funding. Affordability of biologics has resulted in restricted access in the public health sector and even in the private sector to more expensive medical aid plans.

Application for funding requires registration with SABIO with informed consent and approval by a peer-reviewed biologics panel, consisting of experienced rheumatologists. Screening for LTBI is strongly advocated by the South African Rheumatism and Arthritis Association (SARAA). The primary aim of this study was to evaluate the TB rate of biologic users for rheumatic diseases in South Africa, and secondary aims to evaluate our LTBI programme, risk factors for TB (age, race, sex, rheumatic disease, geographic location, comorbidities and concomitant medications) and outcome.

METHODS

SABIO, a national prospective observational registry, was established in 2008, recruiting all patients starting their first ever biologic.[18 19] The initial purpose of the registry was to monitor biologic use and collect TB data including LTBI screening and treatment in RA, ankylosing spondylitis (AS), psoriatic arthritis (PsA) and juvenile inflammatory arthritis (JIA). Eligibility was based on SARAA criteria for the use of biologics. Those commenced on a biologic prior to 2008 including all TB cases were also encouraged to be registered. In January 2013, the registry was upgraded to optimise data quality and included six monthly patient telephonic calls to document the biologic start date and serious adverse events in RA, AS and PsA. In addition a biologic naive cohort was started in January 2013 consisting of patients fulfilling criteria for recruitment onto the registry but declining biologic treatment for reasons including funding, side effect concerns, pregnancy and logistical reasons. A total of 4982 patients from 2008 to July 2017 were included in the analysis to assess the TB incidence rate, LTBI screening and treatment. A subset of 1587 patients derived from the upgraded registry was used to determine baseline characteristics and TB risk factors in biologic exposed cases. In this subset we excluded JIA. Confirmation and evaluation of TB diagnosis, time lines between the start of a biologic and acquiring TB, treatment, outcomes and subsequent biologic use were recorded. Diagnosis was based on microbiological (culture, Gen Probe PCR or smear acid fast bacilli (AFB)), histological or radiographic confirmation or on clinical suspicion (constitutional symptoms of fever, weight loss, night sweats, elevated erythrocyte sedimentation rate, suspicious X-ray findings and response to TB therapy). Treatment of active TB was as per standard protocols with isoniazid (INH), rifampicin, ethionamide and pyrazinamide for 2 months and INH with rifampicin for the remaining four. TB is a notifiable disease in SA.

Reactivation TB was defined as TB diagnosed within 18 months of starting a biologic and new infections as TB cases after 18 months. Screening and treatment for LTBI was based on SARAA recommendations and included a chest X-ray (CXR) and TST or IGRA. TST of ≥5 mm induration, positive IGRA, granulomas on CXR or radiographic presence of TB was considered a positive test. Retesting was not undertaken in LTBI negative screens or contact with active TB. LTBI treatment with INH monotherapy for 9 months or combined with rifampicin for 3 months, starting at least 1 month before biologic initiation is recommended if any of the screening tests are positive or if the patient has a high TB exposure risk. LTBI treatment failure was defined as developing reactivation TB despite INH/rifampicin therapy and LTBI screen failure as those who screened negative for LTBI but developed reactivation TB.

There was no patient and public involvement in the design, conduct, publishing or dissemination of this research.

Baseline

All patients provided written consent to be entered onto the registry and to have their data analysed for study purposes. A standardised baseline form was completed by the rheumatologist. Data included demographics, rheumatic disease, rheumatoid factor, anti-cyclic citrullinated peptide, simple disease activity index, human leukocyte antigen B27 (HLAB27), comorbidities, LTBI screening tests and treatment, concomitant DMARDs and corticosteroid.

Follow-up

A standardised follow-up questionnaire on disease activity, adverse events and treatment changes was completed 6 monthly by the rheumatologist. Both biologic exposed and naive cohorts had telephonic follow-up 6 monthly from 2013, specifically addressing adverse events and treatment changes. Patient reported side effects including TB were only included if verified by the rheumatologist. In addition TB cases were also cross-referenced with pharmaceutical companies.

Statistical methodology

The number of patient years was calculated per biologic. The total sum of exposure was calculated from the first to the last day of biologic use which was assumed to be 1 June 2017. The total number of years that a patient used a biologic was referred to as the exposure, in patient years, to that drug. If a patient switched biologics, the actual time each biologic was used was attributed to that specific biologic.

On starting a biologic (biologic naive), TB was attributed to the drug if diagnosed any time from start to 6 months after stopping. The same rule applied to subsequent biologics used (except if starting within 6 months of the preceding biologic, in which case the former biologic would be deemed causative).

The TB incidence rate was calculated by dividing the number of TB cases per biologic, by the sum of exposure, in years, for each biologic. To get the rate per 100 000 person years, the incidence rate was multiplied by 100 000. The incidence rate ratio (IRR) was calculated by dividing the relevant incidence rates by each other. The Fisher exact test was used to calculate the corresponding 95% CI and p-values. Significance was tested at 0.05.

A general linear model, with binomial distribution and log-odds (or logit) link function, was used to determine the risk factors associated with acquiring TB in biologic patients. Univariate and multivariate analysis was performed. Patients with disease category JIA were excluded from all univariate and multivariate analyses. During multivariate analysis, stepwise backward elimination was used to predict the final model using the Akaike information criterion to choose the best fitting model. Patients with missing or unknown data points were removed from the data set before performing multivariate analyses. Patients with race indicated as Asian were removed from multivariate analyses, as no patients in the TB group had race specified as Asian. Certain data sets from the new registry (starting 2013) were extrapolated to the upgraded registry (starting 2008) to obtain

Table 1 Diagnosis of TB cases (n=96)

Microbiologic (n=49)		Histological (n=17)		Radiographic (n=23)		Constitutional (n=6)
Sputum/washings		Lung	4	Miliary	7	Constitutional symptoms
Culture +ve	15	Pleura	4	Fibrocavitation	2	PPD +ve, elevated ESR
PCR +ve	7	Synovium	3	Pleural effusion		response to treatment
Smear+ve	18	Lymph node	3	ADA*+ve	2	
Lymph node	2	Bone	1	ADA*−ve	2	
Synovium	1	Bowel	1	Pleuro-pulmonary	8	
Pleura	2	Peritoneum	1	Pott's disease	2	
Peritoneum	1					
Liver	2					
CSF	1					Unknown (n=1)

*Adenosine deaminase.
CSF, cerebrospinal fluid; ESR, erythrocyte sedimentation rate; PPD, purified protein derivative; TB, tuberculosis.

total percentages. The p-value for the proportions switching biologics was calculated using a test of equal proportions.

RESULTS

A total of 4830 (7742 person years) were included in the biologic cohort and 152 (463 person years) in the biologic naïve cohort. There were 95 patients in the TB cohort (two separate events in one patient) with a total of 96 events: RA 55 (57%), AS 27 (28%), PsA 4 (4%) and JIA 10 (10%). Reactivation TB occurred in 45/96 (47%) cases, new onset TB in 50/96 (52%) cases and 1 was undetermined due to insufficient data. Pulmonary TB occurred in 60/96 (62.5%) cases while 36/96 (37.5%) had extra-pulmonary, including disseminated (7/96) disease. The diagnosis was proven microbiologically in 49, histologically in 17, radiographically in 23 and clinically in 6, while the exact details in one could not be verified (table 1). The rate of TB was 1240/100 000 person years for all biologic users combined, compared with the biologic naive arm of 0/100 000 years (n=0) with an incidence rate difference of 0.0124 (95% CI 0.007 to 0.018, p<0.0001). TB occurred in all seven biologics: adalimumab 48, infliximab 15, golimumab 3, etanercept 19, tocilizumab 2, abatacept 5 and rituximab 4. The incidence rate was highest for monoclonal TNF inhibitors (1683/100 000 person years) compared with etanercept (861/100 000 years) and non-TNF inhibitors (681/100 000 years). The IRR for monoclonal inhibitors compared with etanercept was 1.96 (95% CI 1.16 to 3.45, p=0.005) and 2.47 (95% CI 1.29 to 5.19, p=0.002) compared with non-TNF inhibitors. There was no significant difference between non-TNF inhibitors and etanercept (IRR 0.79; 95% CI 0.34 to 1.75, p=0.336). TB rates of individual biological agents are represented in table 2. The incidence of TB cases peaked in 2011/2012 and subsequently declined over the ensuing years (figure 1). More cases of reactivation TB occurred with monoclonal anti-TNF agents compared with other biologics whereas new TB cases are associated with all biologic DMARD's (online supplementary figure 1).

Table 2 TB rates of individual biologic agents (SABIO)*

Biologic	Sum of exposure (years)	TB cases/drug (n=96)	TB rate/100 000 person years
Adalimumab	2954	48	1625
Infliximab	694	15	2160
Golimumab	273	3	1099
Etanercept	2207	19	861
Abatacept	546	5	916
Rituximab	803	4	498
Tocilizumab	265	2	754

*South African Biologics Registry.
TB, tuberculosis.

Outcome

From the 96 TB events, 86 recovered fully including two with drug resistant TB. Two recovered with sequelae (deafness, pulmonary aspergillosis), while six died. There was insufficient data to determine outcome in 2. After TB treatment, 17 patients continued on their original biologic, 46 switched and 25 stopped completely.

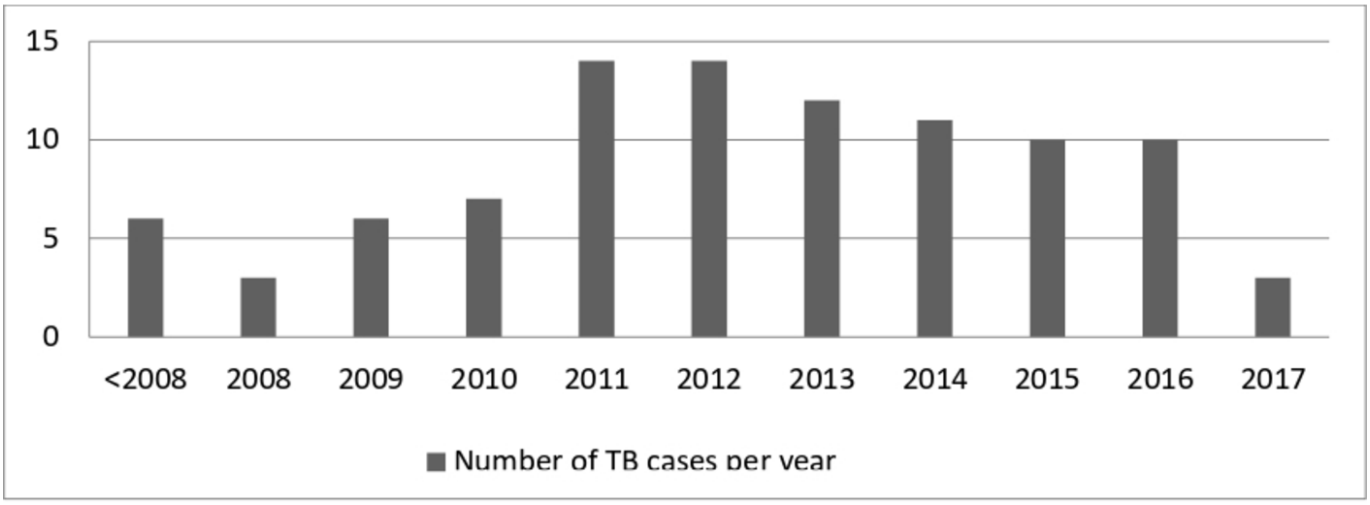
LTBI screening results

LTBI screening was undertaken in 98.3% of cases with CXR combined with TST (n=3719; 77%) and/or IGRA (n=1526; 32%). Adherence to SARAA treatment recommendations was 71%. TST was positive in 7% (n=260) and IGRA in 25% (n=387). In total 647 (12.9%) from 4830 screened LTBI positive. From 14 that developed TB infection despite screening LTBI positive, 13 took INH/rifampicin treatment; however, 4 failed to complete the course. Of the 13 patients, 9 developed reactivation TB (treatment failure) and 4 new onset TB. Seventy seven TB cases screened negative, of which 33 developed reactivation TB (screen failures). Empiric INH was prescribed for 7 of the 77 due to high background risk; yet three still developed reactivation TB (treatment failure). LTBI screening was not done in 5/96 cases. LTBI treatment failure therefore occurred in 12 cases (figure 2). The overall TB risk for all biologic users with a rheumatic disease was 2%. The risk of developing reactivation TB was 1.4% if LTBI screened positive (despite treatment) and 0.7% if screened negative. The negative predictive value for both TST and IGRA was 99%, and the positive predictive value, although numerically superior for TST, was not statistically different. LTBI screening with CXR was unhelpful with 84 X-rays reported as normal, 6 showing chronic non-specific changes and 6 reports not available. No granulomas signifying LTBI were reported.

Tb risk factors

Table 3 shows baseline characteristics of the TB cohort, biologic exposed and biologic naïve cohorts, used to evaluate TB risk factors. Table 4 compares baseline characteristics of monoclonal TNF inhibitors, soluble receptor and non-TNF inhibitors.

Both univariate and multivariate analysis of risk factors for TB infection showed black race (p=0.029, OR 2.13), younger



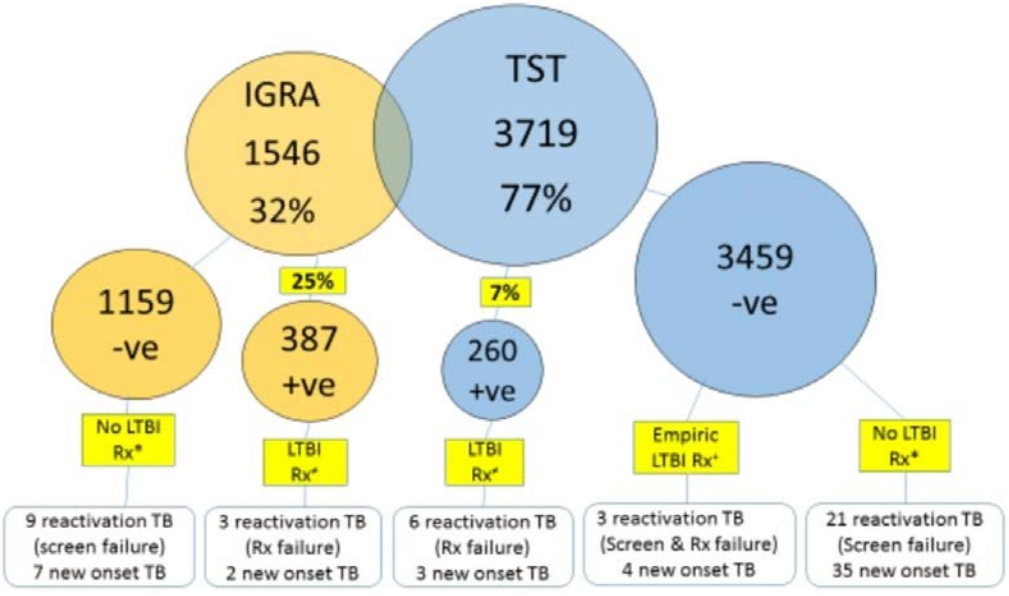
*South African Biologics Registry

Figure 1 Annual incidence of TB (SABIO)*.

age (p<0.001) and male sex (p=0.03, OR 1.93) to be statistically significant. Multivariate analysis showed residence in the Western Cape to have an increased risk (p=0.045, OR 3.05) compared with other provinces. Underlying rheumatic disease and comorbidities showed no statistical difference (table 5 and online supplementary table 1). Methotrexate use at the time of TB diagnosis (76%) was statistically less (p<0.001) than at baseline (90%) and approximates real life registry data from biologic registries and US claims databases, indicating that 30% of patients take biologics as monotherapy.[20–28] Similarly steroid use at time of TB diagnosis (53%, median 9.45) was numerically less than at baseline (60%, median 9.6) and equates well with the Australian Rheumatology Association Database which reports steroid attrition from 55% to 39% over a 10-year period (p<0.001).[29] The association with methotrexate and steroid use and risk of acquiring TB is therefore unlikely. Use of more than one biologic prior to TB diagnosis occurred in 29% (25/86) of TB cases compared with 26.5% in the SABIO registry with no statistical difference (p=0.597).

DISCUSSION

This study highlights differences to other registries and adds new insight from our active LTBI screening programme and large TB cohort. We showed that all biologics increase the risk of TB, especially monoclonal inhibitors, but importantly also



*Patients screened IGRA / TST negative and did not receive treatment for latent TB infection
‡Patients screened IGRA / TST positive and received treatment for latent TB infection
†Patients screened TST negative and received empiric treatment for latent TB infection
5 TB patients were not screened (3 reactivation TB, one new infection and one not determined). 2 patients (both new onset TB) screened both TST and IGRA negative

Figure 2 Relative tuberculin skin test and IGRA usage and outcome (n=4830).

Table 3 Baseline characteristics

Characteristics	TB cohort (n=86)	Biologic exposed (n=1587)	Biologic naïve (n=152)
Mean age (years)	45.2	52.0	50.7
% female	56	71	67
RA, n (%)	55 (64)	1085 (68)	103 (68)
AS, n (%)	27 (31)	379 (24)	37 (24)
Psoriatic arthritis, n (%)	4 (5)	123 (8)	13 (8)
Geographic area, n (%)			
Gauteng	38 (44)	935 (59)	74 (49)
Western Cape	31 (36)	364 (23)	38 (25)
Kwazulu Natal	12 (14)	192 (12)	32 (21)
Free State, E. Cape	5 (6)	96 (6)	8 (5)
Ethnicity, n (%)			
White	60 (70)	1069 (67)	97 (63)
Black	11 (13)	92 (6)	21 (14)
Coloured	5 (6)	102 (6)	5 (3)
Indian	10 (12)	135 (9)	17 (11)
Asian	0 (0)	73 (5)	13 (9)
Unknown	0 (0)	116 (7)	0 (0)
RF/ACPA in RA (%)	72	62	42
HLAB27 in AS (%)	78	65	88
SDAI, mean (range)	N/A	45.4 (1.3–88.5)	40.9 (2–80)
BASDAI, mean (range)	N/A	6.6 (0.5–9.75)	6.1 (3.1–9.1)
BASFI, mean (range)	N/A	6.6 (0.5–9.7)	6.5 (2.85–10)
CXR (% abnormal)	7	3.7	2
TST +ve, n (%+ve)	9 (10)	260 (7)	104 (12)
IGRA +ve, n (%+ve)	5 (6)	387 (25)	64 (23)
MTX* % (mean dose; range)	N/A	90 (19.4; 5–40)	88 (20.2; 20–25)
Steroid % (mean dose; range)	N/A	60 (9.6; 5–60)	67 (10.3; 7.5–15)

*Methotrexate.
ACPA, anti-citrullinated protein antibody; AS, ankylosing spondylitis; BASDAI, bath ankylosing spondylitis disease activity index; BASFI, bath ankylosing spondylitis functional index; CXR, chest X-ray; HLAB27, human leukocyte antigen B27; IGRA, interferon gamma release assay; RA, rheumatoid arthritis; RF, rheumatoid factor; SDAI, simple disease activity index; TB, tuberculosis; TST, tuberculin skin test.

Table 4 Baseline characteristics of biologic subsets

Characteristics	Monoclonal's (n=703)	Soluble receptor (n=498)	Non-TNF (n=386)
Mean age in years	49.9	51.1	56.5
% female	66	68	82
% ever smoked	22	21	20
% employed	79	81	74
Mean comorbidities	1.3	1.4	2
Ethnicity (%)			
White	77	79	64
Black	6	4	13
Coloured	4	7	7
Indian	10	8	10
Asian	3	2	6
% Rheumatoid arthritis	54	64	100
% RF/ACPA+ve	56	57	72
Mean SDAI	41.5	43.0	46.7
Mean HAQ	1.6	1.9	1.9
% ankylosing spondylitis	35	26	0
BASDAI (mean)	6.9	6.4	N/A
% psoriatic arthritis	11	11	0
Methotrexate % (mean dose)	88 (19,2)	89 (19.6)	95 (19.8)
Steroid % (mean dose)	61 (9.7)	53 (9.6)	67 (9.3)

ACPA, anti-citrullinated protein antibody; BASDAI, bath ankylosing spondylitis disease activity index; HAQ, health assessment questionnaire; RF, rheumatoid factor; SDAI, simple disease activity index; TNF, tumour necrosis factor.

non-TNF inhibitors with a risk not statistically different to etanercept. South Africa is a TB endemic country with the 2010 WHO report showing a prevalence rate of 798/100 000, one of the highest in the world.[30 31] Therefore it is not surprising that the TB incidence rate among biologic users is around ten times higher than European countries (table 6). LTBI is much more prevalent than active TB and LTBI screening rates from SABIO (12.9%) are not reflective of the broader SA population. The highest prevalence of LTBI estimated at 88% has been found among people in the age group 30–39 years old living in townships and informal settlements.[32 33] SABIO LTBI screening data account for an economically privileged population subset and the rate in the general rheumatology population is still to be determined, but is estimated to be far in excess.

Post-marketing surveillance studies from Japan and a study from Taiwan have reported on the increased risk of TB associated with anti-TNF.[34–36] The Brazilian Registry (Registro Brasileiro de Monitoração de Terapias Biológicas—BiobadaBrasil) reported the incidence rate of tuberculosis was 287/100 000 patient years among anti-TNF users (adalimumab: 443/100 000; etanercept: 192/100 000 and infliximab: 182/100 000) while no cases of tuberculosis occurred in the non TNF group (abatacept, rituximab and tocilizumab).[37]

The number of patients accessing biological therapies in South Africa is increasing yearly (online supplementary figure 2) and so too is the annual incidence of TB in the general population (online supplementary table 2). The recent annual decline in

Table 5 Risk factors for TB using univariate analysis

	Registry data		TB cases		Univariate analyses	
	N	%	N	%	P-value	OR
Gender	**1587**		**86**			
Female	1126	71	48	56	Reference	1
Male	461	29	38	44	0.003	1.93
Disease category	**1587**		**86**			
AS	379	24	27	31	Reference	1
PsA	123	8	4	5	0.151	0.46
RA	1085	68	55	64	0.160	0.71
Race	**1587**		**86**			
White	1069	67	60	70	Reference	1
Coloured	102	6	5	6	0.776	0.87
Black	92	6	11	13	0.029	2.13
Indian	135	9	10	12	0.433	1.32
Asian	73	5	0	0	Excluded from univariate analyses due to 0 cases in TB arm	
Unknown	116	7	0	0		
Region	**1587**		**86**			
E.Cape and Free State	96	6	5	6	Reference	1
Gauteng	935	59	38	44	0.611	0.78
Kwazulu-Natal	192	12	12	14	0.739	1.20
Western Cape	364	23	31	36	0.321	1.64
Comorbidities	**1587**		**86**			
0	494	31	38	44	Reference	1
>=1	857	54	47	55	0.133	0.71
Unknown	236	15	1	1	0.004	0.06
Age	**1351**		**83**			
Mean	52.01		45.20		<0.001	

AS, ankylosing spondylitis; PsA, psoriatic arthritis; RA, rheumatoid arthritis; TB, tuberculosis.

the TB rate among biological users was therefore not expected, but is perhaps due to greater vigilance in LTBI screening, judicious biologic selection and empiric LTBI treatment for high risk patients.

Screening and treatment of LTBI prior to biologic commencement markedly reduces reactivation TB.[11] The British registry, British Society for Rheumatology Biologics Register (BSRBR), recorded 40 TB cases with no data on LTBI screening.[12] From the 69 TB patients recorded in the French registry, French Research Axed on Tolerance of Biotherapies (RATIO), TST was done in 45 and was positive (>5 mm) in 15. There was a history of TB exposure in 10 and past history in 4. None of the patients received correct treatment according to French recommendations (9 months of INH or 3 months with two TB drugs including rifampicin).[13] The Spanish registry, BIOBADASER, recorded 15 cases of TB infection and used a two step skin test as their protocol for detection of LTBI. They concluded that the probability of developing TB was seven times higher when recommendations were not followed.[11] Despite our LTBI programme, approximately 50% developed reactivation TB mainly due to LTBI screen failures (33/77). Poor intradermal injection technique, inaccuracies in reading the TST and under-reporting of granulomas on CXR may be some of the reasons. We were unable to demonstrate any statistical advantage of TST over IGRA as a screening tool. LTBI treatment failure occurred in 12, possibly due to poor adherence, drug resistance or starting a biologic too soon after initiating LTBI treatment. Importantly, the remaining 50% of TB cases were new infections for which LTBI screening will not impact. Continuous INH treatment, avoiding high TB risk spaces such as public transportation and institutions and tapering biological therapy where clinically appropriate may be mitigating factors.

There were differences across registries concerning clinical presentation. Our data show 40% were extra-pulmonary and 60% pleuro-pulmonary, compared with BSRBR: 25/40 (62%) extra-pulmonary, 11 disseminated and RATIO: 42/69 (61%) extra-pulmonary.[12 13]

Table 6 Comparison of TB rates in biologic users across registries

National registry	SABIO*	BSRBR†	BIOBADASER‡	RATIO§	US National Data Bank
Total number of patients	4830	10 712	5198	N/A	6460
Number of TB cases	96	40	15	69	4
TB rate for anti-TNF per 100 000 patient years	1387	106	172	116	5
TB rate for non-TNF per 100 000 patient years	681	N/A	NA	N/A	N/A

*South African Biologics Registry.
†British Society for Rheumatology Biologics Register.
‡Base de Datos de Productos Biólogicos de la Sociedad Española de Reumatología.
§French Research Axed on Tolerance of Biotherapies registry.
TB, tuberculosis; TNF, tumour necrosis factor.

Most patients responded well to TB treatment including two drug resistant cases. We recorded 6 deaths from 96 cases (6%) compared with BSRBR: 10 from 40 (25%) and RATIO: 2 from 57 (3.5%) with missing data in 12.[12 13] After treatment completion, 63/96 (66%) of our patients elected to continue on a biologic.

Univariate analysis of risk factors for TB in biologic users showed that male sex, black race and younger age were statistically significant risk factors, all possibly attributable to exposure in the workplace. Multivariate analysis showed that residence in the Western Cape increases TB risk (p=0.045, OR 3.05) coinciding with a higher prevalence of TB in the province.[38] There was no increased risk for underlying rheumatic disease (RA, AS, PsA) despite RA having an inherent risk.[39 40] The use of methotrexate, corticosteroids and previous biologic therapies were also not risk factors. Twenty five from 86 cases (29%) received more than one biologic prior to acquiring TB compared with BSRBR; 7 from 40 (17.5%) and RATIO; 11 from 69 (16%).[12 13]

A limitation of this study is that our registry data capture the economically advantaged who have the least exposure and risk of developing TB and cannot be extrapolated to the general rheumatology population. Even in this privileged cohort however, the risk of TB remains high. Further limitations include the assumption that early onset TB, defined as occurring within the first 18 months of starting a biologic, is reactivation TB and late onset TB occurring after this time period are new infections. Other limitations are those inherent to registries including missing data (confounding variables such as disease activity and duration, conventional synthetic disease-modifying antirheumatic drugs (csDMARDs), cumulative steroid dose, smoking and pre-existing pulmonary disease) and reliance on data accuracy from treating rheumatologists. Rheumatologists in private practice make use of the registry as a prerequisite to gain access to funding, whereas rheumatologists in the state sector are not incentivised as funding can be obtained from government hospitals, although limited.

CONCLUSION

Patients on biological therapies for rheumatic diseases have a considerable risk of developing reactivation and new onset TB in South Africa (2%), compared with other countries. All biologics evaluated, particularly monoclonal therapies but also non-TNF inhibitors were implicated. Reactivation of latent TB is driven at large by monoclonal anti-TNF therapies whereas new TB cases are associated with all bDMARDs. Black race, male sex, younger age and residence in the Western Cape are risk factors for acquiring TB while concomitant steroids, methotrexate, multiple biological use and underlying rheumatic disease were not. TB risk stratification prior to commencement, screening for LTBI and adherence to SARAA treatment recommendations are strongly advocated. Benefits of continuous INH therapy require further study, considering that 50% of cases are new infections.[11] The majority of biologic users in SA are socioeconomically advantaged; however, prescribing these agents in higher TB risk communities is concerning and therefore managing their disease remains an unmet need.

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REFERENCES

1 WHO. Global Tuberculosis report; 2015. http://www.who.int/tb/publications/global report/en/
2 Cantini F, Nannini C, Niccoli L, *et al*. Risk of tuberculosis reactivation in patients with rheumatoid arthritis, ankylosing spondylitis, and psoriatic arthritis receiving non-anti-TNF-targeted biologics. *Mediators Inflamm* 2017;2017:1–15.
3 Selwyn PA, Hartel D, Lewis VA, *et al*. A prospective study of the risk of tuberculosis among intravenous drug users with human immunodeficiency virus infection. *N Engl J Med* 1989;320:545–50.
4 Cantini F, Niccoli L, Goletti D. Adalimumab, etanercept, infliximab, and the risk of tuberculosis: data from clinical trials, National registries, and postmarketing surveillance. *J Rheumatol Suppl* 2014;91:47–55.
5 Cantini F, Nannini C, Niccoli L, *et al*. Guidance for the management of patients with latent tuberculosis infection requiring biologic therapy in rheumatology and dermatology clinical practice. *Autoimmun Rev* 2015;14:503–9.
6 Cantini F, Lubrano E, Marchesoni A, *et al*. Latent tuberculosis infection detection and active tuberculosis prevention in patients receiving anti-TNF therapy: an Italian nationwide survey. *Int J Rheum Dis* 2016;19:799–805.
7 Solovic I, Sester M, Gomez-Reino JJ, *et al*. The risk of tuberculosis related to tumour necrosis factor antagonist therapies: a TBNET consensus statement. *Eur Respir J* 2010;36:1185–206.
8 Bumbacea D, Arend SM, Eyuboglu F, *et al*. The risk of tuberculosis in transplant candidates and recipients: a TBNET consensus statement. *Eur Respir J* 2012;40:990–1013.
9 Harries AD, Kumar AMV, Satyanarayana S, *et al*. Addressing diabetes mellitus as part of the strategy for ending TB. *Trans R Soc Trop Med Hyg* 2016;110:173–9.
10 Ronacher K, Joosten SA, van Crevel R, *et al*. Acquired immunodeficiencies and tuberculosis: focus on HIV/AIDS and diabetes mellitus. *Immunol Rev* 2015;264:121–37.
11 Gómez-Reino JJ, Carmona L, Ángel Descalzo M, *et al*. Risk of tuberculosis in patients treated with tumor necrosis factor antagonists due to incomplete prevention of reactivation of latent infection. *Arthritis Rheum* 2007;57:756–61.
12 Dixon WG, Hyrich KL, Watson KD, *et al*. Drug-Specific risk of tuberculosis in patients with rheumatoid arthritis treated with anti-TNF therapy: results from the British Society for rheumatology biologics register (BSRBR). *Ann Rheum Dis* 2010;69:522–8.
13 Turbach F, Salmon D, Ravaud P, *et al*. Risk of tuberculosis is higher with anti-tumor necrosis factor monoclonal antibody therapy then with soluble tumor necrosis factor receptor therapy. *Arthritis Rheum* 2009;60:1884–94.
14 Ai J-W, Zhang S, Ruan Q-L, *et al*. The risk of tuberculosis in patients with rheumatoid arthritis treated with tumor necrosis factor-α antagonist: a Metaanalysis of both randomized controlled trials and Registry/Cohort studies. *J Rheumatol* 2015;42:2229–37.
15 Askling J, Fored CM, Brandt L, *et al*. Risk and case characteristics of tuberculosis in rheumatoid arthritis associated with tumor necrosis factor antagonists in Sweden. *Arthritis Rheum* 2005;52:1986–92.
16 Hyun L. Quantiferon-TB gold in-tube assay for screening arthritis patients for latent tuberculosis infection before starting anti-tumor necrosis factor treatment. *PLoS One* 2015.
17 Mariette X, Vencovsky J, Lortholary O, *et al*. The incidence of tuberculosis in patients treated with certolizumab pegol across indications: impact of baseline skin test results, more stringent screening criteria and geographic region. *RMD Open* 2015;1:e000044.
18 Dixon WG, Carmona L, Finckh A, *et al*. EULAR points to consider when establishing, analysing and reporting safety data of biologics registers in rheumatology. *Ann Rheum Dis* 2010;69:1596–602.
19 Kearsley-Fleet L, Zavada J, Hetland ML, *et al*. The EULAR Study Group for registers and observational drug studies: comparability of the patient case mix in the

European biologic disease modifying anti-rheumatic drug registers. *Rheumatology* 2015;54:1074–9.
20 Emery P, Sebba A, Huizinga TWJ, *et al*. Biologic and oral disease-modifying antirheumatic drug monotherapy in rheumatoid arthritis. *Ann Rheum Dis* 2013;72:1897–904.
21 Heiberg MS, Koldingsnes W, Mikkelsen K, *et al*. The comparative one-year performance of anti–tumor necrosis factor α drugs in patients with rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis: results from a longitudinal, observational, multicenter study. *Arthritis Rheum* 2008;59:234–40.
22 Soliman MM, Ashcroft DM, Watson KD, *et al*. Impact of concomitant use of DMARDs on the persistence with anti-TNF therapies in patients with rheumatoid arthritis: results from the British Society for rheumatology biologics register. *Ann Rheum Dis* 2011;70:583–9.
23 Listing J, Strangfeld A, Rau R, *et al*. Clinical and functional remission: even though biologics are superior to conventional DMARDS's, overall success rates remain low – results from RABBIT, the German biologics register. *Arthritis Res Ther* 2006;8.
24 Askling J, Fored CM, Brandt L, *et al*. Time-Dependent increase in risk of hospitalisation with infection among Swedish RA patients treated with TNF antagonists. *Ann Rheum Dis* 2007;66:1339–44.
25 Mariette X, Gottenberg J-E, Ravaud P, *et al*. Registries in rheumatoid arthritis and autoimmune diseases: data from the French registries. *Rheumatology* 2011;50:222–9.
26 Lee SJ, Chang H, Yazici Y, *et al*. Utilization trends of tumor necrosis factor inhibitors among patients with rheumatoid arthritis in a United States observational cohort study. *J Rheumatol* 2009;36:1611–7.
27 Pappas D, Reed G, John A, *et al*. Predictors of initiating biologic monotherapy in biologic naïve patients with rheumatoid arthritis (rA) in a US registry population. *Arthritis Rheum* 2012;64:S214.
28 Engel-Nitz N, Ogale S, Kulakodlu M, *et al*. Use of anti-tumor necrosis factor monotherapy and adherence with non-biologic disease-modifying anti-rheumatic drugs in combination with anti-tumor necrosis factor therapy among rheumatoid arthritis patients in a real-world setting. *Arthritis Rheum* 2012;64:S165.
29 Black RJ, Lester S, Buchbinder R, *et al*. Factors associated with oral glucocorticoid use in patients with rheumatoid arthritis: a drug use study from a prospective national biologics registry. *Arthritis Res Ther* 2017;19.
30 World Health Organization. Global tuberculosis control: WHO report 2011; Report No.: WHO/HTM/TB/2011.16. Geneva (Switzerland): WHO Press, World Health Organization; 2011.
31 World Health Organization. Global tuberculosis control: annual report 2007; Report No.: WHO/HTM/TB/2007.376. Geneva (Switzerland): WHO Press, World Health Organization; 2007.
32 Wood R, Liang H, Wu H, *et al*. Changing prevalence of tuberculosis infection with increasing age in high-burden townships in South Africa. *Int J Tuberc Lung Dis* 2010;14:406–12.
33 Pettipher C, Rudolph R, Musenge E, *et al*. A prospective study of anti-tumor necrosis factor therapy in South African rheumatoid arthritis patients. *Int J Rheum Dis* 2016;19:594–9.
34 Koike T, Harigai M, Inokuma S, *et al*. Postmarketing surveillance of safety and effectiveness of etanercept in Japanese patients with rheumatoid arthritis. *Mod Rheumatol* 2011;21:343–51.
35 Watanabe A, Matsumoto T, Igari H, *et al*. Risk of developing active tuberculosis in rheumatoid arthritis patients on adalimumab in Japan. *Int J Tuberc Lung Dis* 2016;20:101–8.
36 Lim CH, Chen H-H, Chen Y-H, *et al*. The risk of tuberculosis disease in rheumatoid arthritis patients on biologics and targeted therapy: a 15-year real world experience in Taiwan. *PLoS One* 2017;12:e.
37 Yonekura CL, Oliveira RDR, Titton DC, *et al*. Incidence of tuberculosis among patients with rheumatoid arthritis using TNF blockers in Brazil: data from the Brazilian registry of biological therapies in rheumatic diseases (Registro Brasileiro de Monitoração de Terapias Biológicas – BiobadaBrasil). *Rev Bras Reumatol* 2017;57:São Paulo.
38 National Strategic Plan on HIV. *STIs and TB 2012-2016, South African National AIDS Council*, 2011. www.gov.za/documents/national-strategic-plan-hiv-stis-and-tb-2012-2016
39 Carmona L, Hernández-García C, Vadillo C, *et al*. Increased risk of tuberculosis in patients with rheumatoid arthritis. *J Rheumatol* 2003;30:1436–9.
40 Yamada T, Nakajima A, Inoue E, *et al*. Increased risk of tuberculosis in patients with rheumatoid arthritis in Japan. *Ann Rheum Dis* 2006;65:1661–3.

Use of composite outcomes facilitate core outcome set uptake in rheumatoid arthritis trials

The WHO and the International League of Associations for Rheumatology set of core outcomes for rheumatoid arthritis (hereafter RA-COS) was adopted in 1993; it comprises eight individual outcomes which aim to assess disease activity, patient-reported outcome and damage.[1 2] In addition, several validated composite outcome measures are available for use in clinical trials to measure both disease activity and state. The two key indices used in RA, the American College of Rheumatology (ACR) 20% and the Disease Activity Score (DAS) 28, represent combined subsets of the core outcome set (COS); they have been widely adopted as primary endpoints, and reporting of one of these, as well as the RA-COS, is recommended in regulatory guidance.[3 4] Non-reporting of the individual core outcomes restricts meta-analyses, might lead to relevant information being missed, to exaggerated perceptions on how well an intervention works, and could lead to suspicion of intentional selective reporting. To protect against these issues, the European League Against Rheumatism (EULAR) and ACR have jointly recommended that single outcomes in the core set should be considered and reported in full.[5] We investigated (1) the adherence to these guideline recommendations on the planned reporting of disease activity in RA trials and (2) the association of composite outcomes on COS uptake.

METHODS

Phase III/IV trials of RA were found on the WHO International Clinical Trials Registry Platform[6] search portal, first registered between 10 May 2005 and 16 October 2018. To be included trials needed to be within the scope of the RA-COS, that is, the study population was exclusive to RA participants, the intervention was pharmacological and the purpose of the study considered efficacy as an endpoint.

For each trial, all composite outcomes listed on the trial registry under the 'outcome measures' section were extracted, alongside any mention of the plan to measure any of the individual core outcomes. To check discrepancies between planned and reported outcomes, final study reports linked through the trial registry entries were identified. In a random sample of 30 registry entries where the ACR criteria were listed as a planned outcome but the full COS was not listed individually, the reported outcomes in the final publication were assessed.

RESULTS

Of the 341 eligible trials identified, 326 (96%) planned to measure at least one composite outcome and in 246 (72%), this was the ACR criteria (table 1). In total, 70% (238/341) of all trials planned to measure the full RA-COS[7] but only 16% (55/341) listed all outcomes separately. In contrast to the ACR composite, uptake was very low in trials specifying only the DAS/EULAR composite as an endpoint with several outcomes missing, most frequently physician global assessment and patient pain. In the sample of 30 trial reports, we found seven papers (23%) reporting on all the core outcomes despite not listing them in the trial registry entry.

Table 1 Count (%) of studies planning to measure a specific composite and RA-COS outcome

	Number (%)
Endpoint	**Total n=341**
Disease Activity Response	
ACR (eg, ACR 20 and ACR 50)	246 (72)
EULAR	77 (23)
Disease Activity State	
DAS	265 (78)
CDAI	63 (19)
SDAI	62 (18)
RA-COS	
Tender joint count	87 (26)
Swollen joint count	86 (25)
Pain	106 (31)
Patient global assessment	88 (26)
Physician global assessment	84 (25)
Disability	210 (62)
Acute phase reactant	86 (25)
Radiological damage (n=125)*	95 (76)
Listed full RA-COS individually	55 (16)

*denominator adjusted for trials>=52 weeks.
ACR 20, American College of Rheumatology 20%;ACR 50, American College of Rheumatology 50%; CDAI, Clinical Disease Activity Index; DAS, Disease Activity Score; EULAR, European League Against Rheumatism;RA-COS, rheumatoid arthritis—core outcome set; SDAI, Simplified Disease Activity Index.

COMMENT

Current RA trials do not fully comply with the EULAR-ACR reporting guidelines despite good uptake of the RA-COS, this is evidently a reporting problem. Furthermore, this study suggests that the successful uptake of the RA-COS is dependent on the use of one composite outcome, the ACR response criteria. It uses results in the assessment of all core outcomes for short-term trials (<52 weeks), where the assessment of radiological damage is not required. In our random sample, we found several papers reporting on the full core set despite an incomplete listing in the registry; therefore, while our results may be seen as a worst-case scenario, they provide empirical evidence of suboptimal quality of trial assessments and registry entries. Poor adherence to the EULAR-ACR reporting guideline is a major shortcoming which requires urgent action from the research community, including journal editors.

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REFERENCES

1 Boers M, Tugwell P, Felson DT, *et al*. World Health organization and international League of associations for rheumatology core endpoints for symptom modifying antirheumatic drugs in rheumatoid arthritis clinical trials. *J Rheumatol Suppl* 1994;41:86–9.
2 Felson DT, Anderson JJ, Boers M, *et al*. The American College of rheumatology preliminary core set of disease activity measures for rheumatoid arthritis clinical trials. *Arthritis Rheum* 1993;36:729–40.
3 US Department of Health and Human Services. Food and drug administration: guidance for industry clinical development programs for drugs, devices, and biological products for the treatment of rheumatoid arthritis (rA), 1999. Available: www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM071579.pdf [Accessed 8 Aug 2019].
4 The European Agency for the Evaluation of Medicinal Products. Unit for the evaluation of medicinal products for human use: guideline on clinical investigation of medicinal products other than NSAIDs for treatment of rheumatoid arthritis, 2003. Available: www.emea.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003439.pdf [Accessed 8 Aug 2019].
5 Aletaha D, Landewe R, Karonitsch T, *et al*. Reporting disease activity in clinical trials of patients with rheumatoid arthritis: EULAR/ACR collaborative recommendations. *Ann Rheum Dis* 2008;67:1360–4.
6 World Health Organization. International clinical trial registry platform, 2019. Available: https://www.who.int/ictrp/search/en/ [Accessed 15 Oct 2019].
7 Kirkham JJ, Bracken M, Hind L, *et al*. Industry funding was associated with increased use of core outcome sets. *J Clin Epidemiol* 2019;115:90–7.

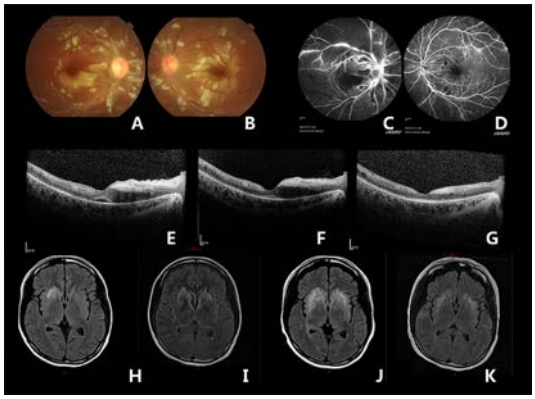

Figure 1 Effects of immunoadsorption therapy on ophthalmic examination and head MR images. Ophthalmological and MR images on admission (A–E, H): funduscopy images (A, B) show the presence of large, multiple cotton wool spots and haemorrhages in both eyes. Fundus fluorescein angiography images (C, D) reveal multiple arteriolar occlusions and thrombosis, fluorescein filling defect, leakage and wall staining; a large non-perfusion area was observed in the posterior pole and macular arch ring structure disappeared in both eyes. OCT image of right eye (E) displays severe macular oedema. Head T2/FLAIR MR image demonstrates bilateral asymmetric hyperintensity in the basal ganglia. After one cycle of high-intensity immunosuppressive therapy, macular oedema of right eye slightly reduced on OCT image (F) and bilateral T2/FLAIR hyperintensity in the basal ganglia became even larger on head MRI. (I) Lesions in bilateral basal ganglia become more pronounced on MRI (J) after given one cycle of rituximab therapy. After the second immunoadsorption therapy, bilateral hyperintensity in basal ganglia and macular oedema of right eye abated obviously on MRI (K) and OCT image (G). FLAIR, fluid-attenuated inversion recovery; OCT, optical coherence tomography.

Rapid induction of clinical remission by immunoadsorption for refractory lupus retinopathy complicated with life-threatening neuropsychiatric lupus

Central nervous system involvement in systemic lupus erythematosus (SLE), termed neuropsychiatric SLE (NPSLE), is a relatively common manifestation of the disease. A previous study showed that patients with NPSLE with eye involvement are less common, with a prevalence of 0.66%.[1] However, retinopathy occasionally presents as the primary manifestation of NPSLE, making diagnosis and management more challenge.[2] Limited data exist regarding the optimisation of NPSLE treatment.[1 3] Patients with NPSLE are usually treated with high doses of corticosteroids, cyclophosphamide (CTX), mycophenolate mofetil and azathioprine. In the case of refractory NPSLE, rituximab might be an alternative option.[2 3] A few case reports on the use of immunoadsorption as a rescue treatment for patients with life-threatening NPSLE; however, none reported on refractory NPSLE patients with eye involvement.

We report on a patient with refractory NPSLE and progressive retinopathy who was successfully treated with immunoadsorption.

An 18-year-old female patient was first diagnosed with SLE in July 2016 had been treated with oral methylprednisolone (MP) (4 mg) and hydroxychloroquine (200 mg) daily, more recently photophobia in both eyes had developed. Laboratory tests revealed the presence of anti-nuclear antibody positive (1:1280), with antibodies to Smith (+), double-stranded DNA (dsDNA) (47 IU/L), Anti-Ro-52/ sjögren syndrome A antibody (RO-52) (+++), Anti-Ro-60/ sjögren syndrome A antibody (SSA) (+++), anti-UI-ribonucleoprotein antibody (U1-RNP) (+++), Anti–ribosomal-P antibodies (rib-P) (+) and white blood cell count was 2.62×10^9/L. Despite the prompt withdrawal of hydroxychloroquine which was suspected to be the cause of visual loss initially, the patient complained of worsened vision loss a week later. Retinal vasculitis was confirmed by ophthalmological examinations and multiple haemorrhages, multiple arteriolar occlusions, extensive avascular areas and macular oedema in both eyes were detected by fundus fluorescein angiography and optical coherence tomography (OCT) (figure 1A–E). Moreover, headache and manic-depressive disorder appeared. Head MRI demonstrated bilateral asymmetric hyperintensity in the basal ganglia on fluid-attenuated inversion recovery (FLAIR) images (figure 1H). Therefore, the diagnosis of retinal vasculitis was highly active (lupus retinopathy) complicated with NPSLE was confirmed.

According to the European League Against Rheumatism recommendations,[4] pulse of intravenous MP (500 mg/day ×3 days) in combination with intravenous CTX pulse therapy (200 mg every other day ×3 days) was used for considering severe organ-threatening SLE with the low white blood cell count in December 2018. Following two times photocoagulation treatments for both eyes was also performed simultaneously. During the following 6 weeks, the patient received an intrathecal injection of methotrexate 10 mg and dexamethasone 10 mg every week. At the same time,

the patient was given oral prednisone 45 mg every day when the patient did not use MP intravenously. Despite aggressive treatment, neuropsychiatric symptoms and eyesight of the patient develop progressively. Sluggish eyes, gibberish, insomnia and mood disorders appeared (unknown crying and yelling) with bilateral larger T2/FLAIR hyperintensity in the basal ganglia on head MRI image (figure 1I). Thereafter infusions of rituximab (500 mg ×2, every 2 weeks) was performed as a treatment strategy for life-threatening refractory SLE in January 2019.

With rapid progression on neuropsychiatric symptoms and visual acuity in both eyes, the dose of intravenous MP was increased to 1000 mg/day for 3 days. One week later, the patient began to complain about movement disorder (including unstable standing, difficulty walking and unable to swallow), urinary incontinence and her vision was almost total lost. The second following OCT displayed severe bilateral macular oedema still exist for both eyes (figure 1F). MRI showed enlarged T2/FLAIR hyperintensity in bilateral basal ganglia (figure 1J).

As a rescue therapy, the patient received twice immunoadsorption (2 days apart) therapies in February that were well tolerated while CTX was increased to 1200 mg (200 mg every day ×6 days). Her mental symptoms improved instantly after 1 week of therapy and followed MRI revealed rapid regression of the bilateral basal ganglia lesions (figure 1K). Meanwhile, OCT image indicated that macular oedema is significantly abated (figure 1G).

After second immunoadsorption, a significant clearance of circulating IgG (drops from 18.15 g/L to as low as 0.33 g/L), autoantibodies including immune complexes and anti-dsDNA antibodies were noted (figure 2). In order to consolidate the therapeutic effect, the third immunoadsorption was performed 5 days later. Mental symptoms alleviated significantly and vision of the patient remained almost stabilised. No infection complications occurred and white blood cell count maintained in the normal range during follow-up for 4 months (figure 2). A protein A immunosorbent column (KONPIA, Guangzhou KONCEN Biological Technology Co., China) and Fresenius 4008S hemodialysis machine (Fresenius Medical Care AG, Bad Homburg, Germany) was used for the three treatments. Before the treatment, dexamethasone 5 mg was intravenously infused, and then the plasma separator P2S was performed. The first five cycles of immunoadsorption treatment, the total plasma was adsorbed 3000 mL. The second seven cycles of immunoadsorption treatment, the total plasma adsorption was 4200 mL. The third time was six cycles, and the total amount of plasma adsorbed was 3600 mL.

The successful use of immunoadsorption has been reported in a few patients with intractable SLE, such as lupus nephritis, NPSLE and blood abnormalities.[5 6] There is a paucity of literature on the diagnosis and management of retinal disease in patients with NPSLE.[1 2] However, to our knowledge, a report using this rescue approach to therapy for intractable retinopathy combined with refractory NPSLE has not been performed by other investigators. Although it has also been recommended that life-threatening NPSLE or refractory SLE can be managed with intravenous pulse high-dose glucocorticoids, CTX and even rituximab, this patient is ineffective for all of the above treatments, neuropsychiatric symptoms and visual acuity gradually worsened. Therapy of immunoadsorption could rapidly remove autoantibodies and immune complexes from the circulation in this refractory retinopathy combined with NPSLE. Even though this therapeutic option saved the patient's life, the severe vision loss was irreparable. We must admit that the administration of intravenous bolus CTX during immunoadsorption treatment may also play a role in controlling the disease, but from the fluctuation of immunoglobulin, the main therapeutic effect should be immunoadsorption treatment. Therefore, based on this clinical experience, it is imperative to emphasise that immunoadsorption should be applied as early as possible for patients with refractory lupus retinopathy complicated with intractable NPSLE to improve the quality of life and prognosis before severe vision loss.

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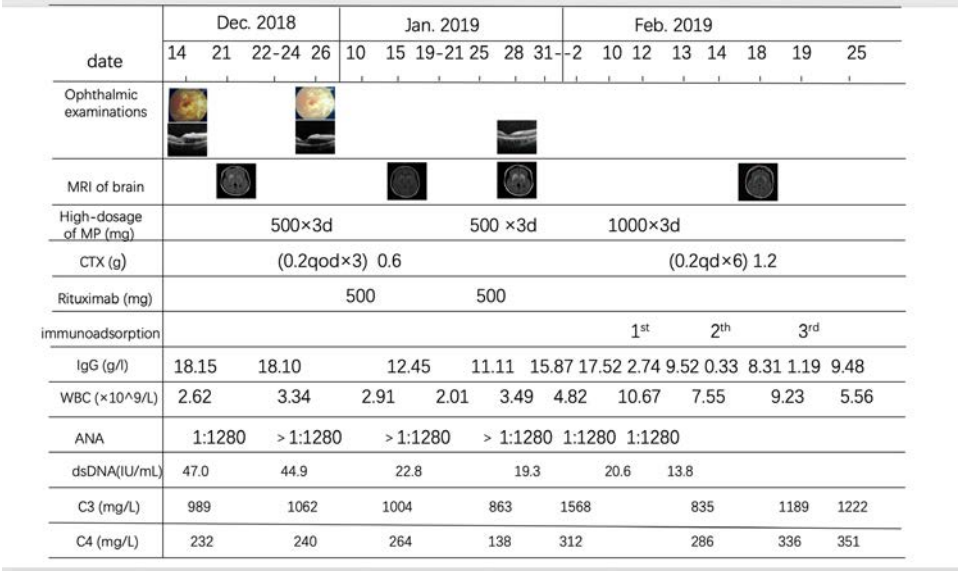

Figure 2 Clinical courses-treatment and examination results of the patient corresponding to the time. ANA is analysed by immunofluorescence (the highest dilution titre in our institution is 1:80, 1:160, 1:320, 1:640, 1:1280. If there is still strong fluorescence at 1:1280, then it is always shown as >1:1280) and the anti-dsDNA assay is performed by ELISA. ANAs, antinuclear antibodies; anti-dsDNA, antibodies against double-stranded DNA; CTX, cyclophosphamide; MP, methylprednisolone.

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REFERENCES

1 Ushiyama O*et al*. Retinal disease in patients with systemic lupus erythematosus. *Ann Rheum Dis* 2000;59:705–8.
2 Mizuno Y, Nishide M, Wakabayashi T, *et al*. OCTA, a sensitive screening for asymptomatic retinopathy, raises alarm over systemic involvements in patients with SLE. *Ann Rheum Dis* 2018:annrheumdis-2018-214751.
3 Schwartz N, Stock AD, Putterman C. Neuropsychiatric lupus: new mechanistic insights and future treatment directions. *Nat Rev Rheumatol* 2019;15:137–52.
4 Fanouriakis A, Kostopoulou M, Alunno A, *et al*. 2019 update of the EULAR recommendations for the management of systemic lupus erythematosus. *Ann Rheum Dis* 2019;78:736–45.
5 Yamaji K. Immunoadsorption for collagen and rheumatic diseases. *Transfusion and Apheresis Science* 2017;56:666–70.
6 Stummvoll GH, Aringer M, Jansen M, *et al*. Immunoadsorption (IAS) as a rescue therapy in SLE: considerations on safety and efficacy. *Wien Klin Wochenschr* 2004;116:716–24.

Effect of belimumab treatment on antiphospholipid antibody levels: post-hoc analysis based on two randomised placebo-controlled trials in systemic lupus erythematosus

The presence of antiphospholipid antibodies (aPL) in patients with systemic lupus erythematosus (SLE) has been associated with increased risk of thrombotic and/or obstetric manifestations.[1] The mechanism of action of belimumab is inhibition of the binding of soluble circulating B lymphocyte stimulator to its target receptors on B cells. Belimumab use was associated with autoantibody reduction, such as antidouble-stranded DNA, anti-Sm and anticardiolipin (aCL) antibodies in some previous reports.[2] The objective of this study was to assess the effect of belimumab 10 mg/kg versus placebo on aPL titres using pooled data from two large randomised SLE-controlled trials (BLISS-76 (NCT00410384) and BLISS-52 trial (NCT00424476)).

Levels of three isotypes of aCL antibodies (IgG, IgM and IgA) were assessed at baseline and at each visit. There we no available data on antiβ2GPI antibodies and lupus anticoagulant. The median (IQR) aCL titre, and the titre change from baseline (Δ titre) at 3, 6 and 12 months was compared between treatment arms by Mann-Whitney U test. At a second step, we undertook a random intercept mixed-effects model with the change of aCL titres from baseline as the dependent variable and treatment arm as a fixed effect. Time was added in the model, while the interaction of treatment and time was also assessed. The models were further adjusted for potential confounders (age, sex, concomitant immunosuppressive therapy, concomitant antimalarials, baseline prednisolone dose). Due to a potential effect of antimalarials on aCL titres, stratified analyses were also performed. We also assessed the proportion of patients in each group who seroconverted from positive at baseline to negative in the last two available visits.

The number of patients tested positive for IgA, IgG and IgM aCL in both trials was 362, 375 and 120, respectively, from a total of 1684 patients (819 and 865 patients with SLE from BLISS-76 and BLISS-52 trial, respectively). The number of patients tested positive for IgA, IgG and IgM aCL in placebo/belimumab 10 mg/kg groups was 125/124, 29/49 and 124/122, respectively. At specific time-points, no significant differences between belimumab and placebo in aCL titres and their change from baseline were found (tables 1A–C) apart from a lower median IgG titre (table 1A) and a greater change of IgA aCL titre only at 12 months (table 1C).

In the mixed-effects model analysis, the main effect of belimumab on the change of IgG and IgM aCL titres versus placebo was non-significant (−1.5 U/mL, p=0.43 and −5.8, p=0.41, respectively) but significant for IgA (−3.4 U/mL, p<0.0001). The effect of treatment over time (figure 1A–I) was statistically significant only for IgA titres (figure 1A and D) and was retained after adjustment for confounders. In the stratified analysis by concomitant antimalarial treatment, a significant effect of belimumab versus placebo on IgG (p=0.03) and IgA aCL (p<0.0001)

Table 1A Median (IQR) IgG aCL titre at baseline, 3, 6 and 12 months and median titre change from baseline at 3, 6 and 12 months for IgG aCL

		IgG aCL		
		Placebo	Belimumab 10 mg/kg	P value
Median (IQR) titre	Baseline	15 (12; 27) n=125	17 (13; 27) n=124	0.35
	3 months	11 (9; 20) n=66	10 (9; 19) n=50	0.49
	6 months	9 (9; 15) n=75	9 (9; 17) n=55	0.77
	12 months	12 (9; 19) n=88	9 (9; 17) n=86	0.02
Median (IQR) change	3 months	−3 (−7; −1)	−6 (−10; −2)	0.11
	6 months	−4 (−13; −2)	−6 (−12; −3)	0.55
	12 months	−4 (−7; −1)	−5 (−12; −1)	0.27

aCL, anticardiolipin.

Table 1B Median (IQR) IgM aCL titre at baseline, 3, 6 and 12 months and median titre change from baseline at 3, 6 and 12 months for IgM aCL

		IgM aCL		
		Placebo	Belimumab 10 mg/kg	P value
Median (IQR) titre	Baseline	21 (14; 43) n=29	19 (12; 38) n=49	0.59
	3 months	11 (9; 33) n=14	10 (9; 26) n=14	0.93
	6 months	11 (9; 29) n=19	10 (9; 24) n=20	0.75
	12 months	23 (20; 37) n=14	30 (12; 44) n=22	0.05
Median (IQR) change	3 months	−7 (−14; −3)	−3 (−9; −1)	0.70
	6 months	−5 (−8; −1)	−4 (−11; −2)	0.57
	12 months	−2 (−7; 5)	−3 (−15; 1)	0.15

aCL, anticardiolipin.

titres over time was observed only in the subgroup of patients treated with antimalarials (figure 1B and C). The proportion of patients who seroconverted in the belimumab versus the placebo arm was 59% versus 47% for IgG aCL, 26% versus 23% for IgM aCL and 37% versus 33% for IgA aCL (all p>0.05). Importantly, the majority of patients in this analysis had a low aCL titre at baseline (191/246, 217/249 and 58/78 for IgA, IgG and IgM, respectively) which makes it easier to convert to negative. Only three and two patients with IgG aCL who seroconverted in belimumab and placebo groups, respectively, started from a medium/high baseline titre (defined as >40 U/mL according to the updated Sapporo classification criteria for antiphospholipid syndrome (APS)), and none from the IgM/IgA groups.

This post-hoc analysis showed no significant effect of belimumab over time on IgG or IgM aCL titre, but an effect on IgG and IgA aCL was shown in patients with concomitant antimalarials, suggesting that concomitant antimalarial treatment may exert a beneficial synergistic effect. Previous studies have shown a modifying effect of antimalarials on aPL titres.[3] The lack of significant aCL level reduction does not necessarily exclude a potential therapeutic implication of belimumab on aPL-related manifestations in the context of SLE or APS, through—other than antibody reduction—B cell-driven effects that need to be elucidated. Rituximab, a B-cell depletion therapy has been off label used for severe APS manifestations and refractory catastrophic APS,[1] although with a variable effect on aPL titres.[4 5] The differential effect observed on IgA aCL levels compared with the other two isotypes is of interest and its clinical significance remains to be further elucidated.

Table 1C Median (IQR) IgA aCL titre at baseline, 3, 6 and 12 months and median titre change from baseline at 3, 6 and 12 months for IgA aCL

		IgA aCL		
		Placebo	Belimumab 10 mg/kg	P value
Median (IQR) titre	Baseline	22 (18; 30) n=124	24 (19; 37) n=122	0.32
	3 months	19 (13; 29) n=120	19 (13; 28) n=116	0.89
	6 months	19 (11; 27) n=121	17 (11; 26) n=112	0.61
	12 months	20 (12; 27) n=87	16 (10; 27) n=94	0.20
Median (IQR) change	3 months	−4 (−9; 2)	−7 (−11; −2)	0.06
	6 months	−6 (−12; 0)	−9 (−14; −4)	0.07
	12 months	−7 (−12; 2)	−10 (−15; −7)	0.0007

aCL, anticardiolipin.

One report has previously demonstrated an effect of belimumab in three refractory cases of APS but with no effect on aPL titres.[6] In two recent case series of 3 and 12 patients with SLE, respectively, treated with belimumab, a progressive reduction of aPL was reported.[7 8] Two out of 3 and 3 out of 12 patients, respectively, were on concomitant antimalarials. A more recent study with 50 patients SLE treated with belimumab, showed a significant decrease of IgG aCL only at 18 months, while 30% of patients seroconverted to negative.[9] A post-hoc analysis on the same trial's data has previously been reported. The main drawback of that analysis was the assessment of the reduction at only one time point (52 weeks), disregarding the time-points between. It is well known that aCL fluctuate with time,[10] which makes it important to collect as many measurements as possible to draw safer conclusions about the effect of a treatment on these autoantibodies. Additionally, it is crucial to include the role of antimalarials as potential effect modifiers.

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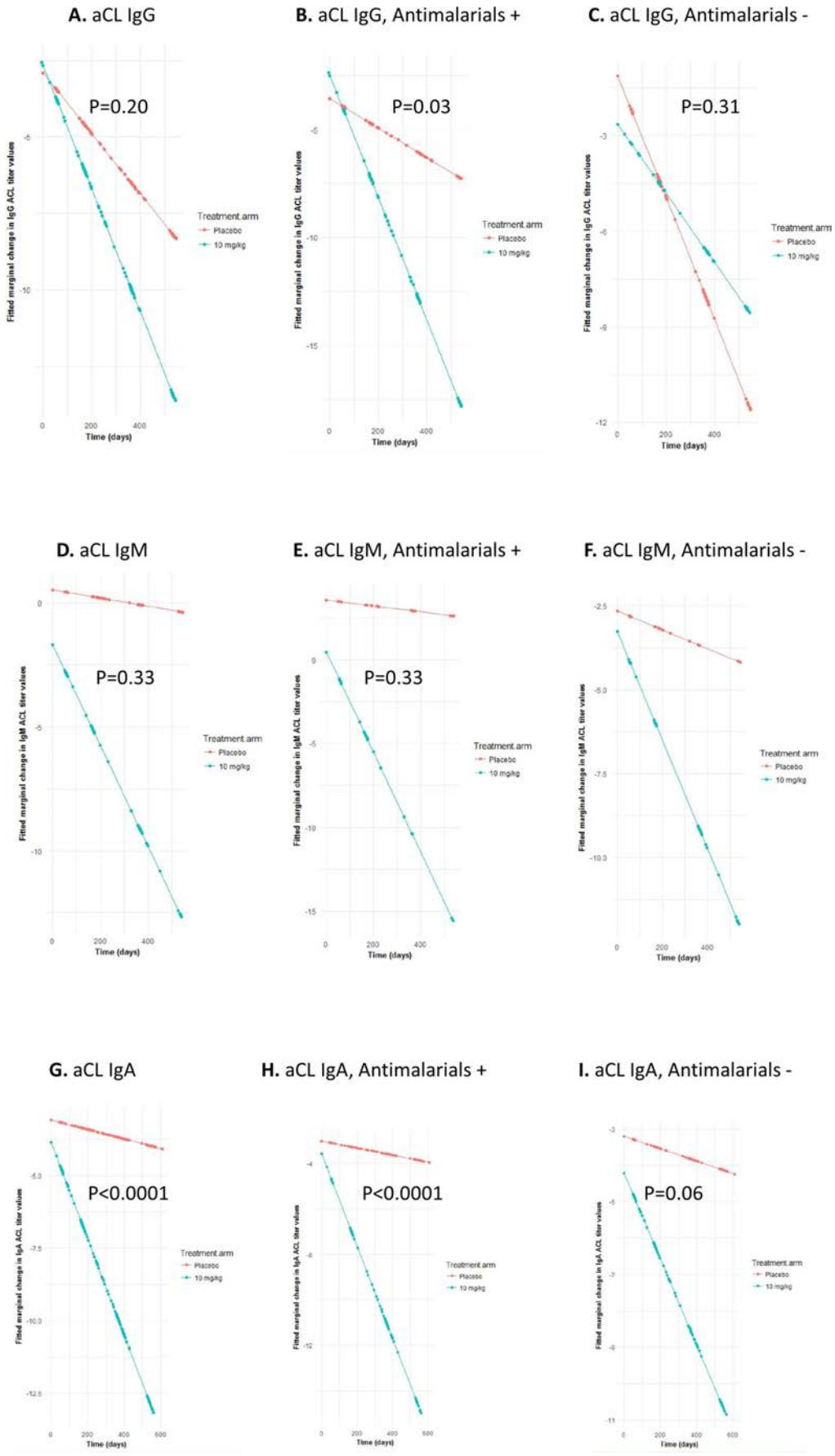

Figure 1 (A–I) Fitted marginal change from baseline for IgA, IgG and IgM aCL in the group of patients tested positive for IgA, IgG and IgM aCL at baseline (figures A, D and G, respectively), as well as in the subgroups of patients treated (B, E and H) and non-treated (C, F and I) with concomitant antimalarial. The p values for the interaction terms are shown. aCL, anticardiolipin.

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REFERENCES

1 Tektonidou MG, Andreoli L, Limper M, *et al*. EULAR recommendations for the management of antiphospholipid syndrome in adults. *Ann Rheum Dis* 2019.
2 Stohl W, Hiepe F, Latinis KM, *et al*. Belimumab reduces autoantibodies, normalizes low complement levels, and reduces select B cell populations in patients with systemic lupus erythematosus. *Arthritis Rheum* 2012;64:2328–37.
3 Broder A, Putterman C. Hydroxychloroquine use is associated with lower odds of persistently positive antiphospholipid antibodies and/or lupus anticoagulant in systemic lupus erythematosus. *J Rheumatol* 2013;40:30–3.
4 Ramos-Casals M, Brito-Zerón P, Muñoz S, *et al*. A systematic review of the off-label use of biological therapies in systemic autoimmune diseases. *Medicine* 2008;87:345–64.
5 Rodríguez-Pintó I, Cervera R, Espinosa G. Rituximab and its therapeutic potential in catastrophic antiphospolipid syndrome. *Ther Adv Musculoskelet Dis* 2015;7:26–30.
6 Yazici A, Yazirli B, Erkan D. Belimumab in primary antiphospholipid syndrome. *Lupus* 2017;26:1123–4.
7 Emmi G, Bettiol A, Palterer B, *et al*. Belimumab reduces antiphospholipid antibodies in SLE patients independently of hydroxychloroquine treatment. *Autoimmun Rev* 2019;18:312–4.
8 Sciascia S, Rubini E, Radin M, *et al*. Anticardiolipin and anti-beta 2 glycoprotein-I antibodies disappearance in patients with systemic lupus erythematosus and antiphospholipid syndrome while on belimumab. *Ann Rheum Dis* 2018;77:1694–5.
9 Cavazzana I, Kumar R, Pozzari C, *et al*. Autoantibodies' titre modulation by anti-BlyS treatment in systemic lupus erythematosus. *Lupus* 2019;28:1074–81.
10 Out HJ, de Groot PG, Hasselaar P, *et al*. Fluctuations of anticardiolipin antibody levels in patients with systemic lupus erythematosus: a prospective study. *Ann Rheum Dis* 1989;48:1023–8.

Increased risk of multiple myeloma in primary Sjögren's syndrome is limited to individuals with Ro/SSA and La/SSB autoantibodies

Primary Sjögren's syndrome is a systemic autoimmune disease characterised by chronic inflammation of exocrine glands, primarily the salivary and lacrimal glands. In the glands, ectopic lymphoid tissue may form, with germinal centre-like structures promoting B-cell DNA rearrangements and Ro/SSA and La/SSB autoantibody production.[1] The presence of autoantibodies correlate with disease severity and influence long-term outcome.[2] High circulating levels of BAFF also contributes to the polyclonal B-cell activation, and increased plasmablast differentiation and hypergammaglobulinaemia are common.[3 4] The autoantibodies can induce production of type I interferons, which further a positive feed-forward loop of chronic B-cell activation.[3]

Patients with primary Sjögren's syndrome have a 5–15 times higher risk of lymphoma than the general population, corresponding to a lifetime risk of 5%–10%.[5] The malignancies are commonly B-cell non-Hodgkin lymphomas, predominantly marginal zone lymphomas. However, whether there is an increased risk of multiple myeloma in Sjögren's syndrome has not been unequivocally defined (see online supplementary text).[6] Considering the abundance and activity of plasmablasts in Sjögren's syndrome, we therefore conducted a cohort study investigating the incidence of myeloma in this chronic B-cell dominated inflammatory disorder. Moreover, we were interested to understand if the risk was different in patients with Ro/SSA and La/SSB autoantibodies, hypothesising that these individuals would be at a greater risk.

Patients with primary Sjögren's syndrome (n=1009; 93% female) diagnosed between 1967 and 2013 at the departments of Rheumatology at the University Hospitals in Gothenburg, Malmö/Lund, Linköping, Örebro and Uppsala, as well as the Karolinska University Hospital in Stockholm, Sweden were included in the study. All patients fulfilled the American–European consensus group criteria. For each patient, 10 controls from the general population (n=9543; matched on sex, age and region of residency) were randomly selected from the Swedish Population Register.

Cases of multiple myelomas were identified using The Swedish Cancer Register, which has a nationwide coverage on all cancers diagnosed from 1958 and onwards. All cases of cancer are classified according to the calendar year-specific version of the International Classification of Diseases. The cohorts were followed from the calendar year after Sjögren's syndrome diagnosis date until the date of multiple myeloma diagnosis, death, emigration or 31 of December 2013, whichever came first. Hazard ratios (HR) and confidence intervals(CI) for disease were calculated using Cox proportional hazard regression.

The median follow-up was 10 years (interquartile range: 5 to 16 years), corresponding to 10 386 and 102 257 person-years for Sjögren's syndrome patients and controls, respectively. During follow-up, 4 cases of multiple myeloma were discovered in the Sjögren's syndrome cohort and 14 in the comparison cohort, corresponding to an HR of 2.9 (95% CI 0.9 to 8.7; table 1). Three of the four myeloma cases occurred within 5 years of follow-up and notably, all cases of myelomas in patients with primary Sjögren's syndrome occurred in individuals with Ro/SSA and/or La/SSB autoantibodies. Three of the four cases occurred

Table 1 Risk of multiple myeloma after one calendar year or more following pSS diagnosis date, overall and stratified by Ro/SSA and La/SSB status at time of diagnosis in the pSS cohort (pSS diagnosed 1967–2013)

	Multiple myeloma, N (%)		Person-years		Incidence rate per 1000 person-years (95% CI)		Risk estimate		Median time to event, years*	
pSS patients	pSS†	Controls‡	pSS†	Controls‡	pSS†	Controls‡	HR	95% CI	pSS†	Controls‡
All	4 (0.4%)	14 (0.2%)	10376	102241	0.4 (0.1 to 1.0)	0.1 (0.1–0.2)	2.9	(0.9–8.7)	3.8	10.2
SSA/SSB double positive§	3 (0.7%)	5 (0.1%)	4588	45739	0.7 (0.2 to 2.0)	0.1 (0.0–0.3)	6.2	(1.5–26.0)	3.9	12.1
SSA/SSB single positive¶	1 (0.4%)	4 (0.2%)	2642	25811	0.4 (0.1 to 2.7)	0.2 (0.1–0.4)	2.5	(0.3–22.5)	3.7	10.2
SSA/SSB negative**	0 (0.0%)	5 (0.2%)	2980	28976	NA	0.2 (0.1–0.4)	NA	NA	NA	8.3

Eighteen primary Sjögren's syndrome patients did not have available records on Ro/SSA and La/SSB antibodies, and are hence not included in subgroup analyses.
*In subjects experiencing the event.
†Primary Sjögren's syndrome patients (n=1009).
‡General population comparators (n=9543), matched on sex, age and area of residency.
§Ro/SSA and La/SSB double-positive pSS patients (n=416).
¶Ro/SSA and/or La/SSB single-positive pSS patients (n=284).
**Ro/SSA and La/SSB negative (n=291).
NA, not applicable; pSS, primary Sjögren's syndrome; SSA, Ro/SSA antibodies; SSB, La/SSB antibodies.

in patients with both Ro/SSA and La/SSB autoantibodies, corresponding to an HR of 6.2 (95% CI 1.5 to 26.0 table 1). Three of the four cases were female.

In conclusion, our results imply a non-negligible risk of multiple myeloma in patients with primary Sjögren's syndrome, for which the presence of Ro/SSA and La/SSB autoantibodies demarks the high-risk population. The risk of lymphoma has been described to increase with disease duration,[5] but our results indicate a relatively shorter timespan between Sjögren's syndrome diagnosis and development of myeloma. Further, and not previously described, we observed that the increased risk of myeloma appears restricted to patients with Ro/SSA and La/SSB autoantibodies. The study has limitations to consider, such as being unable to account for occupation or the presence of monoclonal gammopathy (see online supplementary text).[7 8] Nonetheless, our results stress the importance of personalised follow-up for early identification of malignant transformation in patients with Sjögren's syndrome.

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REFERENCES

1 Stott DI, Hiepe F, Hummel M, *et al*. Antigen-Driven clonal proliferation of B cells within the target tissue of an autoimmune disease. the salivary glands of patients with Sjögren's syndrome. *J Clin Invest* 1998;102:938–46.
2 Mofors J, Holmqvist M, Westermark L, *et al*. Concomitant Ro/SSA and La/SSB antibodies are biomarkers for the risk of venous thromboembolism and cerebral infarction in primary Sjögren's syndrome. *J Intern Med* 2019;286:458–68.
3 Bodewes ILA, Björk A, Versnel MA, *et al*. Innate immunity and interferons in the pathogenesis of Sjögren's syndrome. *Rheumatology* 2019;11.
4 Brauner S, Folkersen L, Kvarnström M, *et al*. H1N1 vaccination in Sjögren's syndrome triggers polyclonal B cell activation and promotes autoantibody production. *Ann Rheum Dis* 2017;76:1755–63.
5 Nocturne G, Mariette X. Sjögren syndrome-associated lymphomas: an update on pathogenesis and management. *Br J Haematol* 2015;168:317–27.
6 McShane CM, Murray LJ, Landgren O, *et al*. Prior autoimmune disease and risk of monoclonal gammopathy of undetermined significance and multiple myeloma: a systematic review. *Cancer Epidemiol Biomarkers Prev* 2014;23:332–42.
7 Tomi A-L, Belkhir R, Nocturne G, *et al*. Brief report: monoclonal gammopathy and risk of lymphoma and multiple myeloma in patients with primary Sjögren's syndrome. *Arthritis Rheumatol* 2016;68:1245–50.
8 Brito-Zerón P, Retamozo S, Gandía M, *et al*. Monoclonal gammopathy related to Sjögren syndrome: a key marker of disease prognosis and outcomes. *J Autoimmun* 2012;39:43–8.

Mandatory, cost-driven switching from originator etanercept to its biosimilar SB4: possible fallout on non-medical switching

We read with great interest the recently published results from DANBIO registry on switching from originator etanercept (ETA) to the biosimilar SB4.[1] The study includes a really impressive number of patients who underwent mandatory switching to SB4, and at first glance the results show a good evidence of efficacy and safety of the procedure. However, in our opinion, how these results may be applied on non-medical switching strategy is rather questionable. A careful reading of the paper raises several concerns related to the demographic and clinical characteristics of switchers and non-switchers, and to the timing of clinical evaluations that may generate misleading biases.

First, switchers had longer previous ETA treatment duration and fewer previous bDMARDs compared with non-switchers suggesting a less severe disease. This seems to be confirmed by the baseline lower disease activity both in switchers with rheumatoid arthritis (RA) and psoriatic arthritis (PsA).

Second, an unbalanced treatment regimen resulted with 124 (43%) out of 286 RA non-switchers who continued to receive ETA at the dose of 25 mg weekly while 887 (95%) of switchers were treated with SB4 50 mg weekly. Similarly, this difference was present in both PsA and axial spondyloarthritis groups (18% vs 1% and 36% vs 1%, respectively).

Beyond the reduced dosage, both in RA and PsA relevant differences between the two groups of treatment were appreciable regarding the concomitant methotrexate (MTX) treatment (60% vs 49% in RA and 48% vs 30% in RA and PsA, respectively). These differences seem to be statistically significant, and may have negatively influenced the results, especially in patients with RA where it has been long recognised that ETA efficacy is superior if combined with MTX with respect to monotherapy.[2] Indeed, considering that the majority of withdrawals were related to lack of efficacy, the higher discontinuation rate in non-switchers (33%) as compared with switchers (18%) may be, at least in part, explained by the different treatment regimens together with the baseline higher disease activity in originator ETA continuers. The significant lower retention rate in non-switcher patients with RA in comparison with ETA historic cohort seems to confirm this issue.

Third, 101 adverse events occurred in switchers, and 39 (38.5%) were unreported. These data, together with the low number of subjective events, support the hypothesis of absence or low impact of the nocebo effect in switchers, in contrast with some recently published data.[3]

Fourth, it is unclear why data on disease activity were limited to 3-month visit and not to the end of follow-up, while the discontinuation rate was evaluated after 1 year.

Fifth, the strength of the results was greatly influenced by the nature of the study itself, that is to say mandatory switching without a well-structured study design, and, per se, these data do not constitute a solid base to ensure the rheumatologist for non-medical switching. In this sense, the DANBIO study does not meet any of the Food and Drug Administration guidance indicating the design elements for non-medical switching study (box 1),[4] thus providing a low level of evidence, not sufficient to support the European League Against Rheumatism recommendation 6 on switching strategy.[5]

In conclusion, due to the above underlined methodological defects, greatly limiting the grade of evidence, the results of DANBIO registry cannot be translated in clinical practice to carry out non-medical switching. As we previously stated,[6] we believe that properly designed clinical studies are required to definitively address the efficacy and safety of switching from originator ETA to its biosimilar SB4.

Box 1 List of Food and Drug Administration (FDA) recommended elements for studies on non-medical switching from biologic originators to the respective biosimilar

- Randomised, double-blind trial ensuring the homogeneity of treatment groups and control bias.
- Adequate control with measurement of different outcomes.
- Adequate statistical powering and proper statistical analysis.
- Multiple switches over the study period.
- Evaluation of immunogenicity-related outcomes.
- Adequate follow-up.
- Assessment of individual patient-level outcomes.

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REFERENCES

1 Glintborg B, Loft AG, Omerovic E, *et al*. To switch or not to switch: results of a nationwide guideline of mandatory switching from originator to biosimilar etanercept. one-year treatment outcomes in 2061 patients with inflammatory arthritis from the DANBIO registry. *Ann Rheum Dis* 2019;78:192–200.
2 Pope JE, Haraoui B, Thorne JC, *et al*. The Canadian methotrexate and etanercept outcome study: a randomised trial of discontinuing versus continuing methotrexate after 6 months of etanercept and methotrexate therapy in rheumatoid arthritis. *Ann Rheum Dis* 2014;73:2144–51.
3 Kravvariti E, Kitas GD, Mitsikostas DD, *et al*. Nocebos in rheumatology: emerging concepts and their implications for clinical practice. *Nat Rev Rheumatol* 2018.
4 US Food and Drug Administration. *Considerations in demonstrating interchangeability with a reference product*. Bethesda, MD: US Department of Health and Human Services, 2018.
5 Kay J, Schoels MM, Dörner T, *et al*. Consensus-based recommendations for the use of biosimilars to treat rheumatological diseases. *Ann Rheum Dis* 2018;77:165–74.
6 Cantini F, Benucci M. Switching from the bio-originators to biosimilar: is it premature to recommend this procedure? *Ann Rheum Dis* 2019;78:e23.

Response to: 'Mandatory, cost-driven switching from originator etanercept to its biosimilar SB4: possible fallout on non-medical switching' by Cantini and Benucci

Thank you for the interest in our publication based on data from the Danish DANBIO registry regarding 2061 patients who were eligible for a mandatory non-medical switch from originator to biosimilar etanercept in routine care.[1 2] The marketing of biosimilars has changed the landscape of the biological drugs with the potential for huge cost reductions, most markedly if patients may be switched from an ongoing successful treatment with the expensive originator to a much cheaper biosimilar. Thus, up to 75% price reduction has been experienced in Denmark so far with no evidence of increased use of health resources.[3] The outcomes of a non-medical switch of etanercept in the real-world setting are, however, largely unknown, since prevous publications on switching in routine care have included limited numbers of patients. The aim of the study was therefore to investigate the effectiveness of a large-scale, non-medical switch—including to characterise the patients who were not switched despite the national guideline, as well as those who switched back to the originator.

This study is an example of how observational studies constitute a valuable supplement to randomised trials and provide insight regarding the performance of a drug in large, unselected patient groups.[4–6] In our publication, the strengths and limitations of the observational design are carefully discussed, which Cantini and Benucci[1] largely ignore in their letter published recently in this journal. For example, the observed differences in the demographic and clinical characteristics of switchers compared with non-switchers illustrate that despite a national guideline, the clinical decision to switch a patient or not was associated with certain patient characteristics. Thus, patients with more comorbidities, higher disease activity and prior failed biological treatments were less likely to be switched. This important finding may reflect uncertainty among patients and rheumatologists on how to implement a newly introduced biosimilar in routine care, and it explains why the patients who maintained treatment with the originator drug had poorer retention to treatment compared with the patients who switched to the biosimilar. Furthermore, subjective negative expectations (the nocebo effect) may affect biosimilar treatment outcomes.[7]

The patients in the study suffered from rheumatoid arthritis, psoriatic arthritis and axial spondyloarthritis, diseases well known for fluctuations in inflammatory activity over time. Even patients in remission may experience flares. Therefore, a time window of 3 months and the use of patients as their own controls were chosen in the evaluation of disease activity prior to and following the switch. Increasing the time windows to 1 year as suggested by Cantini and Benucci[1] would significantly have decreased the ability to attribute a flare to the switch.

A major point raised by Cantini and Benucci relates to the treatment regimen in the non-switchers. Their argumentation is not correct; as stated in the Results section, it was less than 15% that received 25 mg etanercept weekly, not 43% as claimed by Cantini and Benucci.

We fully agree that properly designed randomised controlled clinical switch studies such as the NOR-SWITCH study for infliximab are highly needed.[8] It is reassuring that observational switch data on infliximab from DANBIO were in agreement with those reported in NOR-SWITCH.[9] It is our opinion that our paper is a well-balanced contribution to the ongoing discussion on real-world effectiveness of biosimilar etanercept in patients with inflammatory arthritis. Randomised clinical trials provide strong evidence regarding treatment effects and safety of biosimilars in strictly selected patient groups with tight monitoring and short follow-up. On the other hand, observational studies allow us to explore outcomes in unselected patient cohorts representing the whole disease spectrum and—as discussed above—may also provide knowledge on how the biosimilars are implemented and perform in the real-world setting.[6] At the end of the day, the decision on whether and how to carry out non-medical switching relies on evidence generated from both research methods.

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REFERENCES

1 Cantini F, Benucci M, Mandatory BM. Mandatory, cost-driven switching from originator etanercept to its biosimilar SB_4: possible fallout on non-medical switching. *Ann Rheum Dis* 2020;79:e13.
2 Glintborg B, Loft AG, Omerovic E, *et al*. To switch or not to switch: results of a nationwide guideline of mandatory switching from originator to biosimilar etanercept. one-year treatment outcomes in 2061 patients with inflammatory arthritis from the DANBIO registry. *Ann Rheum Dis* 2019;78:192–200.
3 Glintborg B, Sørensen J, Hetland ML. Does a mandatory non-medical switch from originator to biosimilar infliximab lead to increased use of outpatient healthcare resources? A register-based study in patients with inflammatory arthritis. *RMD Open* 2018;4:e000710.
4 Toussirot E, Marotte H. Switching from originator biological agents to biosimilars: what is the evidence and what are the issues? *RMD Open* 2017;3:e000492.
5 Uhlig T, Goll GL. Reviewing the evidence for biosimilars: key insights, lessons learned and future horizons. *Rheumatology* 2017;56(suppl_4):iv49–iv62.
6 Kay J, Schoels MM, Dörner T, *et al*. Consensus-based recommendations for the use of biosimilars to treat rheumatological diseases. *Ann Rheum Dis* 2018;77:165–74.
7 Kristensen LE, Alten R, Puig L, *et al*. Non-pharmacological effects in switching medication: the nocebo effect in switching from originator to biosimilar agent. *BioDrugs* 2018;32:397–404.
8 Jørgensen KK, Olsen IC, Goll GL, *et al*. Switching from originator infliximab to biosimilar CT-P13 compared with maintained treatment with originator infliximab (NOR-SWITCH): a 52-week, randomised, double-blind, non-inferiority trial. *The Lancet* 2017;389:2304–16.
9 Glintborg B, Sørensen IJ, Loft AG, *et al*. A nationwide non-medical switch from originator infliximab to biosimilar CT-P13 in 802 patients with inflammatory arthritis: 1-year clinical outcomes from the DANBIO registry. *Ann Rheum Dis* 2017;76:1426–31.

Some concerns from Turkey

We read the paper written by Kaneko *et al* with great interest.[1] This study demonstrates the safety and efficacy of tocilizumab in patients with adult-onset Still's disease (AOSD). This randomised placebo-controlled study could be one of the pioneer studies about the use of biological therapy in AOSD. We wanted to ask about some raising concerns.

ACR20, ACR50 and ACR70 responses were used as composite indexes to assess disease activity. In the studies which investigate the effectiveness of a biologic agent in rheumatoid arthritis, general approach is to allow non-steroidal anti-inflammatory drugs (NSAID) if the dose had been stable for a while.[2] However, we could not find any data of NSAIDs in the paper except for a patient with drug eruption related to a painkiller. Did the patients use NSAIDs on demand or a stable dose?

Second, another point that should be clarified is the results about the glucocorticoid sparing effect of tocilizumab. Baseline prednisolone doses, the percentage of decrements and daily dosage of prednisolone at 12th week of tocilizumab and placebo groups are shown in table 1 . If we calculate the decrement mentioned in the paper, there is a disparity in data written in the paper and calculated data. Actually, baseline prednisolone dose of both groups was non-homogeneously distributed. Thus, a median (IQR 25–75) would be more useful to reflect the data better. A non-parametric statistical analysis could be useful in this setting.

Third, it is unclear whether the data of patients who escaped from part 2 of the trial in both study arms integrated into analysis or not. It was shown in the article by Kaneko *et al* that one patient was withdrawn in part 2 of the trial and three patients escaped because of either unmet ACR20 response criteria at the beginning of part 2 or unmet ACR50 response criteria during the part 2 of the trial[1] . In addition to this, the proportion of patients with an ACR50 response was given as 61.5% (8 of 13) at week 12. When considering all these data, we would ask: Were the data of withdrawn patient considered for analysis or excluded? If excluded, ACR50 response rate should be calculated over 12 patients. Also, there should be five patients who did not meet ACR50 response criteria. Even supposing that the withdrawn patient was considered as non-responder, one patient who did not meet ACR50 criteria is missing. Should that patient be another escaper?

Finally, although authors concluded that the investigators must have selected patients who can tolerate placebo to the placebo group, patients in placebo group seem to have more active disease according to the number of swollen joints, ferritin levels, and so on. Considering the outcome measures, we would expect worse results in placebo group because of having more active disease. But except glucocorticoid sparing effect, no differences in outcome measures were obvious between groups in this study. As the baseline disease activity may have an effect on outcome measures, we think a more sophisticated, validated tool or scoring system is needed to determine the disease activity of patients with AOSD.

Thank you again for such great work!

Table 1 Prednisolone data at baseline and 12th week in both groups

	Tocilizumab	Placebo
Baseline prednisolone (mg/day (SD))	23.0 (16.2)	32.5 (20.4)
Decrement at end of 12th week (%)	46.2	21
Mean prednisolone dose (mg/day (SD)) at end of 12th week	9.4 (3.4)	16.3 (6.8)
Calculated mean prednisolone dose (mg/day) at end of 12th week	12.4	25.7

Emre Bilgin,[1] İrem Ozarli,[2] Levent Kılıç,[1] Ertuğrul Çağrı Bölek,[1] Gözde Kübra Yardımcı,[1] Ömer Karadağ[1]

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REFERENCES

1 Kaneko Y, Kameda H, Ikeda K, *et al*. Tocilizumab in patients with adult-onset still's disease refractory to glucocorticoid treatment: a randomised, double-blind, placebo-controlled phase III trial. *Ann Rheum Dis* 2018:1720–9.
2 Gabay C, Emery P, van Vollenhoven R, *et al*. Tocilizumab monotherapy versus adalimumab monotherapy for treatment of rheumatoid arthritis (ADACTA): a randomised, double-blind, controlled phase 4 trial. *Lancet* 2013;381:1541–50.

Response to: 'Some concerns from Turkey' by Bilgin *et al*

We would like to thank Bilgin *et al*[1] for their interest in our paper[2] and asking several questions for clarification.

First, we allowed non-steroidal anti-inflammatory drugs (NSAIDs) in this trial, except for the 6 weeks (from 2 weeks prior to the initiation of trial drugs until 4 weeks) during which NSAIDs were allowed only for fever, in order to assess the status of body temperature appropriately.

Second, the per cent change in glucocorticoid dose were compared between the placebo and tocilizumab groups by means of analysis of covariance model with group as a factor and baseline as a covariate. Therefore, least squares means adjusted for baseline imbalance were presented in our manuscript, which are not consistent with simple summary statistics. We chose this model (not non-parametric one) because of its robustness in terms of the deviation from the normality assumption even with small sample size and prespecified this linear model in statistical analysis plan before the unblinding. We also confirmed that there were no outliers in our data to ensure the appropriateness of the analysis plan.

Third, the primary analysis population for efficacy evaluation in the double-blind phase (parts 1 and 2) was full analysis set, which consisted of patients who received at least one dose of the study medication. Therefore, all randomised patients, except for one patient in the placebo group whose underlying disease turned out to be lymphoma, not adult-onset Still's disease, were included in efficacy analyses. This is consistent with the intention-to-treat principle. The criteria for escape during part 2 in our trial was non-achievement of American College of Rheumatology (ACR) 50 without fever under the initial dose of glucocorticoids while the endpoint at week 12 was ACR50 achievement.

Finally, as Bilgin *et al* pointed out, patients who received placebo had worse disease activity and took higher doses of glucocorticoids, and the efficacy outcomes in the placebo group were better than had been expected. This might be owing to careful patient enrollment or the effectiveness of slightly higher dose of predonisolone in the placebo group. However, this was the first trial using placebo in patients with adult-onset Still's disease, and considering occasional fatal clinical courses of the disease, the investigators were compelled to be cautious.

We totally agree with Bilgin *et al* that establishing more sophisticated, validated tools or scoring systems is essential in trials and the management of adult-onset Still's disease.

Yuko Kaneko,[1] Hideto Kameda,[1,2] Kei Ikeda,[3] Tomonori Ishii,[4] Kosaku Murakami,[5] Hyota Takamatsu,[6] Yoshiya Tanaka,[7] Takayuki Abe,[8] Tsutomu Takeuchi[1]

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REFERENCES

1 Bilgin E, Ozarli İ, Kılıç L, *et al*. Some concerns from turkey. *Ann Rheum Dis* 2020;79:e15.
2 Kaneko Y, Kameda H, Ikeda K, *et al*. Tocilizumab in patients with adult-onset still's disease refractory to glucocorticoid treatment: a randomised, double-blind, placebo-controlled phase III trial. *Ann Rheum Dis* 2018;77:1720–9.

OCTA, a sensitive screening for asymptomatic retinopathy, raises alarm over systemic involvements in patients with SLE

We have read with great interest the letter by Conigliaro *et al*[1] regarding the usefulness of an optical coherence tomography angiography (OCTA) for evaluating retinal microvasculature in patients with systemic lupus erythematosus (SLE). The authors suggested that OCTA is a sensitive tool to detect preclinical ocular changes, and retinal vascular abnormalities are related to renal involvement in patients with SLE. In addition to this, we wish to emphasise that patients with abnormal eye findings should be closely followed, even if it is asymptomatic and in an early phase, keeping in mind the possibility of neuropsychiatric SLE.

Previous study showed that patients with SLE with eye involvement often have neuropsychiatric dysfunction as well,[2] and we here report a case of asymptomatic retinal vasculitis incidentally detected by OCTA during ophthalmological examination prior to the introduction of hydroxychloroquine (HCQ). The patient had no ocular complaints at the onset, but headache and photophobia rapidly developed, which were complicated by neuropsychiatric SLE.

A 37-year-old Japanese man was diagnosed with SLE based on malar rash, wrist arthralgia, leucocytopaenia, and positive antinuclear antibody and antidouble-strand DNA antibody titres. He had no ocular complaints; however, ophthalmological examination prior to HCQ therapy incidentally found retinal vasculitis in both eyes. The patient was initially treated with 30 mg of oral prednisolone. For the next 10 days, he rapidly developed bilateral photophobia. A branch of the retinal vein was occluded and an extensive avascular area was detected by wide-angle OCTA (figure 1A). Moreover, headache and manic-depressive disorder appeared with bilateral macular shadows in the basal ganglia on head MRI fluid-attenuated inversion recovery images. We diagnosed that retinal vasculitis was highly active and exacerbated, complicated with neuropsychiatric SLE. Pulse methylprednisolone therapy (1 g/day ×3 days) and pulse cyclophosphamide therapy (1300 mg per body, equivalent to 750 mg/m^2) were performed, followed by oral prednisolone at 60 mg/day. Headache abated quickly and his mental status stabilised thereafter. His photophobia resolved 5 months after the treatment began. The extent of the avascular area gradually improved, and wide-angle OCTA after 6 months from hospitalisation showed signs of angiogenesis and recanalisation of retinal microvasculature (figure 1B).

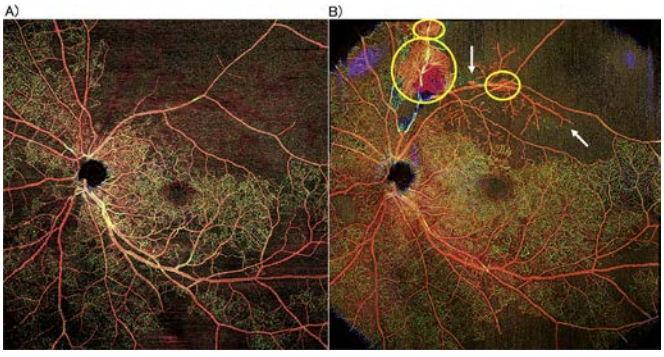

Figure 1 (A) Wide-angle OCTA image at hospitalisation. (B) Wide-angle OCTA image 6 months from hospitalisation. Yellow circles indicate the areas of angiogenesis. White arrows indicate recanalisation. OCTA, optical coherence tomography angiography.

Symptomatic retinopathy has been reported in 0.66% of patients with SLE,[2] whereas when asymptomatic patients are included approximately 10% of patients with SLE are reported to have lupus retinopathy.[3] By using sensitive screening tools such as OCTA, earlier changes of ocular findings can be picked up in patients with SLE. In addition, HCQ is widely used and the number of patients with early-onset or new-onset SLE who undergo ophthalmological screening has been increasing. For these reasons, asymptomatic retinopathy will be found in more patients with SLE. We should once again realise that retinal vasculitis is an important manifestation of SLE, even if the patient is asymptomatic, as in this case. Furthermore, physicians need to be in close contact with ophthalmologists, and patients with abnormal eye findings should be closely followed because they have the possibility of rapid progression of systemic symptoms, including neuropsychiatric SLE.

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REFERENCES

1 Conigliaro P, Cesareo M, Chimenti MS, *et al*. Evaluation of retinal microvascular density in patients affected by systemic lupus erythematosus: an optical coherence tomography angiography study. *Ann Rheum Dis* 2020;79:e20.
2 Ushiyama O, Ushiyama K, Koarada S, *et al*. Retinal disease in patients with systemic lupus erythematosus. *Ann Rheum Dis* 2000;59:705–8.
3 Gao N, Li MT, Li YH, *et al*. Retinal vasculopathy in patients with systemic lupus erythematosus. *Lupus* 2017;26:1182–9.

Response to: 'OCTA, a sensitive screening for asymptomatic retinopathy, raises alarm over systemic involvements in patients with SLE' by Mizuno et *al*

We read with interest the letter titled 'OCTA, a sensitive screening for asymptomatic retinopathy, raises alarm over systemic involvements in patients with SLE' by Mizuno *et al* published in the *Annals of the Rheumatic Diseases*.[1] In the letter, the authors pointed out that retinal vasculitis is an important manifestation of systemic lupus erythematosus (SLE), even if the patient is asymptomatic. Furthermore, they claim that patients with abnormal eye findings should be closely followed. We agree with Mizuno *et al* considering the non-invasive nature of optical coherence tomography angiography (OCTA), the reliability, and that high-resolution images of the retinal vasculature can be obtained approaching histology-level resolution. OCTA seems to be at least as good as invasive dye angiography in different retinal diseases, such as diabetic retinopathy and retinal vein occlusions.[2] An important limitation of OCTA in the evaluation of retinal vascular diseases is the field of view; however, as the authors clearly demonstrated in their case report, this limitation will be likely overcome as commercial systems adopt wide field scan patterns. Actually, there is a strong need for 3D algorithms that will (at least partially) remove both noise and artefacts from OCTA. However, OCTA-based assessment of capillary density and morphology are very similar to histology-based studies, and in our study patients with SLE (without signs of retinopathy according to standard lupus retinopathy classification) displayed a reduced retinal microvascular density compared with normal subjects, in particular those with kidney involvement. Vessel density provided a quantitative metric of capillary network that correlated with age, best corrected visual acuity and clinical features as SLE disease activity.[2] In patients with SLE, as in those with diabetic retinopathy or retinal vein occlusion, the need for frequent examination of retinal vasculature is crucial, mainly in light of therapeutic interventions, but fluorescein and indocyanine green angiography are impractical at that frequencies. Moreover, such angiographies are clearly contraindicated for individual with kidney failure or with a known allergy to fluorescein sodium dye or indocyanine green dye. OCTA may be useful especially for those patients with risk factors for retinopathy such as the presence of antiphospholipid antibodies, kidney or neuropsychiatric involvement.[3] In this context, future studies should address first the sensitivity and specificity of OCTA to detect a vascular retinopathy in patients with SLE when compared with the gold standard technique such as the dye angiography. Then, it should be evaluated in longitudinal studies the predictive value of OCTA alterations in asymptomatic patients in relation to the development of retinal and/or systemic vasculitis. In agreement with data from the literature,[4] OCTA has rapidly gained clinical acceptance and we believe that it will change the practice of the standard of care in patients with SLE. We welcome this report and all others that may appear in the future that will contribute to improve the research agenda in this field.

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REFERENCES

1 Mizuno Y, Nishide M, Wakabayashi T, *et al*. OCTA, a sensitive screening for asymptomatic retinopathy, raises alarm over systemic involvements in patients with SLE. *Ann Rheum Dis* 2020;79:e17.
2 Conigliaro P, Cesareo M, Chimenti MS, *et al*. Evaluation of retinal microvascular density in patients affected by systemic lupus erythematosus: an optical coherence tomography angiography study. *Ann Rheum Dis* 2019;78:287–9.
3 Conigliaro P, Triggianese P, Draghessi G, *et al*. Evidence for the Detection of Subclinical Retinal Involvement in Systemic Lupus Erythematosus and Sjögren Syndrome: A Potential Association with Therapies. *Int Arch Allergy Immunol* 2018;177:45–56.
4 Kashani AH, Chen CL, Gahm JK, *et al*. Optical coherence tomography angiography: A comprehensive review of current methods and clinical applications. *Prog Retin Eye Res* 2017;60:66–100.

Prevention of infections in patients with antineutrophil cytoplasm antibody-associated vasculitis: potential role of hydroxychloroquine

In a recent observational study, Kronbichler *et al* recorded 95 severe/life-threatening infections in 49 of 192 patients (25.5%) with associated vasculitides (AAV) within approximately 2 years following rituximab initiation.[1] Respiratory tract infections were the most common infectious complications. In patients with a positive culture, opportunistic pathogens were frequently seen, though *Pneumocystis jirovecii* was identified in only one case.

Trimethoprim/sulfamethoxazole prophylaxis was administered in 73 of 192 patients (38.0%) and resulted in an impressive reduction in the risk of severe infectious complications by 70%. Approximately half of patients were treated with 480 mg or 960 mg on alternate days. The optimum prophylactic dose of trimethoprim/sulfamethoxazole in patients with non-HIV remains unknown. The current recommendations for the management of AAV encourage prophylaxis against *P. jirovecii* infection with trimethoprim/sulfamethoxazole 960 mg on alternate days or 480 mg daily in all patients being treated with cyclophosphamide, where not contraindicated.[2] There is some evidence suggesting that a lower dose of trimethoprim/sulfamethoxazole may be equally effective and more safe than 480 mg daily. In a randomised controlled 52-week trial involving 183 patients with systemic rheumatic diseases, daily administration of 240 mg of trimethoprim/sulfamethoxazole for the prophylaxis of Pneumocystis pneumonia was as effective as daily single-strength dose of 480 mg and was shown to be superior in safety.[3]

Trimethoprim/sulfamethoxazole can cause serious adverse events. However, in patients with rheumatic diseases exposed to prolonged high-dose glucocorticoid its benefit outweighs a potential harm. In Kronbichler *et al* study, 5 of 73 patients (6.8%) stopped trimethoprim/sulfamethoxazole due to adverse events. Nevertheless, the majority of patients were able to continue trimethoprim/sulfamethoxazole prophylaxis during the 2 year observation (mean 14.7 months).

Trimethoprim/sulfamethoxazole is probably not the only medication that can be routinely used for the prevention of infections in patients with AAV and other rheumatic diseases. Accumulating evidence suggests that antimalarials can have a protective effect against infectious complications in patients with systemic lupus erythematosus (SLE). Feldman *et al* studied the epidemiology of serious infections in a nation-wide cohort of 33 565 patients with SLE.[4] Hydroxychloroquine users had a reduced risk of infection as compared with never users (HR 0.73, 95% CI 0.68 to 0.77). A negative association between duration of antimalarials use and severe infections was also observed in the Spanish cohort of 3658 patients with SLE, though in this study the protective effect was small.[5] In vitro studies indicate that chloroquine/hydroxychloroquine have a broad spectrum of activity against different bacteria, fungi and viruses at clinically achievable plasma concentrations.[6] Although the available data are scarce, the susceptibility of *P. jirovecii* to chloroquine in tested concentrations was shown in the infected human lung fibroblasts,[7] while in a double-blind, randomised clinical trial another antimalarial primaquine in combination with clindamycin were similar in efficacy to trimethoprim-sulfamethoxazole for treatment of mild to moderate Pneumocystis pneumonia in patients with acquired immune deficiency syndrome.[8] We follow a 25-year-old female patient with Takayasu arteritis who developed Pneumocystis pneumonia during treatment with infliximab. Administration of trimethoprim/sulfamethoxazole was complicated by severe bronchial obstruction. Pentamidine or atovaquone is not available in Russia. Two weeks treatment with clindamycin 900 mg daily and hydroxychloroquine 600 mg daily resulted in rapid recovery and radiographic resolution of pneumonia. Subsequently, she continued to take hydroxychloroquine for 2 years.

Hydroxychloroquine may control constitutional symptoms, decrease the risk of lupus flares and organ damage, spare the dosage of corticosteroids, prevent the thrombotic effects of antiphospholipid antibodies and increase the life expectancy of patients with SLE. In a recent meta-analysis of 19 studies involving 19 679 participants, chloroquine/hydroxychloroquine use was associated with a significantly reduced risk of cardiovascular disease.[9] Recently, Casian *et al* reported the successful treatment with hydroxychloroquine in a few patients with AAV and other systemic vasculitides[10] and proposed a phase II 52 week, double-blind, randomised placebo-controlled trial in adult patients with AAV who continue to have active disease after remission-induction therapy (Hydroxychloroquine in antineutrophil cytoplasmic antibody (ANCA) Vasculitis Evaluation—HAVEN). The investigators aim to demonstrate that addition of hydroxychloroquine to standard maintenance therapies may improve vasculitis activity, morbidity and quality of life.

In summary, severe infectious complications are common during treatment with rituximab in patients with AAV. Kronbichler *et al* showed that routine use of trimethoprim/sulfamethoxazole might be justified in rituximab-treated patients. Regarding dosing of trimethoprim/sulfamethoxazole, physicians should probably follow the 2016 European League Against Rheumatism (EULAR)/European Renal Association—European Dialysis and Transplant Association (ERA-EDTA) recommendations for patients being treated with cyclophosphamide, that is, 960 mg on alternate days or 480 mg daily. We speculate that hydroxychloroquine may also decrease the risk of infectious complications and may confer additional benefits to patients with AAV. We suggest that serious infections rate may be an additional secondary endpoint in HAVEN or similar trials. Recently, MAINRITSAN2 study results showed that reduced exposure to rituximab was not associated with an impaired efficacy of maintenance therapy in patients with AAV. Therefore, less intensive regimens of rituximab administration, that is, fever infusions or lower doses, may be another approach to improving safety of treatment.

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REFERENCES

1 Kronbichler A, Kerschbaum J, Gopaluni S, *et al*. Trimethoprim-sulfamethoxazole prophylaxis prevents severe/life-threatening infections following rituximab in antineutrophil cytoplasm antibody-associated vasculitis. *Ann Rheum Dis* 2018;77:1440–7.
2 Yates M, Watts RA, Bajema IM, *et al*. EULAR/ERA-EDTA recommendations for the management of ANCA-associated vasculitis. *Ann Rheum Dis* 2016;75:1583–94.
3 Utsunomiya M, Dobashi H, Odani T, *et al*. Optimal regimens of sulfamethoxazole-trimethoprim for chemoprophylaxis of Pneumocystis pneumonia in patients with systemic rheumatic diseases: results from a non-blinded, randomized controlled trial. *Arthritis Res Ther* 2017;19:7.
4 Feldman CH, Hiraki LT, Winkelmayer WC, *et al*. Serious infections among adult Medicaid beneficiaries with systemic lupus erythematosus and lupus nephritis. *Arthritis Rheumatol* 2015;67:1577–85.
5 Rúa-Figueroa Í, López-Longo J, Galindo-Izquierdo M, *et al*. Incidence, associated factors and clinical impact of severe infections in a large, multicentric cohort of patients with systemic lupus erythematosus. *Semin Arthritis Rheum* 2017;47:38–45.
6 Rolain JM, Colson P, Raoult D. Recycling of chloroquine and its hydroxyl analogue to face bacterial, fungal and viral infections in the 21st century. *Int J Antimicrob Agents* 2007;30:297–308.
7 Bartlett MS, Eichholtz R, Smith JW. Antimicrobial susceptibility of Pneumocystis carinii in culture. *Diagn Microbiol Infect Dis* 1985;3:381–7.
8 Safrin S, Finkelstein DM, Feinberg J, *et al*. Comparison of three regimens for treatment of mild to moderate Pneumocystis carinii pneumonia in patients with AIDS. A double-blind, randomized, trial of oral trimethoprim-sulfamethoxazole, dapsone-trimethoprim, and clindamycin-primaquine. ACTG 108 Study Group. *Ann Intern Med* 1996;124:792–802.
9 Liu D, Li X, Zhang Y, *et al*. Chloroquine and hydroxychloroquine are associated with reduced cardiovascular risk: a systematic review and meta-analysis. *Drug Des Devel Ther* 2018;12:1685–95.
10 Casian A, Sangle SR, D'Cruz DP. New use for an old treatment: hydroxychloroquine as a potential treatment for systemic vasculitis. *Autoimmun Rev* 2018;17:660–4.

Response to: 'Prevention of infections in patients with antineutrophil cytoplasm antibody-associated vasculitis: potential role of hydroxychloroquine' by Novikov *et al*

We thank Dr Novikov *et al* for their letter on the risk of infections of patients with antineutrophil cytoplasm antibody (ANCA)-associated vasculitis and the proposed beneficial effects of hydroxychloroquine (HCQ) to reduce severe infections, as a response to our recently published article 'Trimethoprim-sulfamethoxazole prophylaxis prevents severe/life-threatening infections following rituximab in antineutrophil cytoplasm antibody-associated vasculitis'.[1 2]

Modern therapies and adoption of treatment protocols have improved outcome of patients with ANCA-associated vasculitis. Morbidity and mortality, either attributable to the disease or immunosuppressive measures, remain a challenge for the treating physician. A recent meta-analysis of observational studies found a 2.7-fold increased risk of death with a trend towards improved mortality rates in more recent cohorts.[3] Among patients recruited into the 'early trials' conducted by the European Vasculitis Society (EUVAS), 133 (25%) deaths were recorded over a median follow-up period of 5.2 years. Main causes for death were infections (48%) and active vasculitis (19%) in the first year, while infectious complications remained one of the leading complications leading to mortality (20%) thereafter. Moreover, infections were the leading contributing factor of mortality in this period.[4] Several risk factors leading to infections have been identified in the pre-rituximab era, namely steroid exposure, older age, higher baseline creatinine or dialysis dependency, low lymphocyte count, pulmonary involvement and a rapid fall in the ANCA titre, the latter presumably as direct consequence of a more aggressive immunosuppressive regimen.[5] No such risk factors have been identified in rituximab-treated patients.

Our study[2] identified several independent risk factors to develop severe infections defined as Common Terminology Criteria for Adverse Events (CTCAE) v4.0 grade 3 and above following rituximab initiation. We found an association with older age, endobronchial involvement, chronic obstructive pulmonary disease and prior alemtuzumab use, while trimethoprim-sulfamethoxazole reduced the risk of severe infections by 70%. An overall event rate of 26.06 per 100 person-years was reported with no clear association of an increased risk during the first months of therapy.[2] The high number of infections merits further efforts to reduce infectious complications in patients with ANCA-associated vasculitis.

We agree with Novikov *et al* that trimethoprim-sulfamethoxazole should be used for the prevention of infections in patients with ANCA-associated vasculitis receiving induction treatment,[1] but more data are required to confirm our findings. As mentioned, the ideal dosage of trimethoprim-sulfamethoxazole is unknown and severe adverse events have been reported, including the onset of Stevens-Johnson syndrome, as also discussed in the letter by Wallace *et al*[6] and our response.[7] In patients receiving high doses of steroids, trimethoprim-sulfamethoxazole reduced the risk of *Pneumocystis jirovecii* and its related mortality. Adverse events attributable to trimethoprim-sulfamethoxazole occurred in 32 patients, corresponding to 21.2/100 person-years.[8] A study investigating efficacy of different trimethoprim-sulfamethoxazole doses to prevent *P. jirovecii* assigned patients to either 400/80 mg daily (SS group), 200/40 mg daily (HS group) or an escalation group with a starting dose of 40/8 mg daily and an increase to 200/40 mg daily (ES group). The rate of serious adverse events was similar and the number of deaths numerically higher in the HS group. However, the overall adverse event rate of the SS group was increased compared with the other groups.[9] Although Novikov *et al* argue for the potential of a dose-reduced chemoprophylaxis,[1] firm conclusions from this non-blinded study performed in Japanese patients with rheumatic diseases are not possible to draw.

Antimalarials, namely chloroquine and HCQ, have been recommended in the management of patients with systemic lupus erythematosus.[10] Both agents have anti-inflammatory, immunomodulating, antithrombotic, metabolic, antitumour and anti-infective properties and are currently tested in several clinical trials testing different indications.[11] In a recent systematic review, patients with lupus nephritis and antimalarial exposure showed a lower likelihood to develop end-stage renal failure, hypertension, thrombotic events, infections and deaths.[12] A large study focusing on major infections, defined as disseminated infections, affecting deep organs, requiring hospitalisation or causing death, found a 94% reduction of infectious complications in those receiving antimalarials.[13] Analysis of serious infections, defined as any infection requiring hospitalisation or the use of intravenous antimicrobial agents, found a protective role of HCQ use to reduce infection-related mortality.[14] Feldman *et al* used the Medicaid Analytic eXtract database and identified 33 565 patients, of whom 5078 had 9078 infectious complications. As expected, patients with lupus nephritis were at increased risk. Both groups, those with or without lupus nephritis, had a reduction of infections when HCQ was used.[15]

But how can these data be translated to ANCA-associated vasculitis? No such data exist for ANCA-associated vasculitis. Experience from a single centre on the use of HCQ in eight patients with ANCA-associated vasculitis was recently reported. A benefit was reported by six and two patients were unsure about efficacy of HCQ. No reports regarding safety have been stated by the authors, but a randomised controlled trial of HCQ (HAVEN; EudraCT Number—2018-001268-40) has received funding by the Medical Research Council UK which will address both, safety and efficacy outcomes, in patients with ANCA-associated vasculitis.[16]

We agree that efforts are needed to reduce comorbidities of patients with ANCA-associated vasculitis. Given the pleiotropic effects of HCQ, this agent may have an impact on future treatment protocols of ANCA-associated vasculitis and may reduce the burden of thrombosis, cardiovascular events, malignancy risk and most importantly infectious complications. Randomised controlled trials like the HAVEN study (HCQ in ANCA vasculitis evaluation) are on the way and may pave the way for such protocols.

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REFERENCES

1 Novikov PI, Bulanov NM, Zykova AS, *et al*. Prevention of infections in patients with antineutrophil cytoplasm antibody-associated vasculitis: potential role of hydroxychloroquine. *Ann Rheum Dis* 2020;79:e19.
2 Kronbichler A, Kerschbaum J, Gopaluni S, *et al*. Trimethoprim-sulfamethoxazole prophylaxis prevents severe/life-threatening infections following rituximab in antineutrophil cytoplasm antibody-associated vasculitis. *Ann Rheum Dis* 2018;77:1140–7.
3 Tan JA, Dehghan N, Chen W, *et al*. Mortality in ANCA-associated vasculitis: ameta-analysis of observational studies. *Ann Rheum Dis* 2017;76:1566–74.
4 Flossmann O, Berden A, de Groot K, *et al*. Long-term patient survival in ANCA-associated vasculitis. *Ann Rheum Dis* 2011;70:488–94.
5 Kronbichler A, Jayne DR, Mayer G. Frequency, risk factors and prophylaxis of infection in ANCA-associated vasculitis. *Eur J Clin Invest* 2015;45:346–68.
6 Wallace Z, Choi H, Stone J. Risk of severe infection following rituximab and the efficacy of antimicrobial prophylaxis. *Ann Rheum Dis* 2019.
7 Kronbichler A, Kerschbaum J. Response to: "Risk of severe infection following rituximab and the efficacy of antimicrobial prophylaxis" by Wallace, et al. *Ann Rheum Dis* 2019.
8 Park JW, Curtis JR, Moon J, *et al*. Prophylactic effect of trimethoprim-sulfamethoxazole for Pneumocystis pneumonia in patients with rheumatic diseases exposed to prolonged high-dose glucocorticoids. *Ann Rheum Dis* 2018;77:644–9.
9 Utsunomiya M, Dobashi H, Odani T, *et al*. Optimal regimens of sulfamethoxazole-trimethoprim for chemoprophylaxis of *Pneumocystis* pneumonia in patients with systemic rheumatic diseases: results from a non-blinded, randomized controlled trial. *Arthritis Res Ther* 2017;19.
10 Bertsias GK, Tektonidou M, Amoura Z, *et al*. Joint European League against rheumatism and European renal Association-European dialysis and Transplant Association (EULAR/ERA-EDTA) recommendations for the management of adult and paediatric lupus nephritis. *Ann Rheum Dis* 2012;71:1771–82.
11 Plantone D, Koudriavtseva T. Current and future use of chloroquine and hydroxychloroquine in infectious, immune, neoplastic, and neurological diseases: a mini-review. *Clin Drug Investig* 2018;38:653–71.
12 Olsen NJ, Schleich MA, Karp DR. Multifaceted effects of hydroxychloroquine in human disease. *Semin Arthritis Rheum* 2013;43:264–72.
13 Ruiz-Irastorza G, Olivares N, Ruiz-Arruza I, *et al*. Predictors of major infections in systemic lupus erythematosus. *Arthritis Res Ther* 2009;11.
14 Teh CL, Wan SA, Ling GR. Severe infections in systemic lupus erythematosus: disease pattern and predictors of infection-related mortality. *Clin Rheumatol* 2018;37:2081–6.
15 Feldman CH, Hiraki LT, Winkelmayer WC, *et al*. Serious infections among adult Medicaid beneficiaries with systemic lupus erythematosus and lupus nephritis. *Arthritis Rheumatol* 2015;67:1577–85.
16 Casian A, Sangle SR, D'Cruz DP. New use for an old treatment: hydroxychloroquine as a potential treatment for systemic vasculitis. *Autoimmun Rev* 2018;17:660–4.

Disease progression of Takayasu arteritis in two patients treated with tocilizumab

INTRODUCTION

In recent years there has been growing interest in the use of tocilizumab for the treatment of large vessel vasculitis, particularly giant cell arteritis.[1] Nakaoka *et al* recently suggested that tocilizumab may be of therapeutic benefit in patients with relapsing Takayasu arteritis (TAK).[2] In this journal, a case of aortic ulceration in a patient with TAK while on tocilizumab was described.[3] We report two additional patients who had disease progression despite tocilizumab therapy.

Case 1

A 44-year-old woman with a 20-year history of seropositive rheumatoid arthritis in clinical remission on adalimumab presented with new-onset right upper extremity claudication. A diagnosis of TAK was made after angiography demonstrated arterial thickening of the aorta and arch branches with multifocal narrowing of the subclavian and common carotid arteries. Mycophenolate mofetil and prednisone were added to adalimumab resulting in symptomatic improvement.

A year and a half later the patient experienced worsening upper extremity claudication, paresthesias and jaw discomfort. Erythrocyte sedimentation rate was 87 mm/1 hour and C-reactive protein (CRP) was 37.8 mg/L. Mycophenolate mofetil was continued while adalimumab was switched to tocilizumab 8 mg/kg monthly and prednisone was restarted. Inflammatory markers and symptoms subsequently improved.

After 2 years of tocilizumab therapy, a new diastolic heart murmur was detected. Imaging demonstrated interval progression of the carotid stenosis (figure 1) and development of aortic root dilation and severe aortic valve regurgitation. She underwent ascending aorta, proximal hemiarch and aortic root replacement. Pathology from the surgical specimen demonstrated smouldering aortitis with prominent adventitial fibrosis and mural thickening, consistent with TAK aortitis. Mycophenolate mofetil and tocilizumab were discontinued. She was placed on high-dose glucocorticoids with taper and a 6-month course of cyclophosphamide. One year after surgery, the patient remains in remission on azathioprine 150 mg daily and prednisone 5 mg daily.

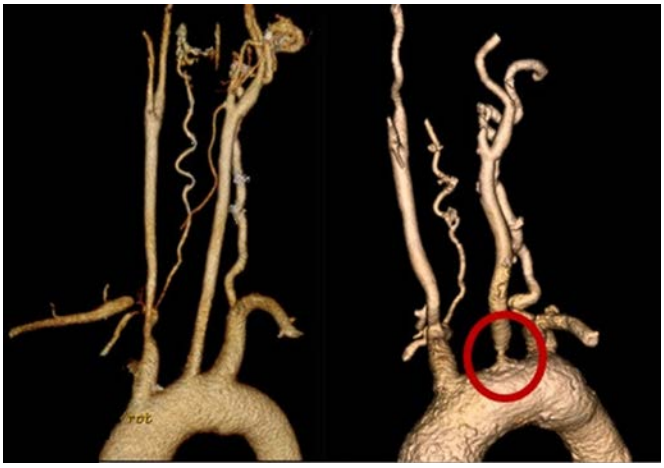

Figure 1 Case 1. Demonstrates progression of the stenosis at the origin of the left common carotid artery.

Case 2

A 25-year-old woman who presented with vision changes, carotidynia and constitutional symptoms was found to have an absent left radial pulse and elevated inflammatory markers. MR angiography demonstrated bilateral carotid artery stenosis and occlusion of left subclavian artery with mural thickening, consistent with TAK. The patient was treated with glucocorticoids and azathioprine. While on azathioprine, carotidynia recurred and inflammatory markers increased, prompting a switch to tocilizumab (8 mg/kg/month). With this, symptoms remitted and inflammatory markers normalised. Fifteen months following treatment with tocilizumab the patient developed recurrent, transient vision loss. A CT angiogram revealed diffuse and severe narrowing of the right common carotid artery, moderate stenosis of the right vertebral and occlusion of the left common carotid, left vertebral and bilateral subclavian arteries. Comparison of repeat angiography to that obtained 1 year prior showed clear evidence of disease progression.

The patient required an ascending aorta to bilateral carotid bypass with transposition of the right vertebral artery. Postoperatively her vision symptoms markedly improved. Currently she remains on azathioprine and low-dose prednisone.

DISCUSSION

Assessment of disease activity in TAK is challenging as inflammatory markers often do not correlate with disease activity.[4] Moreover, tocilizumab directly decreases the synthesis of CRP by inhibiting the biologic activity of interleukin-6, making it difficult to interpret the values of the acute phase reactants.

These two cases clearly illustrate that TAK can progress significantly despite normal inflammatory markers, and despite treatment with tocilizumab. Indeed, the study by Nakaoka *et al* may have underestimated the risk of progression on treatment as standardised imaging was not required in the trial. In summary, monitoring disease activity in TAK with both clinical evaluation and serial imaging studies is of utmost importance.

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REFERENCES

1 Villiger PM, Adler S, Kuchen S, *et al*. Tocilizumab for induction and maintenance of remission in giant cell arteritis: a phase 2, randomised, double-blind, placebo-controlled trial. *The Lancet* 2016;387:1921–7.
2 Nakaoka Y, Isobe M, Takei S, *et al*. Efficacy and safety of tocilizumab in patients with refractory takayasu arteritis: results from a randomised, double-blind, placebo-controlled, phase 3 trial in Japan (the TAKT study). *Ann Rheum Dis* 2018;77:348–54.
3 Liebling EJ, Peterson R, Victoria T, *et al*. Aortic ulceration in a tocilizumab-treated patient with Takayasu arteritis. *Ann Rheum Dis* 2019;78:e116.
4 Salvarani C, Cantini F, Boiardi L, *et al*. Laboratory investigations useful in giant cell arteritis and takayasu's arteritis. *Clin Exp Rheumatol* 2003;21(6 Suppl 32):S23–8.

Paradoxical pulmonary event under tocilizumab treatment for systemic sclerosis-associated usual interstitial pneumonia

We read with interest, the results of the faSScinate trial[1] suggesting tocilizumab had a good safety profile in the treatment of systemic sclerosis-associated interstitial lung disease (SSc-ILD). SSc-ILD is, however, a heterogeneous condition classified according to radiological and histopathological findings. Usual interstitial pneumonia (UIP) is much less frequent than non-specific interstitial pneumonia (approximately 10% and 75% respectively), with no difference in prognosis and survival reported between these two main entities.[2] An earlier report in tocilizumab-treated patients with severe SSc-lung fibrosis reported respiratory function stabilisation in two but slight deterioration in one of the patients.[3] Based on this evidence, we treated one patient in breast cancer remission, with clinical and functional respiratory deterioration and a UIP pattern of SSc-ILD, with off-label tocilizumab. We, hereby, report a reversible life-threatening episode of acute alveolitis that led to treatment discontinuation.

In June 2009, a previously healthy 43-year-old female smoker of Portuguese ancestry experienced Raynaud phenomenon (RP). Three months later, an oestrogen-receptor-positive ductal breast carcinoma was diagnosed and treated by tumorectomy, axillary lymphadenectomy, radiotherapy and tamoxifen. Twelve months after the onset of RP, she developed severely pruritic and diffusely progressive skin thickening, dyspnoea, gastro-oesophageal reflux, recurrent digital ulceration and large joint arthritis. By July 2010, the modified Rodnan Skin Score (mRSS) was 41, and further characterisation revealed interstitial lung disease radiologically categorised by UIP. Initial ratio of first second of forced expiration to forced vital capacity (FVC), absolute and % predicted (p) values for FVC, carbon monoxide diffusing capacity by single-breath technique (DLCO/SB) and DLCO divided by alveolar volume (DLCO/VA) were 94.61%, 1.93 L (70.4%), 1.66 (41.6%) mmol/min/kPa/L and 2.74 (60.2%) mmol/min/kPa, respectively. Resting arterial blood gases on room air were within normal limits. Oesophageal dysmotility and dilatation were documented. There was no echocardiographic evidence of pulmonary artery hypertension. Antinuclear (granular immunofluorescence pattern on HEp2 cells, titre 1/320) and anti-Ro52 kDa antibodies were positive, in the absence of SSc-specific antigens by immunoassay (Euroimmun). Treatment with 6 monthly intravenous cycles of cyclophosphamide 750 mg/m^2 was followed by mycophenolate mofetil (MMF) 2 g/day and prednisolone initially at a dose of 0.5 mg/kg/day for 1 month, subsequently tapered to 10 mg/day. Over the next year, the mRSS decreased to 20 and, despite early deterioration, pulmonary function tests (PFTs) stabilised. She was regularly followed for the next 8 years remaining in cancer remission. During this time, steroids were slowly discontinued. Bosentan (titrated up to 125 mg two times per day) and sildenafil (20 mg three times a day) ameliorated RP and the frequency of digital ulcers. Histamine-2 blockers, proton-pump inhibitors and promotility agents provided symptomatic relief. Recurrent episodes of isolated large joint arthritis (knees and ankles) were observed and treated, with rest and non-steroidal anti-inflammatories. By June 2017, effort-related dyspnoea had become more severe with a progressive decline in the 6-minute walk test (from an initial value of 492 to 300 m, corresponding to 82% and 50% of the expected walking

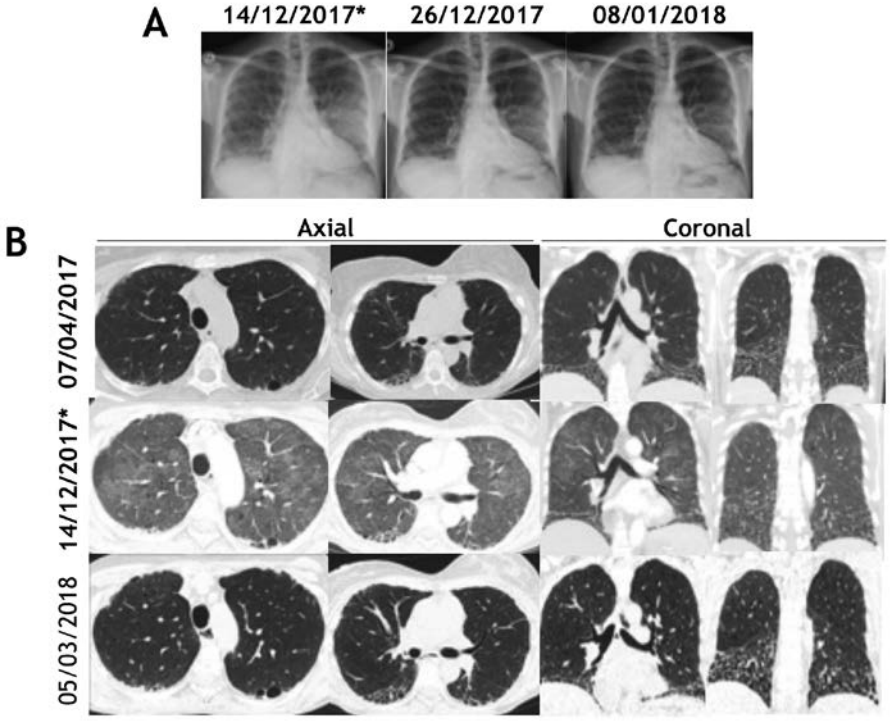

Figure 1 The patient experienced fatigue and self-monitoring revealed an oxygen saturation of 80% on 14 December 2017 (marked by an asterisk). The chest radiograph shows upper lobe opacities bilaterally, substantially reduced 12 and 25 days later (A); HRCT performed prior to the desaturation episode reveal basal interstitial fibrotic changes with an UIP pattern (07 April 2017). Diffuse upper lobe ground glass opacities at the time of desaturation completely resolved on subsequent imaging (B). HRCT, high-resolution computed tomography; UIP, usual interstitial pneumonia.

distances) and a reduction in pDLCO/SB and pDLCO/VA to 28.8% and 50.9%, correspondingly. There was no cardiac anatomical or functional changes and estimated serial pulmonary artery systolic pressures determined by transthoracic echocardiography remained normal. Ambulatory oxygen was provided to meet daily needs.

After a multidisciplinary discussion involving the respiratory, oncology and radiology units, the decision was taken to replace MMF by off-label subcutaneous tocilizumab, 162 mg/week from October 2017. She was symptom free until after the ninth tocilizumab administration when she suddenly experienced fatigue, dyspnoea on minimal effort and self-recorded an oxygen saturation of 80% (room air); hospital evaluation revealed an arterial PaO_2 of oxygen of 56 mm Hg (room air). There were no other prodromal or accompanying symptoms. When compared with previous images, high-resolution computed tomography (HRCT) data showed extensive bilateral ground-glass opacities superimposed on underlying fibrosis. After infection and pulmonary thromboembolism were excluded, she was treated with methylprednisolone pulses (1 g/day for 3 days) followed by prednisolone 0.5 mg/kg/day. Due to rapid improvement, neither bronchoscopy nor lung biopsy were performed. The episode completely resolved with clearance of infiltrates documented by chest radiography and HRCT (figure 1A,B). At the time of event, add-on MMF was not considered a therapeutic option, tocilizumab was switched back to MMF, and prednisolone was tapered over the next 3 months to 10 mg/day. At the last observation, 9 months after the event, there was no deterioration in PFTs.

Our patient experienced a hypoxaemic event, analogous to episodes that are described in the course of idiopathic pulmonary fibrosis, with a similar UIP pattern.[4] Furthermore, adverse event reporting has described drug agents that may paradoxically be associated with interstitial pneumonitis and alveolar damage in other systemic autoimmune conditions such as rheumatoid arthritis.[5] We realise that in the faSSinate trial, the lowest mean predicted % of FVC of baseline patients was 80 (±14), higher than in our patient, who in addition, had advanced lung fibrosis and a longer disease duration. Bearing in mind that the overall effect of tocilizumab therapy is thought to be beneficial in SSc, and notwithstanding, the more severe disease phenotype in our patient, we recommend caution in the follow-up of patients with systemic sclerosis with a UIP form of ILD treated with tocilizumab alone.

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REFERENCES

1 Khanna D, Denton CP, Lin CJF, *et al*. Safety and efficacy of subcutaneous tocilizumab in systemic sclerosis: results from the open-label period of a phase II randomised controlled trial (faSScinate). *Ann Rheum Dis* 2018;77:212–20.
2 Bouros D, Wells AU, Nicholson AG, *et al*. Histopathologic subsets of fibrosing alveolitis in patients with systemic sclerosis and their relationship to outcome. *Am J Respir Crit Care Med* 2002;165:1581–6.
3 Fernandes das Neves M, Oliveira S, Amaral MC, *et al*. Treatment of systemic sclerosis with tocilizumab. *Rheumatology* 2015;54:371–2.
4 Collard HR, Moore BB, Flaherty KR, *et al*. Acute exacerbations of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2007;176:636–43.
5 Perez-Alvarez R, Perez-de-Lis M, Diaz-Lagares C, *et al*. Interstitial lung disease induced or exacerbated by TNF-targeted therapies: analysis of 122 cases. *Semin Arthritis Rheum* 2011;41:256–64.

Predictive factors of pneumocystis pneumonia in patients with rheumatic diseases exposed to prolonged high-dose glucocorticoids

We read with interest the article by Jun Won Park *et al*, describing in a retrospective study the prophylactic effect of trimethoprim-sulfamethoxazole (TMP-SMX) to prevent *Pneumocystis jiroveci* pneumonia (PCP) in patients with rheumatic diseases exposed to prolonged high-dose glucocorticoids.[1] High-dose glucocorticoid is indeed a well-known and major risk factor for PCP supporting the investigation carried out here. The conclusions of the work are that TMP-SMX prophylaxis is very effective and safe at preventing PCP.[2] TMP-SMX was also associated with a reduction in pneumocystis-related mortality, a very important result given that mortality is higher in patients suffering from systemic diseases, in particular in cases of granulomatosis with polyangiitis, than in HIV-infected patients.[3]

We agree with the need to assess the incidence of PCP in patients with rheumatic diseases, especially for those at highest risk. The results from this study are clear and convincing; they also confirm previously published works conducted on smaller cohorts.[4]

However, we believe this study would have been even more compelling had it included an analysis of risk factors associated with the occurrence of PCP in patients without prophylaxis.

Indeed, to our knowledge, no study assessing predictive factors of PCP among patients receiving high-dose glucocorticoids for systemic diseases exists. Yet, for the clinician and particularly the rheumatologist, defining which patients are at highest risk of developing PCP and should subsequently receive prophylaxis is the main concern in a clinical point of view. We believe a subanalysis of your cohort may bring some answers to this often-debated question. Presently, in the absence of randomised studies, recommendations for PCP prophylaxis are limited to patients suffering from granulomatosis with polyangiitis, patients treated by cyclophosphamide or high dose of methotrexate and patients receiving corticosteroids >20mgdaily for at least 30 days or >16 mg daily for at least 60 days associated with at least one of the following risk factors: age >50, malnutrition and lymphopenia $<600/mm^3$.[5]

These criteria remain imprecise and poorly documented. The present study may allow for a more thorough investigation of the risk factors associated with PCP in the population not under prophylaxis. In our view, this additional analysis might add a lot of value to the present work.

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REFERENCES

1 Park JW, Curtis JR, Moon J, *et al*. Prophylactic effect of trimethoprim-sulfamethoxazole for pneumocystis pneumonia in patients with rheumatic diseases exposed to prolonged high-dose glucocorticoids. *Ann Rheum Dis* 2018;77:644–9.
2 Yale SH, Limper AH. Pneumocystis carinii pneumonia in patients without acquired immunodeficiency syndrome: associated illness and prior corticosteroid therapy. *Mayo Clin Proc* 1996;71:5–13.
3 Sowden E, Carmichael AJ. Autoimmune inflammatory disorders, systemic corticosteroids and pneumocystis pneumonia: a strategy for prevention. *BMC Infect Dis* 2004;4:42.
4 Godeau B, Coutant-Perronne V, Le Thi Huong D, *et al*. Pneumocystis carinii pneumonia in the course of connective tissue disease: report of 34 cases. *J Rheumatol* 1994;21:246–51.
5 Khellaf M, Godeau B. [Pneumocystis pneumonia among patients with systemic diseases]. *Presse Med* 2009;38:251–9.

Effectiveness of low-dose radiation therapy on symptoms in patients with knee osteoarthritis

We read with interest the milestone work in the *Annals of the Rheumatic Diseases* by Mahler and colleagues[1] on the effectiveness of low-dose radiation therapy (LDRT) on symptoms in patients with knee osteoarthritis (OA). The authors have conducted a series of studies to investigate the effects of LDRT on OA, which compensate the lack of high-level evidence in the medical literature, and inevitably forcing us to re-examine the true effectiveness of LDRT on OA in real-world clinical practice.[1–3] The group should be applauded for their accomplishments. In this well-designed and well-conducted randomised, double-blinded, sham-controlled trial, they found no substantial beneficial effect on symptoms and inflammatory signs of LDRT in patients with knee OA, and advise against LDRT as routine treatment. However, we have two major concerns and want to discuss them with the authors.

One point that puzzled us is the heterogeneity of the participant population. According to the inclusion and exclusion criteria, patients with Kellgren and Lawrence (K&L) scores ranging from 1 to 3 would be eligible for this trial. We completely agree with the authors about the inclusion of the relevant patient population (basically reflected a real-world population), and a more heterogeneous group of patients with OA has better external generalisability. However, the participants were not stratified by an objective covariate, such as severity of knee OA, but was stratified by a subjective covariate—Numeric Rating Scale for pain.[4] Therefore, the diversity of the individuals can lead to the population who response to LDRT (eg, early stage of knee OA, K&L scores 1–2) rather limited, and we suspect that stratified randomisation by K&L scores might be a better method of allocating patients.

Another aspect is the control of the potential confounding variables. Patients who reported insufficient response to analgesics were encouraged not to change the analgesic regimen, but the possible influence of analgesics could not be ruled out. Furthermore, daily physical activities including walking, standing, squatting, kneeling, climbing, carrying and lifting during the study had not been assessed or adjusted, which would also jeopardise the conclusions of the clinical trial.[5] Overall, LDRT is not as effective as previously thought, and further research is warranted to confirm these results, add evidence to the clinical practice and change the perception of LDRT for knee OA.

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REFERENCES

1 Mahler EAM, Minten MJ, Leseman-Hoogenboom MM, *et al*. Effectiveness of low-dose radiation therapy on symptoms in patients with knee osteoarthritis: a randomised, double-blinded, sham-controlled trial. *Ann Rheum Dis* 2019;78:83–90.
2 Minten MJM, Leseman-Hoogenboom MM, Kloppenburg M, *et al*. Lack of beneficial effects of low-dose radiation therapy on hand osteoarthritis symptoms and inflammation: a randomised, blinded, sham-controlled trial. *Osteoarthritis Cartilage* 2018;26:1283–90.
3 Minten MJ, Mahler E, den Broeder AA, *et al*. The efficacy and safety of low-dose radiotherapy on pain and functioning in patients with osteoarthritis: a systematic review. *Rheumatol Int* 2016;36:133–42.
4 Krebs EE, Carey TS, Weinberger M. Accuracy of the pain numeric rating scale as a screening test in primary care. *J Gen Intern Med* 2007;22:1453–8.
5 Lazaridou A, Martel MO, Cornelius M, *et al*. The association between daily physical activity and pain among patients with knee osteoarthritis: the moderating role of pain catastrophizing. *Pain Med* 2018.

Response to: 'Effectiveness of low-dose radiation therapy on symptoms in patients with knee osteoarthritis' by Wu *et al*

We thank Wu *et al* for their interest in and encouragement of our randomised studies in which we found no substantial beneficial effect on symptoms and inflammatory signs of low-dose radiation therapy (LDRT) in patients with knee and hand osteoarthritis (OA).[1–3] The authors emphasise that our results inevitably force involved clinicians to re-examine the true effectiveness of LDRT on OA in real-world clinical practice. However, they addressed two constructive concerns related to our work, to which we will reply hereby.

The first point addresses the heterogeneity of the participants reflecting a real-world population, that is, patients with clinical diagnosis of knee OA after failure of conservative treatment options. Wu *et al* suggest that randomisation stratified by an objective measure for severity of knee OA (eg, K&L scores) might be preferable to the numeric rating scale for pain to stratify patients. We do not agree on this point because we hypothesised that LDRT would primarily improve clinically relevant OA symptoms including pain and disfunctioning. Accordingly, the primary outcome was the proportion of the OMERACT-OARSI responders, including pain, function and patient global assessment. To prevent bias due to an unbalanced randomisation of a very severe pain score, stratification was performed for pain intensity. Of note, the proportion of patients with K&L score ≥2 in the LDRT and sham group was comparable between groups (LDRT group: 56%; sham group: 61%) as were radiographic scores for joint space narrowing and osteophytes. Additional analyses adjusting for these different kinds of radiographic scores did not modify our results. In conclusion, stratification for pain intensity is in line with the aim of our study to evaluate the effect on symptoms in patients with knee (and hand) OA.

The second concern risen relates to the potential confounding effect of use of analgesics and the ability to perform daily physical activities. We agree that a possible influence of those potential confounders cannot be ruled out due to a potential unbalanced randomisation of these variables considering the limited sample size. However, we think the influence of those two potential confounders is limited. First, analgesic use was allowed, but patients were encouraged not to change it during the study period. Absolute numbers of participants using analgesics at baseline and at all follow-up moments were very low, that is, 36% at baseline and 35% at 3 months did use paracetamol a few days a week or almost daily; for non-steroidal anti-inflammatory drugs this was 20% at baseline and 11% at 3 months, and for tramadol 2% versus 2%, respectively. In addition, these proportions were comparable between the LDRT and sham groups at all time points. Moreover, with regard to daily physical activities as potential confounder in evaluating the relationship between LDRT/sham treatment and symptoms, we argue that we assessed the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) subscale physical function that assesses a diversity of important activities of daily living. At baseline, we did not find an imbalance in this measure between groups. Moreover, sensitivity analysis taking into account the baseline WOMAC subscale physical function as potential confounder yielded similar results. In summary, we think that our conclusion is valid and we do not have any reasons to assume that differences between groups in use of analgesics and physical activities jeopardise the results. However, we agree with the conclusion of the authors that future research with replication of our studies is desirable.

It is disappointing that the 2018 update of the German recommendations for LDRT for benign conditions (DEGRO) continues to advise LDRT for patients with knee OA after failure of non-surgical options and without indication for knee replacement. These criteria mirror the eligibility criteria of our randomised controlled trial. Notwithstanding this, the authors of the guidelines question the validity of our results considering that about half of the patients had duration of symptoms ≤5 years. Sensitivity analysis taking into account the duration of symptoms as potential confounder yielded similar results. Remarkably, by refuting the high-level evidence of absence of clinically significant effect of LDRT on OA from our trials and our previous systematic review,[2–5] the authors of the DEGRO recommendation reverse the burden of proof as they base their recommendation on studies of lower quality, that is, the results of methodologically weaker studies (uncontrolled, retrospective design) and a survey among centres for radiation therapy in Germany. This illustrates that significant efforts are needed to change current beliefs of both patients and involved clinicians into accepting scientific evidence and subsequently to deimplement the use of LDRT for knee and hand OA.

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REFERENCES

1 XD W, KJ H, Xiang BY. eLetter: effectiveness of low-dose radiation therapy on symptoms in patients with knee osteoarthritis. *Ann Rheum Dis* 2020;79:e24.
2 Mahler EAM, Minten MJ, Leseman-Hoogenboom MM, *et al*. Effectiveness of low-dose radiation therapy on symptoms in patients with knee osteoarthritis: a randomised, double-blinded, sham-controlled trial. *Ann Rheum Dis* 2019;78:83–90.
3 Minten MJM, Leseman-Hoogenboom MM, Kloppenburg M, *et al*. Lack of beneficial effects of low-dose radiation therapy on hand osteoarthritis symptoms and inflammation: a randomised, blinded, sham-controlled trial. *Osteoarthritis Cartilage* 2018;26:1283–90.
4 Minten MJ, Mahler E, den Broeder AA, *et al*. The efficacy and safety of low-dose radiotherapy on pain and functioning in patients with osteoarthritis: a systematic review. *Rheumatol Int* 2016;36:133–42.
5 DEGRO. Strahlentherapie gutartiger Erkrankungen. 2018. Available: https://www.degro.org/wp-content/uploads/2018/11/S2-Leitlinie-Strahlentherapie-gutartiger-Erkrankungen-update-2018-Endversion.pdf