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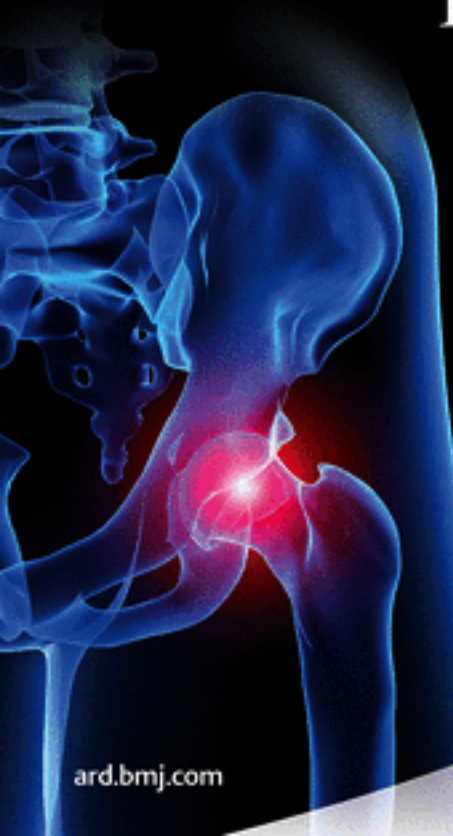

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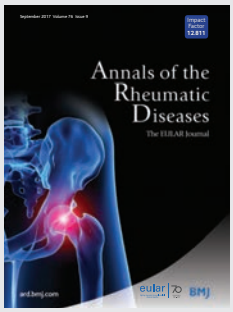




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## Editorial



## Recommendation



## Clinical and epidemiological research



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# The preclinical phase of PsA: a challenge for the epidemiologist

Alexis Ogdie

Psoriatic arthritis (PsA) is a chronic inflammatory arthritis and, like many chronic diseases, often has an insidious onset. The disease likely begins well before patients first present to a rheumatologist and even before they first have symptoms. Several studies have confirmed the presence of subclinical joint and entheseal inflammation in patients with psoriasis.[1–5] However, relatively little is known about the preclinical phase of PsA. The 'preclinical phase' is emerging as an important issue in many rheumatic diseases and is an important area of ongoing research. A preclinical phase in rheumatoid arthritis (RA) has been fairly well described. Current research suggests that, among predetermined or genetically susceptible individuals, an inciting agent (eg, smoking) ignites asymptomatic inflammation. This is followed by asymptomatic synovitis, development of symptoms, a transition to clinically apparent RA and subsequent diagnosis, and then a chronic inflammatory phase.[6–8] The preclinical phase of RA is supported by studies identifying the presence of autoantibodies ≥10 years prior to presentation of clinically apparent disease in RA.[9]

In this issue of the *Annals of Rheumatic Disease*, Kristensen *et al*[10] provide a population-based description of the period leading up to the diagnosis of PsA, potentially the 'preclinical phase' of PsA in their paper 'Societal costs and patients' experience of health inequities before and after diagnosis of psoriatic arthritis'. In this paper, we see that healthcare costs begin to rise approximately 5 years prior to the diagnosis of PsA, peak around the time of diagnosis, improve slightly (likely from institution of therapy and appropriate care) but then remain high (figure 1). Additionally, the investigators demonstrate an elevated prevalence of comorbidities compared with the general population both in the three years before and three years after diagnosis of PsA.

The findings in this study highlight the interesting and often complex issues encountered in designing and interpreting epidemiological studies of PsA. In particular, the elevated prevalence of comorbidities before PsA diagnosis has implications for studies examining comorbidities linked to PsA and studies aiming to identify risk factors for PsA. For both study designs, the critical issue is the definition of when PsA begins and how to manage this potential preclinical or prediagnosis phase of PsA.

## DISENTANGLING 'PRECLINICAL' PSA FROM PSORIASIS AND/OR DELAYED DIAGNOSIS: THE ULTIMATE CHALLENGE

Studying the preclinical phase of rheumatic disease from a population-based perspective is challenging, and this is particularly true for PsA because it is complicated by the coexistence of psoriasis, another disorder associated with systemic inflammation. It is possible that during a subclinical phase of the disease asymptomatic inflammation leads to development of comorbidities and socioeconomic disability, as seen in Kristensen *et al*. Inflammatory states may increase fatigue and malaise, and subsequently, patients miss work, they see the doctor and get more tests and more problems are identified (and thus, this period prior to diagnosis is complicated by potential observation and/or ascertainment bias). However, one wonders whether comorbidities preceding PsA could be in fact attributed to psoriasis. The majority of patients with PsA have psoriasis and the average duration of psoriasis at the time of PsA diagnosis is approximately 7–10 years.[11] Even mild psoriasis is associated with comorbidities including cardiovascular disease.[12 13] Alternatively, these findings may be explained by delayed diagnosis of clinically apparent (rather than clinically asymptomatic) PsA, as reported in previous studies.[14 15] Regardless of the explanation of the findings in Kristensen *et al*, the interval from start of asymptomatic inflammation to diagnosis is particularly important for the design and interpretation of PsA epidemiology studies.

## DEFINING RISK FACTORS FOR THE DEVELOPMENT OF PSA: MANAGING THE TROUBLING PRECLINICAL PHASE

The most challenging aspect of the preclinical phase of PsA is managing its potential existence in studies of risk factors for PsA. In some ways, PsA is the perfect disease for which to identify risk factors. There is a known 'risk pool' (patients with psoriasis) that may develop the disease. And theoretically, if we could identify risk factors for the disease, we could either diagnose the disease earlier (and improve long-term outcomes) or potentially even mitigate the risk for developing the disease by removing the risk factor (eg, smoking cessation). We have previously discussed more general methodological considerations in risk factor studies in PsA.[16] However, this study raises a new concern. The risk assessment window typically used in risk factor studies (shown in figure 1) may be biased: if in the 3–5 years preceding a PsA diagnosis the patient actually has preclinical PsA, we may identify 'risk factors' that are part of the disease rather than true aetiological or causal factors. Case–control studies (which start at diagnosis and look back for exposures) and studies using time-updated exposures/covariates would be particularly at risk for this bias. (An aside: Time-updated exposures are used to acknowledge and account for the fact that people may change exposure status over time during long observation periods. Cox proportional hazards models can account for these changes over time. Time-updated exposures also can be problematic for other reasons if you suspect that the value of the exposure at one time point influences its value at a subsequent time point. We'll set that aside for now.[17]) Let's use socioeconomic status (SES) as an example. Kristensen *et al* found that SES effectively worsens in the 3–5 years prior to PsA diagnosis. If we use a case–control design (or a cohort study using SES as a time-updated risk factor), we will come to the conclusion that lower SES is a risk factor for PsA because, as long as the data are available, the risk factor will be assessed closest to diagnosis. However, it may be that lower SES is instead the result of preclinical PsA. This is problematic if we are aiming to establish causality. Potential ways to address this problem include (a) cohort study designs in which risk factors are identified at baseline only (eg, at psoriasis diagnosis or start of follow-up) or (b) sensitivity analyses restricting the window during which risk factors are assessed (eg, closing the risk factor assessment window 3–5 years prior to diagnosis). On the other hand, if we are looking for factors that may signal onset of clinical symptoms and we aim to detect these patients earlier, then potentially the window should be limited to the

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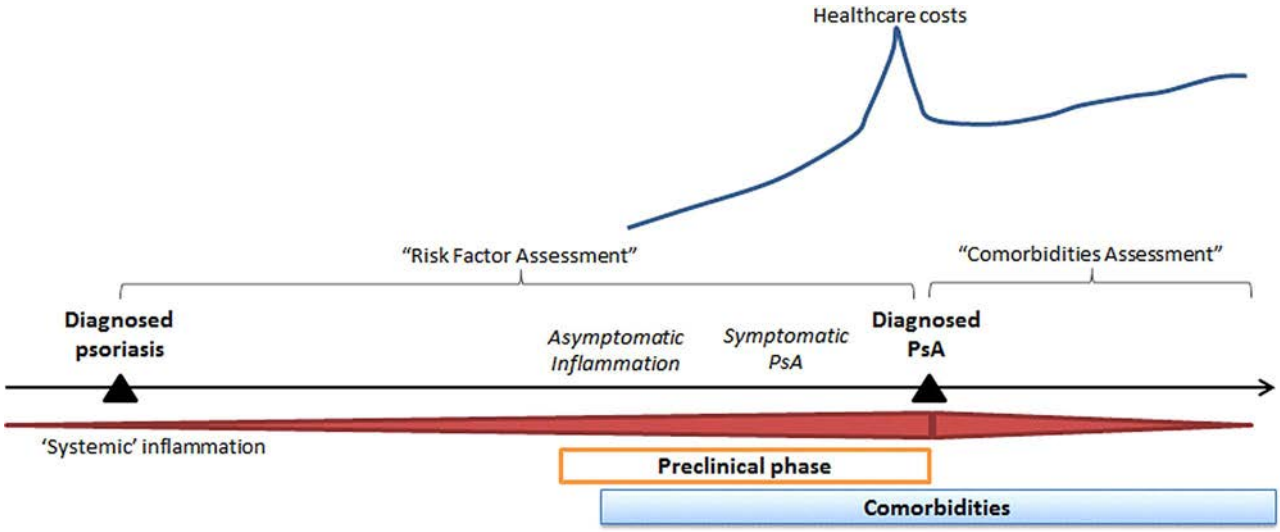


**Figure 1** A preclinical phase of psoriatic arthritis (PsA) likely includes an asymptomatic inflammatory phase followed by development of symptoms and ultimately diagnosis. Kristensen *et al* demonstrated increasing comorbidities and societal economic costs in the 3 and 5 years prior to diagnosis, respectively. Disentangling the 'preclinical phase' of PsA from psoriasis is challenging. Furthermore, this preclinical phase makes assessment of comorbidities and risk factors challenging by impacting the interval over which risk factors and comorbidities may be assessed.

3–5 years prior to diagnosis. This issue reinforces the importance of having a prespecified question (eg, are we seeking potentially causal/aetiological factors or preclinical factors for earlier disease identification?) and designing the study accordingly.

Now, to flip the coin: maybe comorbidities and SES are truly causal risk factors for the development of PsA among patients with psoriasis and this study provides evidence for these risk factors (although a comparison cohort of patients with psoriasis who didn't develop PsA is not included in the study). Obesity and hyperlipidaemia have been identified as risk factors for psoriasis and PsA.[18–21] Biological plausibility exists: comorbidities may be associated with inflammation, an altered endocrine state or mechanical/sheer forces (in the case of obesity)[22]—so, these are possible legitimate triggers for disease onset. In this case, these factors should theoretically be positively associated regardless of the window during which they are assessed (although with potentially differing effect sizes). Thus, investigators should consider assessing both windows in separate analyses to confirm the association.

## IMPLICATIONS FOR EXAMINING RISK OF DEVELOPMENT OF COMORBIDITIES AMONG PATIENTS WITH PSA

This preclinical phase also makes understanding relationships between the disease and comorbid conditions difficult. In assessing the risk for comorbidities in patients with PsA, we often 'start' follow-up time at the diagnosis of PsA ('comorbidities assessment' window in figure 1). We do this in order to examine whether comorbidities can be attributed to the disease rather than pre-existing conditions. Additionally, as incidence is calculated as the number of new cases in the population at risk for the disease, patients with pre-existing comorbidities are not included in this calculation. The study by Kristensen *et al* suggests that if we start the follow-up time at the diagnosis of PsA, we may be falsely assuming a lower incidence of comorbidities by excluding the initial cases when, in fact, these initial cases may actually have been caused by the preclinical phase of the disease. This concept is known as 'depletion of the susceptibles'—the patients most at risk for developing these comorbidities may be excluded (depleted) from the study because they have already developed the comorbidity before study follow-up begins. Following only patients without the disease at baseline may lower the HR relating the risk of an incident comorbidity in patients with PsA compared with the general population. In a recent study examining the risk of fracture among patients with PsA, we managed this potential bias by including a sensitivity analysis in which patients with a fracture prior to start date were included and we adjusted for previous fracture to determine whether it substantially changed the HR.[23] Investigators could also consider moving back the start date by 3 years, for example. Standard analyses are still informative and do appropriately answer the clinically relevant question, 'For this patient with a diagnosis of PsA, what is the likelihood of this patient developing a particular comorbidity?' The bigger problem is attribution; in calculating attributable risk of fracture related to PsA, we will have missed some of the new fractures that could in fact be related to the disease (in the preclinical phase) but occurred prior to formal diagnosis.

## FUTURE CONSIDERATIONS

In summary, the paper by Kristensen *et al* raises many important questions about the years prior to PsA diagnosis and how to best study both the risk factors for PsA and the long-term outcomes related to PsA. Population-based studies of PsA have several strengths; studies addressing risk factors for the development of PsA and long-term outcomes would not be possible without large generalisable populations of patients with physician-diagnosed PsA, physician recording of important covariates (diagnoses, lifestyle habits and medications) and several years of follow-up. However, care should be taken to interpret findings from epidemiology studies of PsA in light of this potential preclinical phase, and sensitivity analyses to address this window should be considered. In the bigger picture, this study by Kristensen *et al* suggests that this disease, once thought to be a relatively benign condition, has costly outcomes. It also suggests, however, that there may be a crucial period during which we can identify and intervene upon patients with early PsA in order to improve outcomes.[24]

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# EULAR recommendations for the use of imaging in the clinical management of peripheral joint osteoarthritis

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## ABSTRACT

The increased information provided by modern imaging has led to its more extensive use. Our aim was to develop evidence-based recommendations for the use of imaging in the clinical management of the most common arthropathy, osteoarthritis (OA). A task force (including rheumatologists, radiologists, methodologists, primary care doctors and patients) from nine countries defined 10 questions on the role of imaging in OA to support a systematic literature review (SLR). Joints of interest were the knee, hip, hand and foot; imaging modalities included conventional radiography (CR), MRI, ultrasonography, CT and nuclear medicine. PubMed and EMBASE were searched. The evidence was presented to the task force who subsequently developed the recommendations. The strength of agreement for each recommendation was assessed. 17 011 references were identified from which 390 studies were included in the SLR. Seven recommendations were produced, covering the lack of need for diagnostic imaging in patients with typical symptoms; the role of imaging in differential diagnosis; the lack of benefit in monitoring when no therapeutic modification is related, though consideration is required when unexpected clinical deterioration occurs; CR as the first-choice imaging modality; consideration of how to correctly acquire images and the role of imaging in guiding local injections. Recommendations for future research were also developed based on gaps in evidence, such as the use of imaging in identifying therapeutic targets, and demonstrating the added value of imaging. These evidence-based recommendations and related research agenda provide the basis for sensible use of imaging in routine clinical assessment of people with OA.

## INTRODUCTION

Osteoarthritis (OA) is a major cause of pain and disability worldwide. Although conventional radiography (CR) is the most commonly used technique to evaluate structural features of OA, significant advances have been made in the field of imaging over the last decade, allowing a more accurate evaluation of both bone and soft-tissue abnormalities. While newer modalities such as MRI and ultrasound have increased the understanding of the multiple pathologies contributing to the OA phenotype, it is not clear how they should be used in routine care. The role of imaging in clinical practice for OA diagnosis, management and follow-up has not been clearly defined. Despite this limitation, the increased availability of modern imaging has expanded its use, with possible excesses[1] leading to increased costs. A European League Against Rheumatism (EULAR) task force was therefore created to develop evidence-based recommendations on the use of imaging in the management of symptomatic, peripheral joint OA, for clinicians who treat OA in their clinical practice.

## METHODS

A group selected from a range of expertise (rheumatologists, radiologists, primary care physicians, methodologists and patients) and representing nine countries was included in the task force. During the first meeting, the focus of the recommendations (symptomatic OA affecting the knee, hip, hand or foot) was clarified. Clinically relevant questions on the application of imaging in OA were proposed and nine research questions were selected by consensus to guide a detailed systematic literature review (SLR). Two questions that covered the same area were subsequently combined. The areas of diagnosis, prognosis, follow-up and treatment were covered. The questions were rephrased according to the population, intervention, comparison, outcome (PICO) (see online supplementary file S1 research questions).

An SLR was performed by one of the authors (GS), with checking of all extractions by one of three other authors experienced in SLRs. The search strategies were based on both MeSh terms and free text. The searches were performed separately for each joint (see online supplementary file S2 search strategies). The titles and abstracts of the references that were retrieved were screened by the same author according to predefined inclusion and exclusion criteria, based on the PICO for each question, and potentially relevant articles were evaluated in their full text. Studies in English including adults (≥18) with symptomatic OA of the knee, hip, hand and foot were eligible for inclusion. Imaging modalities included were CR, MRI, ultrasonography (US), CT and nuclear medicine techniques (scintigraphy, positron emission tomography). Randomised controlled trials (RCTs), systematic reviews and meta-analyses, controlled clinical trials, case–control studies, cross-sectional studies and cohort studies were eligible for inclusion. Studies had to examine the role of imaging in

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the following: in making a diagnosis of OA; in detecting OA elementary lesions; for differential diagnosis; in the management of OA; in predicting outcome and therapeutic response; for follow-up of disease course and to guide treatment. The same articles could be included in more than one search. Due to the variety of joint sites and imaging and the expectation of a strong degree of heterogeneity across studies, meta-analyses were not prespecified before study selection and extraction. The methodological quality of the included studies was not assessed by quality scores, but some aspects were considered for all studies, together with design-specific indicators. For all studies, study design, sample size and setting sampling were considered. For RCTs allocation concealment, drop-out rate as well as the presence of funding, for diagnostic studies the adequacy of the reference standard and for cohort studies the presence of adjustment for confounders were also evaluated. Each aspect was evaluated separately as leading to high, low or unclear risk of bias.

During the second meeting, the results of the literature review were presented and the experts developed 'over-arching' statements (background statements to preface the recommendations) and drafted seven recommendations through a process of discussion and consensus. The number of recommendations emerged through the discussion after the presentation of the literature. To explore the presence of additional evidence concerning two recommendations, two more research questions on (1) the different performance of various radiographic views in detecting OA features and (2) the accuracy of imaging-guided compared with blind joint injections were added to the original eight, with two additional literature searches (see online supplementary file S1, research questions and S2, search strategies). After evaluation of these results, the Task Force confirmed the final wording of the recommendations and scored the perceived level of agreement (LOA) for each statement using a 0–10 numeric rating scale (0=fully disagree; 10=fully agree), reflecting both literature evidence and expert opinion. Recommendations for further research were then developed based on gaps in the SLRs.

## RESULTS

The searches in the electronic databases (PubMed, EMBASE) were performed up to the end of January 2015 for the main searches and December 2015 for the additional searches. The initial search resulted in 6858 records (615 duplicates). Of the remaining 6243 articles, 4926 were excluded based on the title and abstracts, leaving 1317 articles for detailed review. All full-text articles were retrieved, 986 articles were excluded after reviewing the full text, leaving 331 articles for inclusion (see online supplementary file S3). The hand search of the references of the included studies identified 33 additional articles, leading to a total of 364 studies finally analysed. Articles that were relevant to more than one research question were used for each question as appropriate. The number of articles included for each site and imaging is shown in online supplementary figure S4. The complete results of the SLR with references are reported in the online supplementary file S5.

The additional search on the comparison of different radiographic views resulted in 4774 articles (225 duplicates). Of the remaining 4549, 4496 were excluded based on the title and abstracts, leaving 53 articles for detailed review. Twenty-three articles were excluded after reviewing the full text, leaving 30 articles for inclusion. The hand search identified one additional article for inclusion, leading to a total of 31 articles finally included (see online supplementary file S6).

The additional search on the added value of imaging to guide intra-articular procedures resulted in 5379 articles (834 duplicates). Of the remaining 4545, 4520 were excluded based on the title and abstracts, leaving 25 articles for detailed review. Nineteen articles were excluded after reviewing the full text, leaving six articles for inclusion. The hand search identified two additional articles for inclusion, leading to a total of eight articles finally included (see online supplementary file S7). The complete results of the additional searches with references are reported in the online supplementary file S8.

### Recommendations

Table 1 summarises the seven recommendations with their corresponding level of evidence and LOA. Each recommendation is presented in detail below.

#### Overarching statements

1. These recommendations pertain only to symptomatic OA.
2. Imaging abnormalities of OA are commonly seen especially with increasing age.

**Table 1** Recommendations, levels of evidence and level of agreement (LOA)

| Recommendation | Level of evidence | LOA, mean (95% CI) |
|---|---|---|
| 1. Imaging is not required to make the diagnosis in patients with typical* presentation of OA. | III–IV | 8.7 (7.9 to 9.4) |
| 2. In atypical presentations, imaging is recommended to help confirm the diagnosis of OA and/or make alternative or additional diagnoses. | IV | 9.6 (9.1 to 10) |
| 3. Routine imaging in OA follow-up is not recommended. However, imaging is recommended if there is unexpected rapid progression of symptoms or change in clinical characteristics to determine if this relates to OA severity or an additional diagnosis. | III–IV | 8.8 (7.9 to 9.7) |
| 4. If imaging is needed, conventional (plain) radiography should be used before other modalities. To make additional diagnoses, soft tissues are best imaged by US or MRI and bone by CT or MRI. | III–IV | 8.7 (7.9 to 9.6) |
| 5. Consideration of radiographic views is important for optimising detection of OA features; in particular for the knee, weightbearing and patellofemoral views are recommended. | III | 9.4 (8.7 to 9.9) |
| 6. According to current evidence, imaging features do not predict non-surgical treatment response and imaging cannot be recommended for this purpose. | II–III | 8.7 (7.5 to 9.7) |
| 7. The accuracy of intra-articular injection depends on the joint and on the skills of the practitioner and imaging may improve accuracy. Imaging is particularly recommended for joints that are difficult to access due to factors including site (eg, hip), degree of deformity and obesity. | III–IV | 9.4 (8.9 to 9.9) |

Categories of evidence: Ia, evidence for meta-analysis of randomised controlled trials; Ib, evidence from at least one randomised controlled trial; IIa, evidence from at least one controlled study without randomisation; IIb, evidence from at least one other type of quasi-experimental study; III, evidence from non-experimental descriptive studies, such as comparative studies, correlation studies and case–control studies; IV, evidence from expert committee reports or opinions or clinical experience of respected authorities, or both LOA: 0–10 numerical rating scale.
*Typical features include usage-related pain, short duration morning stiffness, age >40, symptoms affecting one or a few joints.
OA, osteoarthritis; US, ultrasonography.

3. Joint symptoms are also common and increase with age. Symptoms are not always causally related to imaging abnormalities.
4. Full history and examination is always required before considering the need for investigations, including imaging.
5. Modern imaging modalities provide the capability to detect a wide range of soft tissue, bony and cartilage pathology in OA. However, the increased information provided has not yet had any influence on clinical decision-making with respect to management.

### Making a diagnosis of OA

**Recommendation 1: Imaging is not required to make the diagnosis in patients with typical[i] presentation of OA.**

Level of evidence: III–IV. LOA (95% CI) 8.7 (7.9 to 9.4)

Although many studies applied imaging for diagnostic purposes, there was a lack of studies in which imaging was applied in addition to clinical findings to evaluate its additional impact on the certainty of diagnosis, which was a predefined criterion for inclusion.

A single study examined the added value of US of hand and feet over clinical findings in a cohort of patients with suspected or confirmed arthritis. When US was added to clinical findings, the diagnostic confidence in differentiating OA from inflammatory arthritis significantly increased.[2] Due to the absence of strong evidence supporting the use of different imaging modalities at different anatomical sites, the systematic use of imaging in the diagnostic process was not recommended in cases with typical clinical presentation. However, based on the joint site and clinical presentation, imaging might be considered when diagnoses other than OA are suspected. This aspect has been taken into account in Recommendation 2.

**Recommendation 2: In atypical presentations, imaging is recommended to help confirm the diagnosis of OA and/or make alternative or additional diagnoses.** Level of evidence: IV. LOA (95% CI) 9.6 (9.1 to 10)

Studies were eligible for inclusion if they investigated the added value of imaging for differential diagnosis over clinical evaluation. Among studies evaluating the application of imaging for differential diagnosis, no study evaluated the impact of the addition of imaging above clinical findings. The possible application if imaging in atypical clinical scenarios was however recognised by the experts, which included this point in the recommendation.

### Monitoring disease

**Recommendation 3: Routine imaging in OA follow-up is not recommended. However, imaging is recommended if there is unexpected rapid progression of symptoms or change in clinical characteristics to determine if this relates to OA severity or an additional diagnosis.** Level of evidence: III–IV. LOA (mean, 95% CI) 8.8 (7.9 to 9.7)

A specific question addressed the use of imaging for the follow-up. The 117 studies (mostly cohort studies) retrieved covered all joint sites except the foot and all imaging modalities except CT (see online supplementary figure S9). Most of the 83 included studies focused on sensitivity to change.[3–86] The remaining studies investigated the trajectories of changes of elementary lesions detected by imaging when following OA natural history or described the parallel changes between different abnormalities detected by different imaging modalities.[40 51 53 87–101] Only a minority of studies examined the correlation between the change in imaging features and symptoms or relevant clinical outcomes (table 2) and only four US studies evaluated the change of imaging after treatment (see online supplementary file S10).[102–111]

Moreover, there were no studies comparing clinical follow-up with imaging follow-up or strategies adding imaging to clinical management.

The impact of imaging in the management of OA was also specifically addressed by the literature search. Three studies addressed this point. One RCT evaluating the impact of MRI in patients with knee pain assessed in a general practice setting showed that MRI led to an increase in therapeutic confidence but no significant changes in management.[112] A cross-sectional study in an orthopaedic setting investigating the impact of CR over management decisions in knee OA showed that CR led to the change in the opinion in 166/400 cases.[113] A similar study evaluating the impact of CR in the assignment of priority for surgery in hip OA showed a relative risk (95% CI) of 1.98 (1.23 to 3.19) for an earlier assignment in patients with more severe radiographic scores.[114] No studies evaluated the impact of imaging for the management of hand or foot OA and no studies specifically addressed the issue of non-surgical management.

**Recommendation 4: If imaging is needed, conventional (plain) radiography should be used before other modalities. To make additional diagnoses, soft tissues are best imaged by US or MRI and bone by CT or MRI.** Level of evidence: III–IV. LOA (95% CI) 8.7 (7.9 to 9.6)

The performance of imaging in the detection of OA elementary lesions was addressed by the SLR and highlighted heterogeneity in the use of imaging modality, lesions considered and reference standard. In fact, physical examination was frequently taken into account as reference standard, while surgery was considered in a minority of studies. Online supplementary file S11 summarises the studies with surgery as the reference standard.[115–136] As expected, the use of CR was mainly to detect bone and indirectly cartilage loss, MRI was used for bone, cartilage and soft tissues, with a single study assessing US for the evaluation of cartilage.

In general, CR was the imaging modality that was most frequently used for diagnostic, prognostic and follow-up purposes. However, no studies of the cost-effectiveness of each imaging modality or their sequence were found. In the absence of appropriate literature, the experts decided to emphasise the role of the most easily available and less costly imaging modality, proposing as second-level investigations techniques that, due to their characteristics, are more suitable for the detailed assessment of soft tissues (MRI and US) or bone (CT).

**Recommendation 5: Consideration of radiographic views is important for optimising detection of OA features; in particular for the knee, weightbearing and patellofemoral views are recommended.** Level of evidence: III. LOA (95% CI) 9.4 (8.7 to 9.9)

This topic was addressed by an additional research question, evaluating the optimal combination of radiographic views in OA. Twenty-seven studies comparing different views for knee OA were included. In this context, all studies involving the tibiofemoral compartment considered weightbearing views, both in extension and various degrees of flexion.[7 8 10 17 25 118 123 137–147 188–191] Studies comparing fully extended and flexed views in general showed a moderate to good agreement between the two projections and similar sensitivity and specificity in detecting cartilage damage, considering arthroscopic findings as reference.[117 138 139 148 149] The flexed views demonstrated

[i]Typical features include usage-related pain, short duration morning stiffness, age >40, symptoms affecting one or a few joints.

superiority in detecting joint space narrowing, a greater sensitivity to change and reproducibility compared with extended views.[8 17 140 141 143 144]

Concerning the assessment of the patellofemoral compartment, skyline views had a greater inter-reader and intra-reader reliability and sensitivity to change compared with lateral projections.[24 143 144 149] With surgery as reference standard, the skyline view had greater sensitivity and specificity to detect cartilage damage at the patellofemoral joint.[150]

There were five studies assessing the hip. Three studies compared weightbearing and supine anteroposterior (AP) views of the pelvis, one of them showing greater average and maximal

**Table 2** Studies correlating changes in imaging findings with symptoms, function or clinical outcome

| Study | N | Site | Study design | Imaging | Outcome | |
|---|---|---|---|---|---|---|
| Fukui *et al*., 2010[103] | 68 | Knee | Cohort | CR | Correlation between radiographic progression and pain and function scores | Progressors had more pain and disability compared with non-progressors |
| Eckstein *et al*., 2014[104] | 189 | Knee | Case–control | MRI | Cartilage loss in patients undergoing TKA vs controls | OR (95% CI) for cartilage loss in patients undergoing TKA vs controls: 1.36 (1.08 to 1.70) |
| Kornaat *et al*., 2007[105] | 182 | Knee | Cohort | MRI | Change in BMLs/change in WOMAC pain and function | No significant differences in WOMAC pain and function depending on the changes of BMLs |
| Phan *et al*., 2006[106] | 34 | Knee | Cohort | MRI | Cartilage and BMLs/WOMAC | No significant correlation between cartilage loss, BMLs and WOMAC changes |
| Zhang *et al*., 2011[107] | 651 | Knee | Cohort | MRI | Change in pain status according to change in BMLs and effusion/synovitis score | Changes in BMLs and synovitis severity (worsening or improving) significantly related to the risk of frequent knee pain (p=0.006 for worsening BMLs and p=0.045 for improving BMLsNo significant correlation with changes in effusion severity |
| Haugen *et al*., 2013[108] | 190 | Hand | Cohort | CR | Radiographic progression/incident tenderness | Joints with progression had higher odds for tenderness, joints with incident KLG 3 or 4 had higher odds for tenderness |

BMLs, bone marrow lesions; CR, conventional radiography; KLG, Kellgren and Lawrence grade; N, number of participants; TKA, total knee arthroplasty; WOMAC, Western Ontario MacMaster Universities Arthritis Index.

**Table 3** Summary of studies evaluating imaging in the prediction of response to treatment: systemic treatment

| Study | N | Site | Study design | Imaging | Outcome | |
|---|---|---|---|---|---|---|
| Gudbergsen *et al*., 2012[156] | 192 | Knee | RCT | CR MRI | mJSW, alignment and MRI scores/pain reduction in response to very-low-energy diet or low-energy diet | Among all radiographic and MRI parameters, only effusion score was significantly related to a reduction in pain |
| Gudbergsen *et al*., 2011[157] | 30 | Knee | RCT | CR MRI | KLG and MRI score/change in WOMAC pain and function during weight reduction at 32 weeks | No significant association between KLG and MRI score and WOMAC |
| Hellio le Graverand *et al*., 2013[14] | 1452 | Knee | RCT | CR | KLG/structural progression in patients treated with cindunistat or placebo at 96 weeks | No significant difference between KLG2 and KLG3 in terms of progression of joint space narrowing in both cindunistat and placebo group |
| Case *et al*., 2003[158] | 82 | Knee | RCT | CR | KLG and medial JSN/WOMAC response to diclofenac vs paracetamol at 12 weeks | Patients with KLG 1–2 and not 3–4 and JSN grade 0–1 compared with 2 had a better response to diclofenac vs both placebo and paracetamol |
| Sawitzke *et al*., 2008[159] | 375 | Knee | RCT | CR | KLG/radiographic progression during treatment with glucosamine, chondroitin sulfate and celecoxib at 24 months | OR for radiographic progression compared with the placebo group was <1 in patients with KLG 2 knees in all treatment groups, whereas it was >1 in patients with KLG 3 knees in all treatment groups |
| Mazzuca *et al*., 2010[160] | 379 | Knee | RCT | CR | Alignment/radiographic progression in doxycycline vs placebo at 30 months | Varus knees exhibited a greater loss of JSW than non-varus knees in patients receiving doxycycline |
| Knoop *et al*., 2014[164] | 91 | Knee | Cohort | MRI | MRI/change in WOMAC function in response to exercise programme at 12 weeks | The severity of the patellofemoral damage was significantly related to less improvement |
| Wenham *et al*., 2012[168] | 65 | Hand | RCT | MRI | MRI/response to prednisolone 5 mg at 12 weeks | The baseline number of joints with definite synovitis or effusion did not correlate with OARSI response |
| Lequesne *et al*., 2002[84] | 163 | Hip | RCT | CR | JSW/structural progression in patients treated with avocado soybean at 2 years | In patients with smaller JSW treated with avocado soybean, the reduction of JSW was half than in the placebo group; no differences in patients with more JSW |
| Rozendaal *et al*., 2009[171] | 222 | Hip | RCT | CR | KLG/WOMAC pain and function, JSN in patients taking glucosamine at 2 years | Significantly better WOMAC function response in patients with KLG 1 compared with KLG 2; no differences in WOMAC pain and JSN |
| Hoeksma *et al*., 2005[172] | 103 | Hip | RCT | CR | KLG/Harris Hip score and range of motion in response to manual therapy vs exercise | Better response in terms of range of motion in lower compared with higher radiographic grades |

CR, conventional radiography; JSN, joint space narrowing; JSW, joint space width; KLG, Kellgren and Lawrence grade; mJSW, minimal joint space width; N, number of participants; OARSI, Osteoarthritis Research Society International; RCT, randomised controlled trial; WOMAC, Western Ontario MacMaster Universities Arthritis Index.

joint space width detected by the weightbearing view, the remaining showing inconsistent results.[151–155] Two studies comparing pelvis, hip and oblique views projections in terms of reliability and sensitivity to change demonstrated similar reliability for views dedicated to the hip and views including all the pelvis, with comparable sensitivity to change.[72 75] No studies assessing the hand and the foot were found.

### Role in prognosis

**Recommendation 6: According to current evidence, imaging features do not predict non-surgical treatment response and imaging cannot be recommended for this purpose.** Level of evidence: II–III. LOA (95% CI) 8.7 (7.5 to 9.7)

Two specific research questions addressed the role of imaging in prognosis, referring to both the prediction of the natural history and to the prediction of non-surgical treatment outcomes. A number of studies addressed the issue of the prognostic value of imaging as predictor of the natural history of OA (see online supplementary figure S12), while only a minority of studies, evaluating all joint sites, investigated the role in predicting treatment response. Due to the heterogeneity in populations, interventions, treatment and study design, a meta-analysis was not possible. In addition, progression of some imaging pathologies may have limited clinical significance. Tables 3 and 4 summarise the results of the 28 primary studies in which imaging was applied to predict treatment response.[14 84 156–176] Moreover, an existing SLR was available, without a quantitative synthesis.[177] The results on the prediction of response were mostly inconsistent across studies; for this reason the use of imaging for this purpose was not recommended.

### Treatment (imaging-guided procedures)

**Recommendation 7: The accuracy of intra-articular injection depends on the joint and on the skills of the practitioner and imaging may improve accuracy. Imaging is particularly recommended for joints that are difficult to access due to factors including site (eg, hip), degree of deformity and obesity.** Level of evidence: III–IV. LOA (95% CI) 9.4 (8.9 to 9.9)

A search addressing the impact of imaging to guide intra-articular injections was run specifically for OA in the beginning. Including only studies comparing imaging-guided to blind procedures, four primary studies were found for the knee and one for the hand, and a qualitative SLR for the knee (table 5). The added value of US was addressed by four studies, while fluoroscopic guidance was tested in a single study.[179–183]

**Table 4** Summary of studies evaluating imaging in the prediction of response to treatment: intra-articular treatment

| Study | N | Site | Study design | Imaging | Outcome | |
|---|---|---|---|---|---|---|
| Barrett *et al*., 1990[178] | 248 | Knee | Cohort | CR | Radiographic severity/response to intra-articular HA at 6 months | Patients with less severe radiographic grade had a better response in terms of pain at rest, at walking and at night |
| Gaffney, 1995[189] | 84 | Knee | RCT | CR | OA severity 0–3/response to intra-articular triamcinolone vs placebo at 3 weeks | No association between improvement in VAS pain and radiographic score |
| Toh *et al*., 2002[161] | 60 | Knee | Cohort | CR | Alignment, sclerosis, cysts, osteophytes, JSN/WOMAC response to intra-articular HA at 12 weeks | Patients with lateral and medial JSN had less WOMAC response compared with patients without |
| Pendleton *et al*., 2008[176] | 86 | Knee | Cohort | US | US/WOMAC response to intra-articular methylprednisolone | Higher baseline US scores: significant improvements in all WOMAC subscales at 1 and 6 weeks |
| Chao *et al*., 2010[162] | 67 | Knee | RCT | US | US inflammation/WOMAC response to triamcinolone at 12 weeks | Statistically significant improvement in pain subscales among without inflammatory abnormalities at US patients compared with the remaining patients |
| Anandacoomarasamy *et al*., 2008[163] | 32 | Knee | Cohort | MRI | Cartilage volume/response to intra-articular HA at 6 months | No correlation between baseline MRI measures and clinical response |
| Drakonaki, 2011[190] | 51 | Foot | Cohort | CR US | Positive therapeutic response (intra-articular. methylprednisolone) at 12 months | No differences in terms of response in patients showing degenerative changes only on US and those showing changes in both US and CR |
| Han *et al*., 2014[165] | 40 | Foot | Cohort | CR | Response to intra-articular HA (VAS pain) at 12 months | Patients with early radiographic stage had a better response compared with those with advanced radiographic stage at 3 and 6 months, but not at 12 months |
| Sun *et al*., 2011[166] | 46 | Foot | Cohort | CR | KLG 2 and 3/AOS, AOFAS scores in response to intra-articular HA | No significant difference in the AOS, AOFAS or clinical balance test scores between KLG 2 and 3 at any time point |
| Mallinson *et al*., 2013[167] | 31 | Hand | Cohort | CR US | CR and US/response to intra-articular triamcinolone at 6 weeks | No significant association between treatment response and grade for osteophytes, joint space narrowing and capsule thickness |
| Atchia *et al*., 2011[169] | 77 | Hip | RCT | US | Synovitis/response to intra-articular methylprednisolone at 6 weeks | The presence of synovitis significantly predicted the response |
| Rennesson-Rey *et al*., 2008[170] | 55 | Hip | Cohort | CR US | Effusion and KLG/OARSI response to HA at 6 months | Patients with KLG 1–2 had a better 1 month response compared with grades 3–4; non-differences at 3 and 6 months, no differences in patients with or without effusion |
| Deshmukh *et al*., 2011[173] | 220 | Hip | Cohort | CR | KLG/pain relief after methylprednisolone injections at 2 weeks | Patients with KLG 3–4 had more frequently delayed relief compared with KLG 2 |
| Robinson *et al*., 2007[175] | 120 | Hip | Cohort | CR US | US osteophytes and capsular thickening, KLG/WOMAC response to intra-articular CS at 12 weeks | No baseline US or radiographic variable predictive of the outcome |

AOFAS, Australian Orthopedic Foot and ankle society; AOS, ankle osteoarthritis score; CR, conventional radiography; CS, corticosteroids; HA, hyaluronic acid; JSN, joint space narrowing; KLG, Kellgren and Lawrence grade; N, number of participants; OA, osteoarthritis; OARSI, Osteoarthritis Research Society International; RCT, randomised controlled trial; US, ultrasonography; VAS, visual analogue scale; WOMAC, Western Ontario MacMaster Universities Arthritis Index.

**Table 5** Studies comparing imaging-guided to blind injections in OA

| Study | N | Site | Study design | Imaging | Outcome | |
|---|---|---|---|---|---|---|
| Bum Park, 2012[191] | 99 | Knee | RCT | US | Accuracy of HA injection vs blind injection | OR (95% CI) for an accurate injection with US compared with blind: 4.68 (0.94 to 23.30) |
| Im *et al*., 2009[179] | 99 | Knee | RCT | US | Accuracy of HA injection vs blind injection | Accurate injections: 95.5% (US-guided) vs 77.2% (blind); p=0.01 |
| Jang *et al*., 2013[180] | 126 | Knee | RCT | US | Accuracy of US-guided in plain injection, US-guided out-of-plane injections and blind injection of triamcinolone hexacetonide | Accuracy: US-guided in plain 95.1%; US-guided out-of-plain 97.7%; blind 78% p<0.05 blind vs US-guided injections |
| Sibbitt *et al*., 2011[181] | 92 | Knee | RCT | US | US-guided vs blind triamcinolone in terms of pain relief, pain related to the injection, reinjection rate and cost | Significant decrease in pain only in patients treated with US-guided injection; US-guided procedure was related to lower pain and reinjection rate, but higher costs |
| Karalezli *et al*., 2007[182] | 16 | Hand | Cohort | CR | Fluoroscopy-guided vs blind injections of HA in the trapezio-metacarpal joint in terms of pain related to the injection | VAS pain related to the procedure: fluoroscopic guide: 4.1 (range 3–6), anatomic guide 5.6 (range 3–7); p<0.005 No significant difference in terms of safety |

CR, conventional radiography; HA, hyaluronic acid; N, number of participants; OA, osteoarthritis; RCT, randomised controlled trial; US, ultrasonography; VAS, visual analogue scale.

In order to retrieve further information on this topic, an additional search was performed (see online supplementary file S1 for search strategies), including studies comparing blind to guided injections in OA and also in other conditions. This search found eight studies, of which three were already included in the previous results (see online supplementary file S13).[184–188] Most of the studies were focused on the knee, with some studies on the hand and the foot, while no studies were found for the hip. All the additional studies investigated the impact of US. Accuracy was found to be better in imaging guided compared with blind procedures; however, the results on the clinical outcomes of the injection were less consistent across studies. For these reasons, the systematic use of imaging to drive injections was not recommended, leaving this tool to drive injection in specific situations, identified by the experts. Although the imaging modality is not specified in the recommendation, there is published evidence for the use of US, and imaging allows for real-time evaluation of injection placement.

### Future research agenda

The most important topics to drive future research were selected by the Task Force based on the (often considerable) gaps in the evidence and the needs arising from clinical practice (table 6).

**Table 6** Future research agenda

| | |
|---|---|
| 1 | There is a need for methodologically robust studies to explore the added value of imaging (any modality) to clinical diagnosis or differential diagnosis. |
| 2 | What is the cost-effectiveness of imaging in osteoarthritis clinical practice? |
| 3 | Is imaging able to help in identification of subgroups/phenotypes that may have different trajectories and enable targeted treatment based on these subgroups? |
| 4 | There is a need to understand if using imaging to measure response to therapy is of clinical benefit. This may require evaluation of novel imaging technologies that are able to sensitively detect change in relevant joint structures. |
| 5 | Quality studies are required to explore imaging (any modality) features that predict response to specific therapies. |
| 6 | There is a need for more research concerning the benefits of imaging in less commonly studied osteoarthritis sites such as the foot and shoulder. |
| 7 | Specifically for hip osteoarthritis, what is the added value of weightbearing vs non-weightbearing X-rays? |
| 8 | What are the benefits of imaging guidance in improving the efficacy of treatments? |

## DISCUSSION

Although a number of recommendations have been made on how to use imaging in OA clinical trials, these are the first recommendations on the use of imaging in OA in clinical practice. The development of the recommendations started from questions of clinical relevance selected by a task force of experts, with the aim to focus on topics of interest for clinical practice rather than research. The literature review identified a large number of studies, covering most joint sites. However, a possible limitation of this work is that we used a search term of 'osteoarthritis' and not 'pain', and it is possible we missed studies that imaged painful sites without specifically mentioning OA; this may explain the paucity of foot pain studies included. Although CR was still the most frequently applied technique, a substantial number of studies focused on modern imaging, MRI and US in particular.

However, despite the amount of data available in the literature, only a small part of this information was relevant for clinical practice. For this reason, many areas needing further investigation were identified. In particular, there was a lack of strategic studies investigating the additional value of imaging over clinical findings in making a diagnosis of OA, in the management and the follow-up of the disease, and inconsistent results dealing with the prediction of the outcome of non-pharmacological treatments. The absence of good study information in these areas did not enable the Task Force to recommend systematic imaging in all these areas. A research agenda was therefore generated in order to address these topics in the future research.

In conclusion, seven recommendations covering different areas in the routine management of OA were developed. These are based on both available scientific evidence and expert opinion to provide a valuable and sensible guide for the use of imaging in clinical practice.

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EXTENDED REPORT

# Societal costs and patients' experience of health inequities before and after diagnosis of psoriatic arthritis: a Danish cohort study

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## ABSTRACT

**Objectives** To comprehensively study the comorbidities, healthcare and public transfer (allowance) costs in patients with psoriatic arthritis (PsA) before and after diagnosis.

**Methods** Nationwide cohort study, using data from Danish registries from January 1998 through December 2014. A total of 10 525 patients with PsA and 20 777 matched general population comparator (GPC) subjects were included. Societal costs, employment status and occurrence of comorbidities in patients with PsA both before and after diagnosis were compared with GPC subjects.

**Results** At baseline, patients with PsA had significantly more comorbidities, including cardiovascular disease (OR 1·70 95% CI 1·55 to 1·86), respiratory diseases (OR 1·73 95% CI 1·54 to 1·96) and infectious diseases (OR 2·03 95% CI 1·69 to 2·42) compared with GPC subjects. At all time points, patients with PsA had higher total healthcare and public transfer costs; they also had lower income (p<0.001) and incurred a net average increased societal cost of €10 641 per patient-year compared with GPC subjects following diagnosis. The relative risk (RR) for being on disability pension 5 years prior to PsA diagnosis was 1·36 (95% CI 1·24 to 1·49) compared with GPC subjects. The RR increased to 1·60 (95% CI 1·49 to 1·72) at the time of diagnosis and was 2·69 (95% CI 2·40 to 3·02) 10 years after diagnosis, where 21·8% of the patients with PsA received disability pension.

**Conclusions** Our findings are suggestive of health inequity for patients with PsA and call for individual preventive measures and societal action.

## INTRODUCTION

Psoriatic arthritis (PsA), a chronic inflammatory disorder, is associated with skin psoriasis (PsO).[1] PsA affects approximately 30% of patients with PsO, the typical onset of PsA occurring during the fourth decade of life.[2–4] The clinical presentation of PsA is heterogeneous, but primary characteristics include peripheral joint inflammation, nail involvement, axial skeleton disorders, enthesitis, tenosynovitis and dactylitis.[5] Approximately 40%–60% of patients with PsA may develop erosive and deforming joint complications, and the disease may lead to progressive disability and pain.[5 6] Furthermore, PsA is associated with several severe comorbidities, including depression, anxiety, reduced quality of life, obesity, type II diabetes, osteoporosis, malignancy and cardiovascular diseases.[1 7] Thus, the awareness regarding cost and health economic aspects of PsA have increased.[8 9] The proportion of work disabled patients with PsA has been reported to be approximately 40%.[7 10]

Few studies to date have focused on the inequities of PsA from a social and economic perspective, comparing patients with PsA with the general population. Likewise, the total burden of PsA with regard to timing and impact of all comorbidities has been scarcely studied.[11–16] Health inequities are systematic differences in the health status of different population groups, and there is abundant evidence that the lower an individual's socioeconomic position, the higher their risk of poor health.[17] However, the causality is often bidirectional; poor health also leads to significant individual, social and economic costs, creating a classic downward spiral.[18] In a nationwide population-based cohort study, based on prospectively recorded register data, we address the hypothesis that patients with PsA face health inequity by studying the healthcare and public transfer (allowance) costs, employment status as well as personal income 5 years before and 10 years after a diagnosis of PsA. Also, we hypothesise that the burden of various comorbidities will be higher in PsA compared with the general population.

## METHODS

### Study design and participants

To ascertain the inequities of PsA from an individual, social and economic perspective, our investigation used a nationwide cohort study with data from Danish registries from January 1998 through December 2014. Our study was conducted in accordance with the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) statement and according to a prespecified protocol (see online supplementary file S1) available and published as open-access at the official website of the Parker Institute (http://www.parkerinst.dk). Data handling and ethical approval for the study were granted by the Regional Ethics Committee and the Danish Data Protection Agency, Copenhagen, Denmark (approval number: 2013-54-0410). No informed consent was applicable as the study

involved only linkage of registry-based data, with no actual interaction with patients. The ethics committee approved this consent procedure.

Some background on the Danish healthcare system and information infrastructure follows, as it is necessary to explain our methods. On 31 December 2014, Denmark had a population of approximately 5.7 million. Health and demographic information on all citizens is updated annually in a series of national registries, with a very high degree of completeness.[19] Linkage of data from these registries is possible using the 10-digit personal identification number automatically assigned to all Danish citizens.[20]

The Danish healthcare system is tax funded and offers universal access. Data on healthcare contacts at inpatient and non-primary outpatient facilities are registered in the Danish Patient Registry (DPR), including date of contact and diagnoses given by the treating physician according to the Danish version of the International Statistical Classification of Diseases (ICD-10 starting 1993).[21] Reporting of data on each single healthcare contact, excluding primary care visits, is required by the state.

Using data from the DPR, we identified a national population-based cohort of patients with PsA, including those patients who had attended an outpatient clinic during the time period 1 January 1998 through 31 December 2014 and who had received at least one ICD-coded diagnosis corresponding to PsA (ie, ICD-10: L40.5, M07.3, M07.0, M07.1, M07.2). A separate validation study done by LEK and LTHJ revealed a validity of >90% of spondyloarthritis diagnoses in a similar cohort.[22]

For each patient with PsA, two general population comparator (GPC) subjects, alive, without PsA and matched on year of birth, gender, time and marital status were identified.

Most patients with PsA are diagnosed by rheumatologists at public outpatient and inpatient facilities.

Information on socioeconomic status was obtained from nationwide registries on employment, educational level, income and pensions. Cost of hospital contacts included costs of hospitalisation weighted by use for separate diagnosis-related groups (tariffs) and cost of specific outpatient treatments (DAGS tariffs) based on data from the Danish Ministry of Health. The cost of medicine was derived from the Danish Drug Prescription Registry and consisted of the retail price of each drug multiplied by prescribed quantity. Information on health costs associated with consultation and treatment in the primary sector was collected from the National Health Insurance Service Registry.

The Civil Registration System (CRS): Since 1968, the CRS has registered deaths and migrations among all Danish citizens.

The PsA population was drawn at the first contact in the DPR after 1998, and the index date was designated as the baseline date. For inpatients, the index date was defined as the date of the first discharge form hospital after January 1998. For outpatients, the index date was defined as the date of the first hospital contact with PsA. Thus, the onset of PsA (index date) is defined as the date of first possible registered PsA diagnosis in DPR. In our cost analysis, subjects had to be eligible for 12 months after the index date; thus, an index date could be no later than 31 December 2013. Consequently, patients with an index date in year 2014 were excluded from our analyses. Healthcare and public transfer (allowance) costs, employment status and personal income 5 years before and 10 years after the index date of patients with PsA were compared with a GPC. Moreover, the burden of various comorbidities was studied 3 years prior to and 3 years after the index date of the patients with PsA. Patients and/or comparators who were registered as deceased were included in the analyses up until the year after their registered date of death. As such, patients/comparators had to be eligible and alive at the beginning of the period but not necessarily alive over the entire period.

Employment status was categorised as regular job/self-employment, unemployment, disability pension, early retirement, age pension retirement, retired on other pensions or not in labour. Average income per patient with PsA and comparators was differentiated into income deriving from employment, social security and unemployment benefit, sick pay, disability pension, early retirement, age pension, other public transfer, other pensions and total income. Very large incomes were not considered valid; income over €270 000/year was set to missing. Yearly healthcare costs for study participants were calculated using information on frequency and cost of hospital contacts (inpatient and outpatient treatments), consultations with general practitioners and other specialists and use and cost of medicine.

Prior to study entry and during follow-up, data on comorbidities registered by physicians in hospital-based inpatient or outpatient somatic care clinics in patients with PsA and GPC subjects were retrieved from the DPR. Comorbidity was pooled on the 22 WHO-chapters (see online supplementary file S2 for definition). We identified all diagnoses 3 years before the baseline date and 3 years after index date (excluding the index date) in the DPR register. Thus, only patients with an index date in the period 2001–2011 were included in the comorbidity analysis. Our study included both main and secondary diagnoses found in the DPR register. The objective and study design were discussed with a patient with PsA after oral and written informed consent and the findings in the current study were shared and discussed with the patient subsequently (see Acknowledgements section for further detail).

### Statistical analysis

Demographic and descriptive data were expressed in crude numbers and fractions (%). The significance of the income and healthcare cost estimates for matched case and comparator groups was assessed by non-parametric bootstrap t-test analysis due to the non-normal distribution of the data.[23] The relative risk (RR) to be unemployed, on disability pension or early retired compared with the background population including the 95% CI were calculated at different time points using crude proportions. ORs with 95% CI were presented for comorbidity diagnoses received up to 3 years prior to baseline and during a 3-year follow-up period after diagnosis of PsA. In all statistical tests, p values <0.05 (two-sided) were considered statistically significant. Calculations were based on observed data, and no imputation of missing data was performed.

## RESULTS

A total of 10 525 patients with PsA and 20 777 matched GPC subjects were included in the study.

Median age of patients with PsA and GPC subjects at study entry was 52 years (IQR 40–60 years), 41% were male. Baseline characteristics of patients with PsA and GPC subjects are presented in table 1. The baseline data on demographics and comorbidities split according to organ systems for the PsA group compared with the general population, presented in table 1, showed that already at the time of diagnosis the group of patients with PsA had significantly more comorbidities including neoplasms (OR 1.25 95% CI 1.11 to 1.41), cardiovascular disease (OR 1.7 95% CI 1.55 to 1.86), respiratory diseases (OR 1.73 95% CI 1.54 to 1.96), infectious diseases (OR 2.03 95% CI 1.69 to 2.42) and haematological diseases (OR 1.94 95% CI 1.55 to 2.43).

**Table 1** Baseline characteristics and comorbidities at the time of diagnosis for patients with PsA and matched general population comparator

| | PsA (n=10 525) | GPC (n=20 777) |
|---|---|---|
| Female, no. (%) | 6222 (59.1) | 12 311 (59.3) |
| Age, no. (%) | | |
| <20 | 201 (1.9) | 403 (1.9) |
| 20–29 | 715 (6.8) | 1414 (6.8) |
| 30–39 | 1707 (16.2) | 3392 (16.3) |
| 40–49 | 2431 (23.1) | 4831 (23.3) |
| 50–59 | 2812 (26.7) | 5572 (26.8) |
| 60–69 | 1686 (16.0) | 3298 (15.9) |
| 70–79 | 765 (7.3) | 1472 (7.1) |
| >80 | 208 (2.0) | 395 (1.9) |
| Married/coliving, no. (%) | 7320 (69.5) | 14 395 (69.3) |
| Comorbidities | PsA (n=7508*) | GPC (n=14 800*) |
| Infections, no. (%) | 251 (3.3) | 249 (1.7) |
| Neoplasms, no. (%) | 502 (6.7) | 805 (5.4) |
| Haematological disorders, no. (%) | 156 (2.1) | 161 (1.1) |
| Endocrine and metabolic disorders, no. (%) | 658 (8.8) | 816 (5.5) |
| Mental disorders, no. (%) | 220 (2.9) | 379 (2.6) |
| Nervous system, no. (%) | 489 (6.5) | 502 (3.4) |
| Cardiovascular disorders, no. (%) | 1060 (14.1) | 1340 (9.1) |
| Respiratory disorders, no. (%) | 522 (7.0) | 613 (4.1) |
| Digestive tract disorders, no. (%) | 965 (12.9) | 1075 (7.3) |
| Skin disorders, no. (%) | 778 (10.4) | 335 (2.3) |
| Musculoskeletal system, no. (%) | 2884 (38.4) | 1936 (13.1) |
| Genitourinary disorders, no. (%) | 796 (10.6) | 1210 (8.2) |

*Please note that comorbidities required at least 3 years of observation prior and after inclusion date.
GPC, general population comparator; PsA, psoriatic arthritis.

### Costs analysis

As illustrated in figure 1, the healthcare costs for the patients with PsA increased from <€2000/year 5 years prior to diagnosis to >€5000/year around the time of PsA diagnosis, reflecting an increased utilisation of healthcare resources associated with reaching a diagnosis. At all time points, the total healthcare costs were higher for patients with PsA compared with the GPC, although the difference was clearly attenuated after time of diagnosis ($p<0.001$). Figure 2 shows that the average yearly income is lower for patients with PsA at all time points from 5 years prior to diagnosis until 10 years after. However, the difference is markedly increased around and after the year of diagnosis. Likewise, the average public transfer payments are higher for the patients with PsA even before time of diagnosis; again, this difference was attenuated after receiving a diagnosis. In table 2, the average yearly costs and income after date of diagnosis for patients with PsA and GPC are summarised, illustrating a net average increased societal cost of €10 641 per patient-year for patients with PsA compared with GPC.

### Socioeconomic status

In figure 3, the proportions of employment (or self-employment), disability pension and other socioeconomic status (ie, student, <16 years, unemployment or retired) can be seen at different time points for the patients with PsA and the matched GPC subjects. A detailed view on all the different socioeconomic status proportions can be seen in online supplementary figure S1. The relative risk for being on disability pension 5 years prior to PsA diagnosis was 1.36 (95% CI 1.24 to 1.49) compared with GPC subjects. This figure increased to RR 1.60 (95% CI 1.49 to 1.72) at the time of diagnosis and was RR 2.69 (95% CI 2.40 to 3.02) 10 years after diagnosis, where 21.8% of the patients with PsA received disability pension. Likewise, the relative risk for being unemployed was 1.21 (95% CI 1.09 to 1.34) for patients with PsA compared with GPC 5 years prior to diagnosis, increasing to RR 1.72 (95% CI 1.58 to 1.87) at the time of diagnosis, where 9.1% of the patients with PsA were unemployed. The RR then decreases to 0.95 (95% CI 0.74 to 1.21). The RR for being employed 5 years prior to diagnosis was 0.95 (95% CI 0.93 to 0.97) compared with GPC subjects. This figure decreased to RR 0.87 (95% CI 0.85 to 0.89) at the time of diagnosis and further decreased to 0.76 (95% CI 0.72 to 0.80) 10 years after diagnosis, where 40.9% of the patients with PsA were working.

### Comorbidities

In table 3, the ORs for various comorbidities in the 3-year period prior to diagnosis and the 3-year period after diagnosis are displayed for subjects diagnosed with PsA and for matched GPC subjects. Subjects diagnosed with PsA have an increased risk of also receiving other diagnoses prior to diagnosis of PsA. However, the ORs are also significantly increased in the 3 years following a PsA diagnosis. Notably, the OR for having mental or behavioural disorders (1.21 95% CI 1.04 to 1.41) became significant after receiving a PsA diagnosis compared with GPC subjects.

## DISCUSSION

This study demonstrates increased healthcare costs, lower income, higher unemployment rates, higher risk for disability pension and more comorbidities for patients with PsA compared with the general population both in the period prior to diagnosis and with accentuating differences in the years following a PsA diagnosis, confirming our prespecified hypothesis of health inequity from a patient's perspective and significant socioeconomic impact of PsA from a societal perspective.

The findings are consistent with previous studies reporting increased comorbidities, costs and work disability.[10 12–16 24] To our knowledge, however, this is the first study to assess healthcare and societal cost as well as comorbidities at large in a population of patients with PsA compared with a matched general population based on nationwide prospective data.

Some potential limitations of the study design should be considered. The DPR consisting of the Inpatient Register and the Outpatient Register is a substantial data source in this study. All physicians in the country working in healthcare units are obliged to report data, including personal identity number and ICD-coded diagnosis, on all inpatient and specialist outpatient visits.

Evaluations of data in the Inpatient Registry have shown validity between 85% and 95% across different diagnoses and coverage of >99%.[19] Regarding data on specialist outpatient visits, the overall coverage of 80% is somewhat lower. This is primarily explained by missing data from private caregivers, whereas coverage from public non-primary care outpatient units is almost 100%.

Thus, nationwide register-based studies like the present have the apparent strength of being population-based reducing the risk of selection bias.[25 26] However, some degree of residual confounding and bias cannot be ruled out.

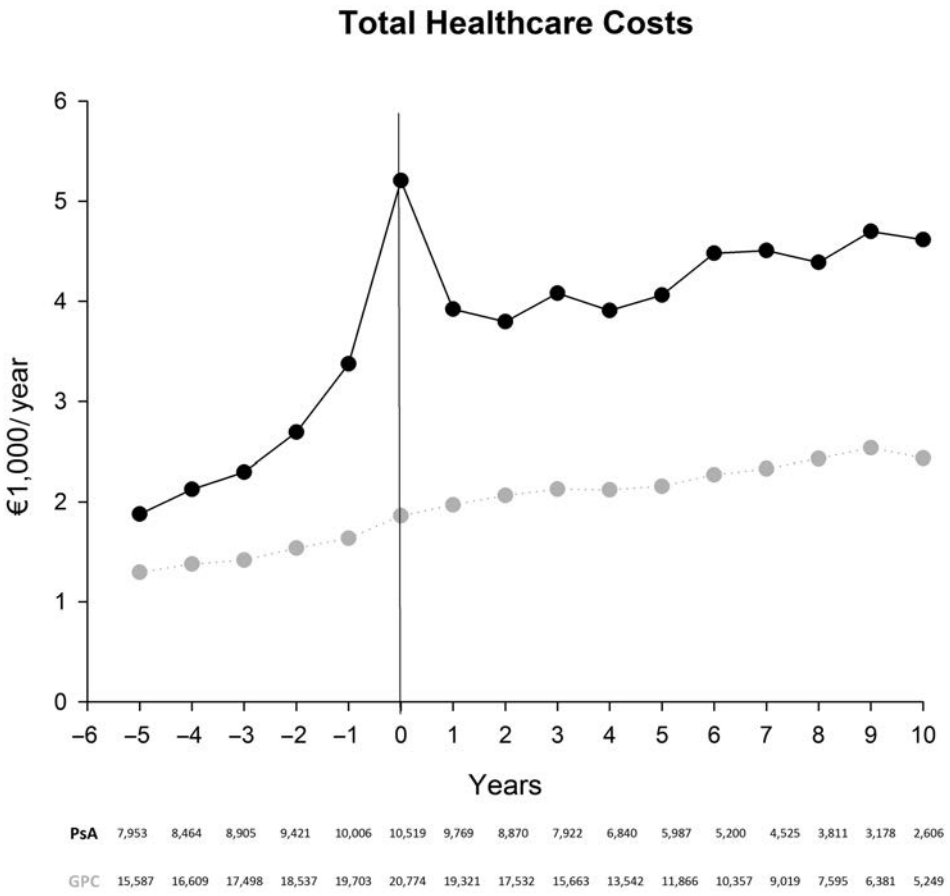


**Figure 1** Illustrates the annual total healthcare costs in Euros for patients with psoriatic arthritis (PsA) and matched general population comparator (GPC) 5 years before diagnosis and 10 years after ($p<0.001$).

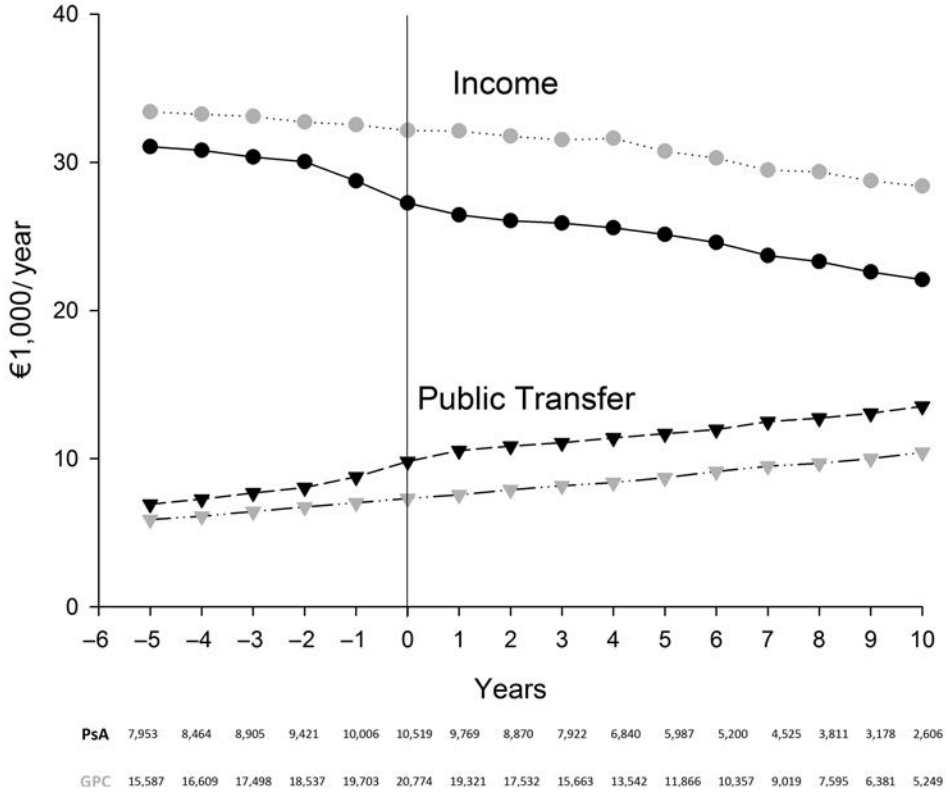


**Figure 2** Illustrates the annual income in Euros from employment and annual public transfer allowance in Euros for patients with psoriatic arthritis (PsA) and matched general population comparator (GPC) ($p<0.001$).

Selection of patients with PsA in this study is based on ICD codes recorded by a selection bias towards more severe cases being included while missing patients with mild disease who are managed entirely at primary care units. However, according to a previous study in Sweden (a Scandinavian country closely resembling Denmark), this is a minor problem and would only increase the number of cases by <4%, at the expense of a larger degree of misclassification.[27] Regarding the case definitions of

**Table 2** Presents average yearly costs and income in Euros for patients with PsA and matched GPC during a 10-year period after date of diagnosis

| | | Patients with PsA | GPC | p Value |
|---|---|---|---|---|
| Number of persons (N) | | 10 525 | 20 777 | |
| Health cost total | | **4336** | **2170** | <0.001 |
| Outpatient services | € | 1074 | 449 | <0.001 |
| Inpatient admissions | € | 1914 | 1062 | <0.001 |
| Prescription drugs | € | 790 | 379 | <0.001 |
| Primary health sector | € | 559 | 279 | <0.001 |
| Home care* | € | 483 | 337 | <0.001 |
| Income | € | **26 429** | **31 879** | <0.001 |
| Income from employment | | 25 083 | 30 673 | <0.001 |
| Other income private pension | | 1346 | 1206 | <0.001 |
| Public transfer income total | € | **11 525** | **8646** | <0.001 |
| Sick pay (public funded) | € | 790 | 357 | <0.001 |
| Disability pension | | 3978 | 1941 | <0.001 |
| Early retirement | | 814 | 1079 | <0.001 |
| Age pension | € | 3974 | 3861 | 0.040 |
| Other public transfers | € | 1970 | 1408 | <0.001 |
| Direct health costs | € | 4336 | 2170 | |
| Home care costs | € | 483 | 337 | |
| Indirect costs, foregone earnings | € | 5450 | | |
| Sum of direct and indirect costs | € | **10 269** | **2507** | |
| Net costs | € | 7762 | | |
| Social transfer payments | € | 11 525 | 8646 | |
| Net costs including transfers | € | **10 641** | | |

*Home care cost data are only available from 2009.
GPC, general population comparator; PsA, psoriatic arthritis.
Bold signifies the value derived from the sum of other values.

PsA used in this study data and results from another group, spondyloarthritis and ankylosing spondylitits, data suggest that misclassification occurs in <10%.[22] Concerning comorbidities such as acute coronary events, misclassification is estimated to be <5%.[19 25]

Moreover, the onset of PsA (index date) is defined as the date of first registered PsA diagnosis, thus introducing a risk of diagnostic delay in the current study. However, the majority of ICD codes comes from outpatient clinic and are registered at the time the patient is seen in the clinic. Moreover, the differences are apparent 5 years prior to the index date and a diagnostic delay of >5 years is highly unlikely.

The increased socioeconomic burden and increased frequency of comorbidities many years prior to diagnosis of PsA raise the possibility that these factors may contribute to the development of PsA. However, it should be noted that patients with PsA often suffers from psoriasis of the skin prior to the joint involvement. Further studies are encouraged in order to clarify these mechanisms and to establish effective prophylaxis. Notably, the differences in socioeconomic and health status are accentuated in the years after diagnosis of PsA, illustrating a potential bidirectional causality. Thus, poor health contributes to significant individual, social and economic costs and the lower an individual's socioeconomic position, the higher their risk of poor health.[17 18] Further studies are needed to disentangle the relative role of poor health and lower socioeconomic position or an interaction of the two with regard to risk for developing PsA. Nonetheless, these mechanisms together create a classic downward spiral. At present, close monitoring and preventive measures for various comorbidities including, but not restricted to, cardiovascular diseases should be undertaken when dealing with patients with PsA in the clinic.[28 29] Moreover, early diagnosis and sufficient and aggressive treatment, including antitumour necrosis factor

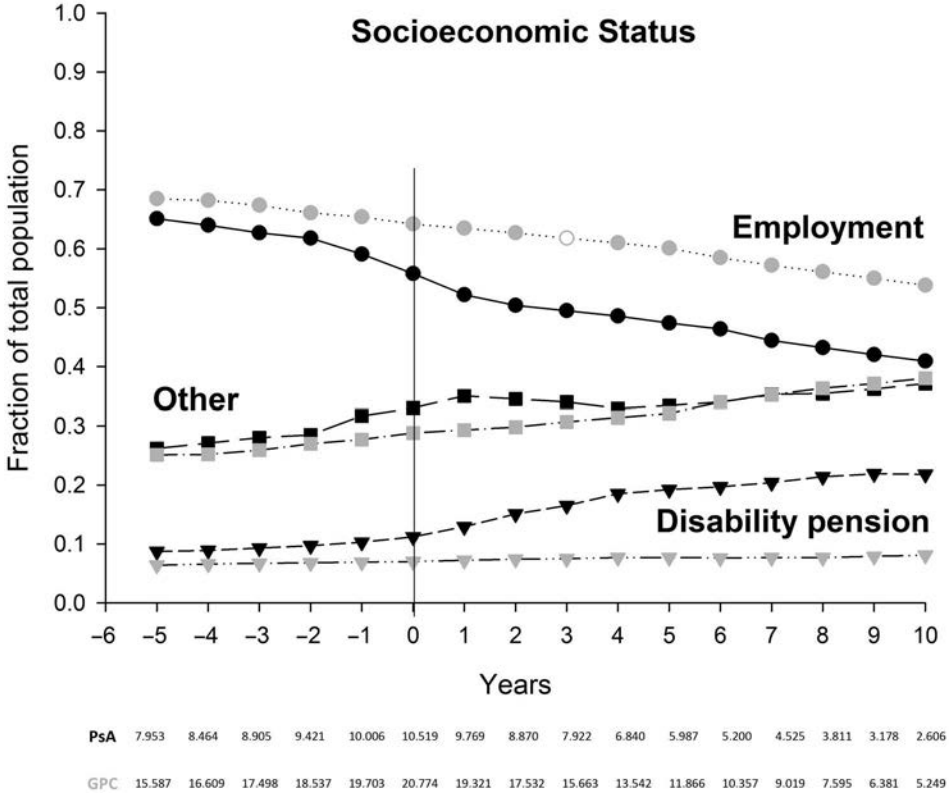


**Figure 3** Illustrates socioeconomic status (ie, employment p<0.001, disability pension p<0.001 and other) for the patients with psoriatic arthritis (PsA) and matched general population comparator (GPC) 5 years prior to diagnosis and 10 years after.

**Table 3** ORs for comorbidities before and after diagnosis of PsA

| Comorbidities | Baseline (PsA: n=7508*; GPC; n=14 800) OR (95% CI) | Follow-up (PsA: n=7508*; GPC: n=14 800) OR (95% CI) |
|---|---|---|
| Infections | 2.03 (1.69 to 2.42) | 2.20 (1.89 to 2.55) |
| Neoplasms | 1.25 (1.11 to 1.41) | 1.26 (1.14 to 1.40 ) |
| Haematological disorders | 1.94 (1.55 to 2.43) | 2.13 (1.77 to 2.56) |
| Endocrine and metabolic disorders | 1.65 (1.48 to 1.84) | 1.89 (1.72 to 2.07) |
| Mental disorders | 1.15 (0.97 to 1.36) | 1.21 (1.14 to 1.40) |
| Nervous system | 1.99 (1.75 to 2.26) | 1.78 (1.58 to 2.00) |
| Cardiovascular disorders | 1.70 (1.56 to 1.86) | 1.70 (1.57 to 1.85) |
| Respiratory disorders | 1.73 (1.54 to 1.96) | 1.75 (1.57 to 1.95) |
| Digestive tract disorders | 1.89 (1.73 to 2.08) | 1.98 (1.82 to 2.16) |
| Skin disorders | 4.99 (4.37 to 5.71) | 10.86 (9.58 to 12.32) |
| Musculoskeletal system | 4.23 (3.94 to 4.54) | 8.37 (7.76 to 9.02) |
| Genitourinary disorders | 1.33 (0.75 to 1.04) | 1.49 (1.36 to 1.63) |

*Please note that comorbidities required at least 3 years of observation prior and after inclusion date.
GPC, general population comparator; PsA, psoriatic arthritis.

therapy, seems to have an impact on the risk for developing work disability and thus diminishing the burden of disease from a patient's perspective and societal perspective.[10 24 30] It is evident from this study that the management of the overall burden of disease in patients with PsA is indeed needed and that a successful holistic handling of patients' health may have an impact on both a personal and societal level.

In conclusion, this is the first study to document increased healthcare costs, lower income, higher unemployment rates, higher risk for disability pension and more comorbidities for patients with PsA compared with the general population both in the period prior to diagnosis and with even larger consequences in the years following a PsA diagnosis. This finding is suggestive of health inequity for patients with PsA and calls for preventive measures for the individual as well as an overall societal action.

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**Contributors** LEK: contributed to study conception and design, literature search, data collection, the analysis and interpretation of data, figures, drafting the manuscript and approving the final version. LEK takes responsibility for all coauthors and the integrity of the work as a whole. TSJ: contributed to study conception and design, the analysis and interpretation of data, figures, revising the manuscript and approving the final version. TSJ had access to data throughout the process and knowledge of roles and responsibilities of each author. HG: contributed to study conception and design, data collection, the analysis and interpretation of data, revising the manuscript and approving the final version. HG had access to data throughout the process and knowledge of roles and responsibilities of each author. LD: contributed to study conception and design, data collection, the analysis and interpretation of data, revising the manuscript and approving the final version. LD had access to data throughout the process and knowledge of roles and responsibilities of each author. RC: contributed to study conception and design, literature search, data collection, the analysis and interpretation of data, revising the manuscript and approving the final version. RC had access to data throughout the process and knowledge of roles and responsibilities of each author. CB: contributed to study conception and design, data collection, the analysis and interpretation of data, revising the manuscript and approving the final version. CB had access to data throughout the process and knowledge of roles and responsibilities of each author. LTHJ: contributed to interpretation of data, drafting and revising the manuscript and approving the final version. LTHJ had access to data throughout the process and knowledge of roles and responsibilities of each author. VS: contributed to interpretation of data, drafting and revising the manuscript and approving the final version. VS had access to data throughout the process and knowledge of roles and responsibilities of each author. PJM: contributed to interpretation of data, drafting and revising the manuscript and approving the final version. PJM had access to data throughout the process and knowledge of roles and responsibilities of each author. JK: contributed to study conception and design, data collection, the analysis and interpretation of data, figures, drafting the manuscript and approving the final version. JK takes responsibility for all coauthors and the integrity of the work as a whole.

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**Data sharing statement** Unidentified and additional raw data making the basis for this work can be requested after proper correspondence with LEK, and under the extent possible according to national Danish law.

**Transparency statement** LEK affirms that the manuscript is honest, accurate and in accordance with the prespecified protocol, which can be accessed in the online supplementary material or as open access at http://www.parkerinst.dk. No important aspects of the study have been omitted in the current manuscript.

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EXTENDED REPORT

# Comparison of MRI with radiography for detecting structural lesions of the sacroiliac joint using CT as standard of reference: results from the SIMACT study

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## ABSTRACT

**Objective** Radiographs of sacroiliac (SI) joints are used for the detection of structural damage in patients with axial spondyloarthritis (axSpA), but are often difficult to interpret. Here, we address the question how the T1-weighted MRI (T1w MRI) sequence compares with radiography for SI joints' structural lesions using low-dose CT as the standard of reference.

**Methods** Radiographs, T1w MRI and low-dose CT of the SI joints from 110 patients (mean age 36.1 (19–57) years, 52% males and 48% females; 53% with axSpA, 21 non-radiographic axSpA and 32% radiographic axSpA, 47% with non-SpA) referred to the rheumatologist because of unclear chronic back pain, but possible axSpA, were scored for structural lesions (erosions, sclerosis, joint space changes and an overall impression of positivity).

**Results** Using low-dose CT as the standard of reference, T1w MRI showed markedly better sensitivity with significantly more correct imaging findings compared with radiography for erosions (79% vs 42%; p=0.002), joint space changes (75% vs 41%; p=0.002) and overall positivity (85% vs 48%; p=0.001), respectively, while there were no differences between X-rays and MRI-T1 sequence regarding specificity (>80% for all scores). Only for sclerosis, MRI-T1 was inferior to radiography (sensitivity 30% vs 70%, respectively), however, not statistically significant (p=0.663).

**Conclusions** T1w MRI was superior to radiography in the detection of structural lesion of the SI joints in patients with axSpA. Future studies should focus on finding an agreement on the definition of MRI-T1 positivity.

## INTRODUCTION

The current Assessment of SpondyloArthritis International Society (ASAS) classification criteria for axial spondyloarthritis (axSpA) and the European League Against Rheumatism (EULAR) recommendations on the use of imaging in axSpA ask for radiographs to detect structural lesions and for MRI to detect active inflammatory lesions in the sacroiliac (SI) joints.[1 2] However, several investigators have pointed out major problems with the use of radiography for SI joint evaluation. One concern is the large inter-reader variability in the interpretation of SI joint radiographs[3] and the failure to improve agreement by training of rheumatologists and radiologists.[4] The problems in evaluating radiographs of the SI joints are understandable because pelvic anatomy is complex, the SI joints have an oblique orientation and overlying bowel gas hampers visualisation. These disadvantages can be overcome by cross-sectional imaging techniques such as MRI and CT.[5–7]

While MRI has become generally accepted over recent years for the detection of active inflammatory lesions using T2-weighted sequences with fat suppression, such as the short-tau inversion recovery (STIR) sequence, its value for the scoring of structural lesions such as erosions, sclerosis, joint space variations and fatty lesions using T1-weighted images is still under debate.[8] Recent EULAR recommendations on the use of MRI in SpA state that this imaging modality should be considered for the detection of both active inflammatory lesions and structural lesions,[1] while the recent ASAS update on the definition of a positive SI joint MRI focuses on active sacroiliitis without an in-depth discussion of the value of MRI for the scoring of structural lesions.[9] Another approach investigated whether the additional evaluation of structural lesions, such as erosions, on T1-weighted images can supplement the evaluation of inflammatory lesions on a STIR sequence and whether a combination of both would be more sensitive and/or specific for the detection of non-radiographic axSpA, in addition to the presence of subchondral bone marrow oedema, however, again without an in-depth discussion of whether T1-weighted MRI could replace radiography for the detection of structural lesions.[10]

Previous work found an acceptable performance of T1-weighted MRI for the detection of structural lesions of the SI joints in comparison with radiography; however, an imaging gold standard was not used in most of these studies.[11 12] While CT is generally regarded as the method of choice for the detection of structural SI joint lesions, it is not widely used because of high radiation exposure.[13] Previous studies directly comparing radiography and CT indicate that radiographs have limited sensitivity and specificity.[14] In the current study, we used low-dose CT (ldCT) as the gold standard, with a radiation exposure similar to radiography,[15–18] and compared this with MRI and X-rays in 110 patients who were referred to one rheumatology department because of unclear low back pain and possible

SpA. The aim was to investigate whether T1-weighted MRI has an acceptable sensitivity and specificity for the detection of structural lesions in comparison with ldCT and performs the same or even better compared with radiography of the SI joints.

## MATERIALS AND METHODS

### Subjects

The patients analysed in this study were derived from the population of the sacroiliac joint MRI and CT study SacroIliac MAgnetic resonance Computed Tomograph (SIMACT). This prospective cross-sectional single-centre study included 110 patients being referred to the local rheumatology department, with chronic low back pain of unknown aetiology, however, with the suspicion of SpA. All patients were under the age of 60 and had no contraindications to MRI, for example, cardiac pacemakers. We also included 18 healthy age and sex matched controls that underwent MRI only (mean age 34.6 years, 9 male and 9 female). The final diagnosis was established by an expert rheumatologist using a published algorithm and taking into account all clinical data, laboratory results and imaging findings.[19] The local ethics committee approved this study, and all patients gave written informed consent. The ethics committee waived the need for additional approval by the German Federal Office for Radiation Protection.

### Imaging procedures

All patients for whom no current radiograph (6 months or less) was available underwent a pelvic radiograph with a 30° caudal tilt (Ferguson view) on a conventional skeletal X-ray device (DigitalDiagnost, Philips Medical Systems, Best, The Netherlands) as part of the standard diagnostic procedure. This is the local standard radiographic examination preferred by the rheumatology department because it displays the SI joints with less superimposition than the anterior–posterior beam path.

Thereafter, all patients underwent a CT scan of the SI joints on a 64-row dual-source scanner (Somatom Definition Flash, Siemens, Erlangen, Germany) using a low-dose protocol with a tube voltage of 100 kVp, tube current of 60 mAs, a collimation of 0.6 mm and a spiral pitch factor of 0.8. Dose modulation during scanning was performed to allow the lowest radiation dose possible with acceptable image quality. The ldCT volume dataset was reconstructed using a standard soft tissue and bone kernel. Additional reconstructions with a soft tissue kernel were done in an oblique coronal view parallel to the axis of the second sacral vertebra at 0.6 mm slice thickness.

Directly after the ldCT, all patients underwent MRI at 3.0 Tesla (Magnetom Skyra, Siemens, Erlangen, Germany) using a spine coil. The MRI protocol included a conventional T1-weighted spin echo and STIR sequence in oblique coronal slice orientation. The parameters for the T1-weighted sequence were: repetition time 652.0 ms, echo time 11.0 ms, slice thickness 3 mm, flip angle 156° and spatial resolution of 410×512.

### Radiation exposure

The estimated effective dose was calculated from the dose–area product using a conversion factor of 0.339 for the radiographs[20] and from the dose–length product for ldCT, applying a conversion coefficient of 0.011.[21] The accuracy of this method was tested in a random sample of 15 patients using a special software for radiation exposure calculation (CT-Expo; SASCRAD, Buchholz, Germany).

### Image scoring

Imaging data were anonymised separately for radiography, ldCT and MRI using OsiriX 6.4 (Pixmeo SARL, Bernex, Switzerland) and scored on a workstation with a high-resolution monitor. The three readers had different experience in musculoskeletal image interpretation (reader 1: a research student (JG) with 1 year of experience in SI joint reading; reader 2: a junior radiologist (TD) with 5 years of experience; reader 3: a senior radiologist (K-GAH) with 15 years of image interpretation experience, specialised in rheumatology imaging). All readers performed a specific training including a teaching session and consensus scoring of test cases. The readers were blinded to patient characteristics, clinical data and the findings of other imaging modalities. Radiographs were scored independently by all three readers; however, ldCT and MRI reading were done by readers 1 and 2, while reader 3 scored only a random sample of 20 cases to test for inter-rater reliability.

We used a scoring system based on previous work of our group.[12] Scoring of the radiographs included erosions (0–3), joint space alterations (0–4), sclerosis (0–2)—as shown in table 1 and figure 1—and grading of sacroiliitis according to the modified New York criteria (mNYC) (0–4). In the evaluation of ldCT and MRI, special care was taken to record the location of lesions within the SI joint. This was done by dividing each joint into four quadrants and three positions (anterior, middle, posterior). Thus, each SI joint was divided into 12 locations to accurately localise lesions in three dimensions (see figure 2). The scoring of ldCT and MRI included the same score for erosions, joint space alterations and sclerosis that was used in radiography. In a consensus scoring exercise, a set of 10 randomly selected test cases was used to train the three readers, test the preliminary definitions and adjust the scoring system. Thereafter, five randomly selected samples were assessed independently to further refine agreement between the readers. A scoring atlas was established based on those 15 cases (see figure 1).

The scoring results of the different readers were not averaged for statistical purposes: radiographs were scored by all three readers, and thus agreement of two out of the three readers was used for the statistical analysis. MRI and ldCT were scored by only two readers. Therefore, agreement of both readers for an imaging finding was necessary to count for the analysis (see also below).

### Definitions of structural lesions

An *erosion* was defined as a focal, usually ill-defined lucency on radiographs of the cancellous bone with a clear interruption of the cortical bone in the cartilaginous compartment. This type of lesion appears hypodense relative to trabecular bone on ldCT and hypointense relative to normal bone marrow signal on T1-weighted images. A small erosion was defined as having a

**Table 1** Scoring system for joint space, erosions and sclerosis

| Joint space | | Erosions | | Sclerosis | |
|---|---|---|---|---|---|
| 0 | No joint space changes | 0 | No erosions | 0 | No sclerosis |
| 1 | Questionable widening or narrowing | 1 | Small isolated erosions (1–2) or questionable single erosion | 1 | Questionable or little sclerosis (5 mm or more) |
| 2 | Pseudowidening | 2 | Definite erosions (3–5; <3 mm) or larger single erosion (>3 mm) | 2 | Evident sclerosis (≥10 mm) |
| 3 | Partial ankylosis | 3 | Multiple (>5) or confluent erosions | | |
| 4 | Extensive/total ankylosis | | | | |

Erosions and sclerosis were scored per joint in radiographs and per region (12 for each joint) on low-dose CT and MRI (T1).

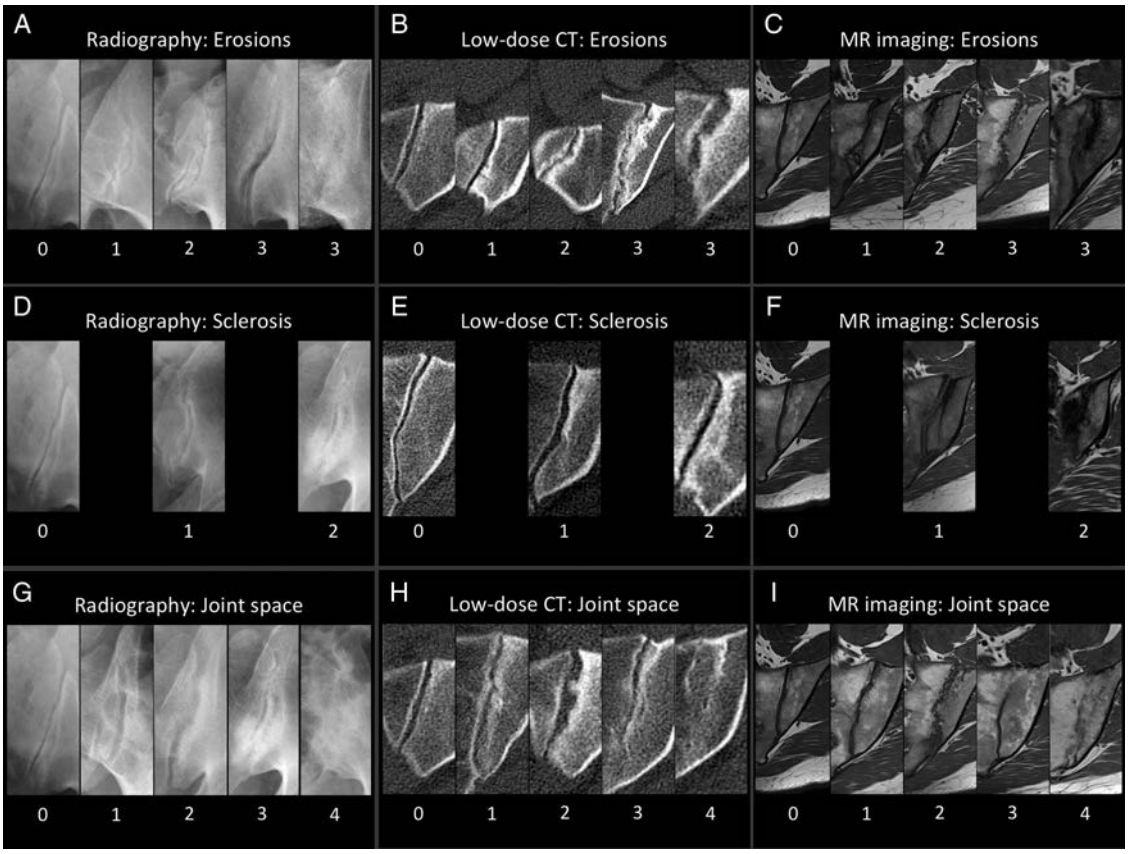


**Figure 1** Scoring atlas for erosions (A–C), sclerosis (D–F) and joint space alterations (G–I) seen on radiography (A, D and G), low-dose CT (B, E and H) and MRI (C, F and I). A detailed description of the different scoring items is shown in table 1.


**Figure 2** Three-dimensional localisation of all 24 regions. The first eight quadrants capture changes in the anterior aspects of both sacroiliac joints, which are anterior to the slices depicting the sacral neuroforamina (<180° of the circumference of S2 is visualised). The true pelvis is seen in the centre of the image. The second eight quadrants (numbered 9–16) subdivide the central portion of both sacroiliac joints, defined by the depiction of the anterior sacral foramina. The remaining quadrants (numbered 17–24) represent the posterior part of the joints, which is recognised by visualisation of the entheseal joint compartment in the middle, and stretching to the posterior and inferior aspect of the joint. Proper oblique coronal slice orientation (parallel to the axis of the S2 vertebra) is crucial for this scoring system. Also, normal anatomical variants must be considered when using it.

diameter below 3 mm, and a large erosion as equal or above 3 mm. Confluent erosions were defined as a loss of the normal cortical border over a length of at least 6 mm parallel to the joint space (see figure 1A–C).

*Sclerosis* was defined as a sharply or ill-defined opacity on radiographs, a hyperdense—clearly more dense than normal cancellous bone—lesion on ldCT, or a very hypointense (black) lesion on all MRI sequences of the cancellous bone adjacent to the joint space with a minimum diameter of 5 mm measured perpendicular to the joint (see figure 1D–F).

*Pseudowidening* was defined for radiography as increased bone-to-bone distance clearly attributable to joint destruction due to confluent erosions affecting at least a quarter of the joint. For ldCT or MRI, the bone-to-bone distance had to be increased in the major part of a region (see figure 1 for definition of regions). *Ankylosis* was defined as an opacity within the joint space suggesting complete bony bridging on radiography. For ldCT, ankylosis was defined as a dense lesion within the joint space consistent with the bone. For T1-weighted MRI, ankylosis was defined as a lesion in the joint space bridging the joint with an increased signal intensity equal to or greater than that of normal bone marrow. Extensive ankylosis was defined as affecting at least half of the joint. Imaging examples of pseudowidening and ankylosis are shown in figure 1G–I.

### Definition of positive imaging findings

A scoring item (ie, erosions, sclerosis and joint space changes) with a score of 2 or higher was defined as unequivocally positive for all modalities (see table 1). On the *joint level*, mNYC grade II or higher was defined as a positive radiograph. Global positivity on ldCT was defined as having an erosion score and/or joint space score of 2 or higher in any of the 12 regions. This definition complies with a grade II, according to the mNYC with unequivocal erosive changes or pseudowidening/ankyloses. Sclerosis was considered non-specific, because it is also frequently

present in other conditions such as osteoarthritis or osteitis condensans. Therefore, it was not included in our definition of a positive SI joint for ldCT or MRI. On the *patient level*, grade II bilaterally or grade III–IV unilaterally, according to the mNYC, was defined as a positive radiograph. For ldCT and MRI, we defined an erosion score and/or joint space score of 2 or higher in any of the 24 regions of both joints as positive.

For statistical analysis, joints and items were counted as positive only if both readers agreed about the presence of the pathological imaging finding.

### Statistical analysis

We performed a Kruskal-Wallis and $\chi^2$ test for significant differences in patient characteristics between the groups of patients with axSpA and non-axSpA. A contingency table analysis was conducted to compare overall positivity of radiographs and MRI with ldCT, calculating sensitivity and specificity on a joint-based level and a patient level using ldCT as standard of reference (SOR). Furthermore, the McNemar test was used to search for significant differences in correct and incorrect findings on radiography and MRI. The same analysis was performed for each structural scoring item (erosions, sclerosis and joint space alterations). Cohen's κ was calculated comparing ldCT with MRI and radiography and each reader pair separately. Cohen's κ was interpreted according to Landis and Koch.[20] The Spearman correlation coefficient was calculated for sum scores of radiography, ldCT and MRI. The Wilcoxon signed-rank test was performed to test for significant differences of sum scores of ldCT and MRI. A value of $p<0.05$ was considered significant.

Analyses were performed with SAS V.9.2 (SAS Institute, Cary, North Carolina, USA) and GraphPad Prism (V.6.0 for MacOS, GraphPad Software, La Jolla, California USA).

## RESULTS

### Subjects

A total of 110 patients presenting with unclear low back pain from September 2012 to January 2014 were included. CT and MRI were tolerated well by all patients.

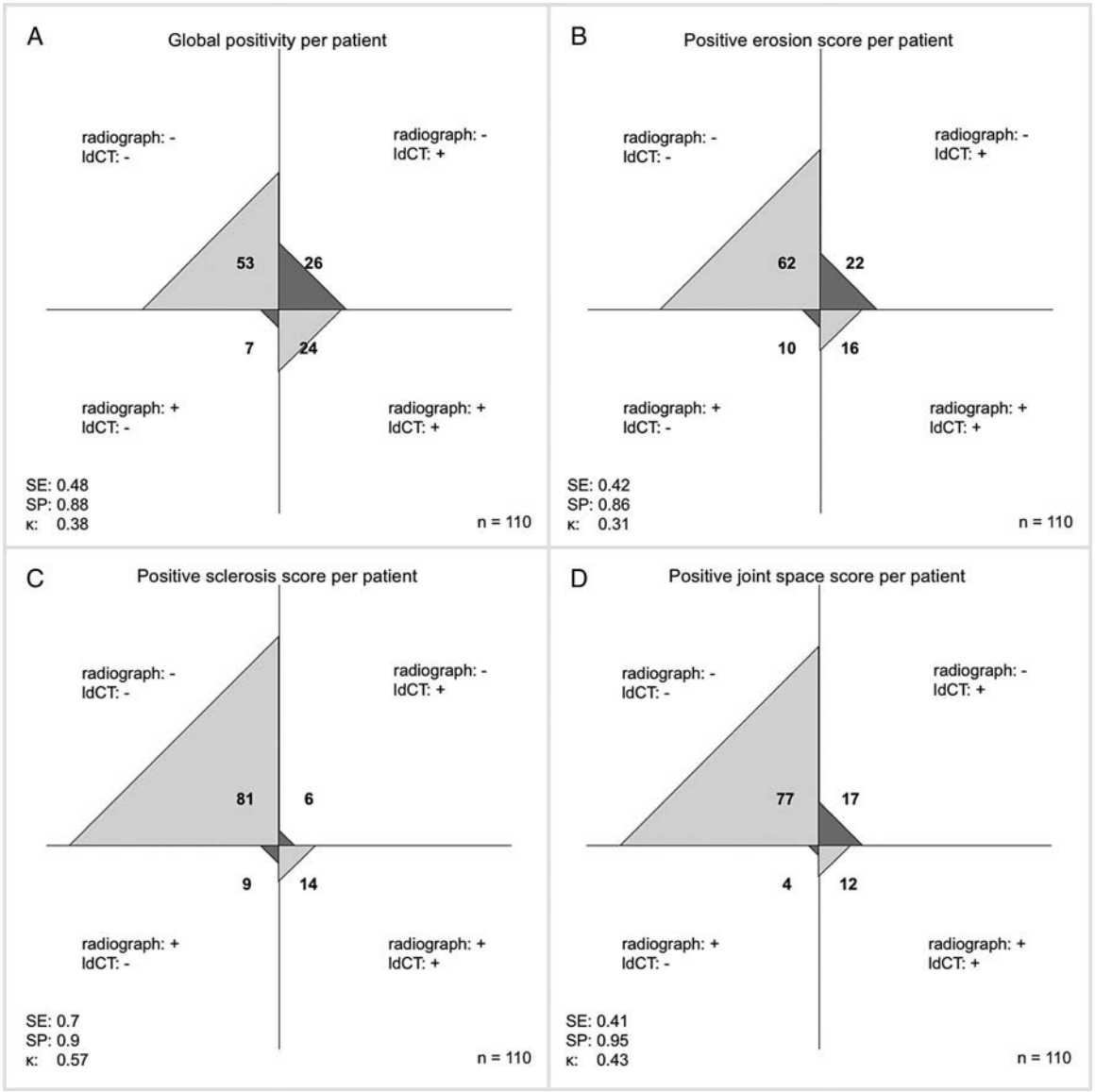


**Figure 3** Contingency analysis radiography versus low-dose CT (ldCT) on the patient level. (A) Global positivity as defined for radiography and ldCT (erosion and/or joint space score >1). (B) Positive erosion score. (C) Positive sclerosis score. (D) Positive joint space score. For definitions of positive findings and scoring items, see Methods section of the text. κ, Cohen's κ for agreement of the two modalities; SE, sensitivity; SP, specificity, using ldCT as the standard of reference.

A total of 58 patients were finally diagnosed with axSpA (AS and nr-axSpA) by the expert rheumatologist (35 men and 23 women; mean age, 34.8 years; 46 human leukocyte antigen (HLA)-B27 positive; mean symptom duration, 94 months), 52 with other diagnoses, for example, osteitis condensans or osteoarthritis (18 men and 34 women; mean age, 37.4 years; 24 HLA-B27 positive; mean symptom duration, 70.2 months). In the axSpA group, 35 patients were diagnosed with nr-axSpA and 23 patients with AS based on a centralised and standardised reading of the radiographs as described above.

### Radiation exposure

The mean radiation exposure of radiography was calculated as 0.52 mSv (SD 0.48) with a maximum of 3.44 mSv. For 10 examinations performed elsewhere, exposure could not be calculated because the parameters of the X-ray machines were not available. The mean radiation exposure of ldCT was calculated to be 0.51 mSv (SD 0.18) with a maximum of 1.46 mSv, including the topogram for planning the examination.

### Scoring results

Applying the definition of positivity for structural changes, 31 patients were positive on radiography, 50 on ldCT and 45 on MRI. Figure 3 provides the contingency graph for the comparison of radiography and ldCT on the joint level based on the contingency table. It also includes sensitivities and specificities and Cohen's κ for positive findings and each scoring item. Figure 4 presents the same information for the comparison of MRI and ldCT. All values were calculated using ldCT as SOR. Figure 5 provides an overview of sensitivities and specificities comparing radiography and MRI, showing that the sensitivity is clearly better for MRI compared with radiography for most of the variables investigated, with the exception of sclerosis for which radiography performed better. However, there was no clear difference in the specificity. Using ldCT as SOR, overall positivity was correct in 70.0% (60.8%–77.8%) of cases in radiography and 89.1% (81.9%–93.7%) in MRI (p=0.0005). We found also a significant difference for erosions with 70.9% (61.8%–78.6%) correct findings on radiography and 88.2% (80.8%–93.0%) on MRI with a p value of 0.0023 and for joint space alterations with 80.9% (72.6%–87.2%) correct findings on radiography and 92.7% (86.3%–96.3%) on MRI with a p value of 0.0019, respectively (see also figure 5). However, for sclerosis, the difference with 86.4% (78.7%–91.6%) correct findings on radiography and 83.6% (75.6%–89.4%) on MRI was not significantly different (p=0.6625).

Regarding inter-rater reliability of readers 1 and 2 for *global positivity*, we found fair agreement for radiography (κ=0.36 on the joint level and 0.33 on the patient level) and substantial agreement for both ldCT (κ=0.69 on the joint level and 0.62

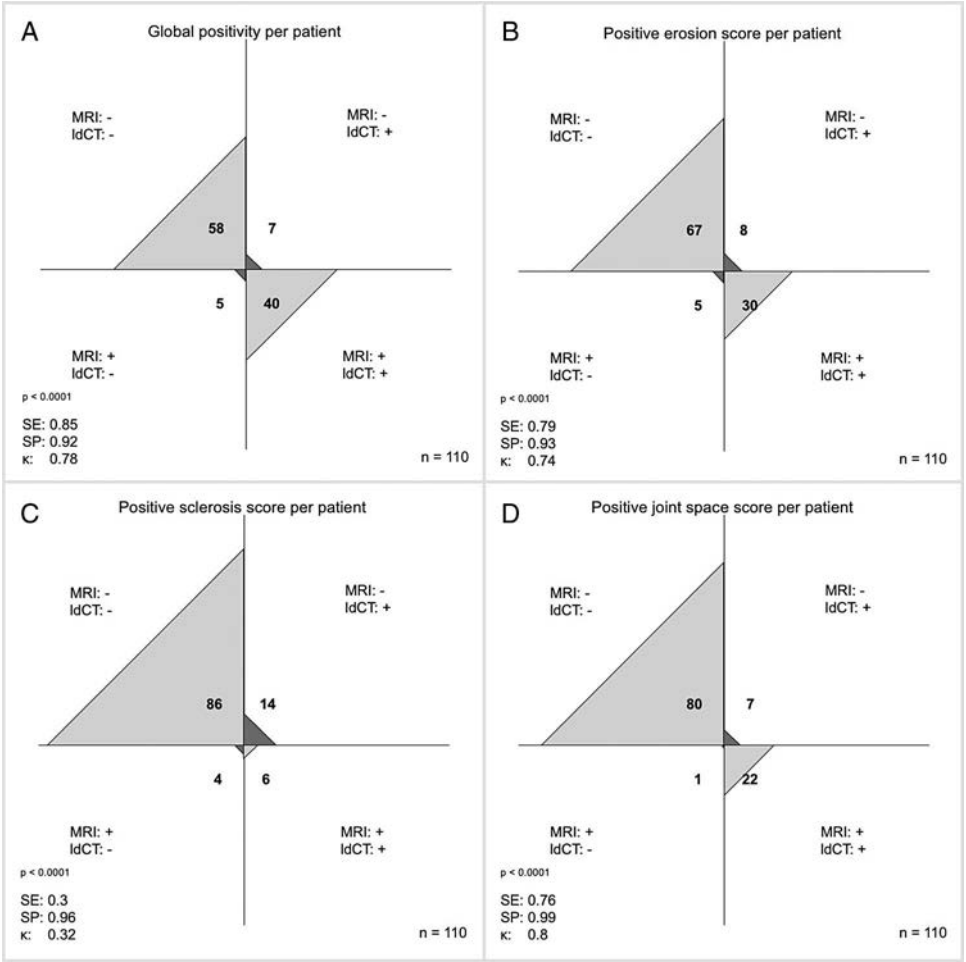


**Figure 4** Contingency analysis. MRI versus low-dose CT (ldCT) on the patient level. (A) Global positivity as defined for radiography and MRI (erosion and/or joint space score >1). (B) Positive erosion score. (C) Positive sclerosis score. (D) Positive joint space score. For definitions of positive findings and scoring items, see Methods section of the text. κ, Cohen's κ for agreement of the two modalities; SE, sensitivity; SP, specificity, using ldCT as the standard of reference.

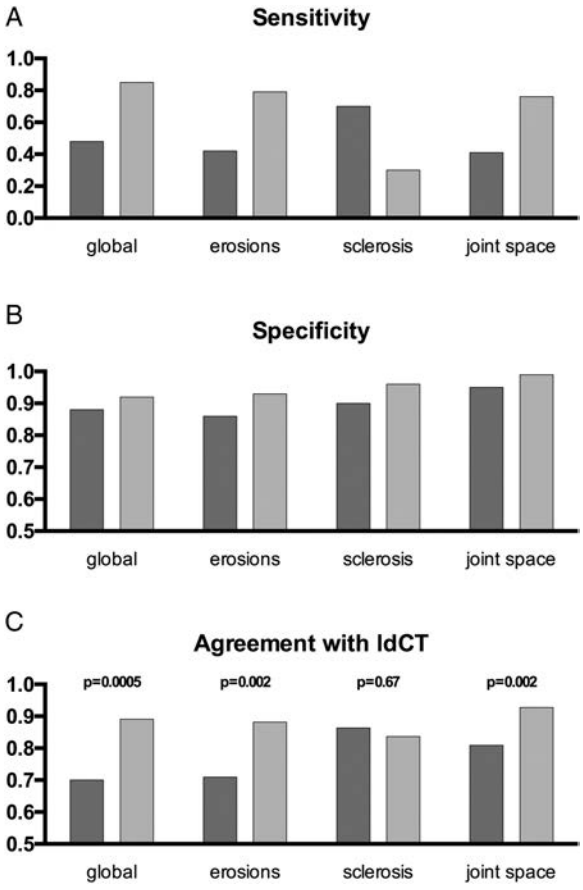


**Figure 5** Summary and comparison of the sensitivity (A), specificity (B) and agreement with low-dose CT (ldCT) on the presence/absence of findings displayed as parts of the whole patients' collective (C) for radiography and MRI for global impression and structural scoring items using ldCT as standard of reference. The p values (C) are calculated using McNemar test. Radiography shows lower sensitivities for all scoring items except sclerosis when compared with MRI. Specificities for radiography are comparable with those for MRI, however, slightly inferior for each item. MRI shows a significantly better agreement to ldCT than radiography for global impression—as defined in the Methods section—erosions and joint space alterations.

on the patient level) and MRI (0.68 on the joint level and 0.62 on the patient level).

Analysing the results for each reader separately, we found for the *erosion* score weak correlation between radiography and ldCT for reader 1 (Spearman correlation coefficient of 0.33) and moderate correlation for reader 2 (0.56). The correlation of the erosion sum scores between MRI and ldCT was strong for reader 1 (0.74) and reader 2 (0.79). Inter-rater agreement for the presence of an erosion score >1 was fair for radiography ($\kappa$=0.34 on the patient level), but substantial for ldCT ($\kappa$=0.77) and moderate for MRI ($\kappa$=0.54).

For *sclerosis*, we found moderate correlation between radiography and ldCT for reader 1 (Spearman correlation coefficient of 0.51) and moderate correlation for reader 2 (0.58). The correlation of ldCT and MRI was moderate for reader 1 (0.42) and reader 2 (0.6).

Inter-rater agreement for the presence of a sclerosis score of 2 was fair for radiography ($\kappa$=0.4 on the patient level) and MRI ($\kappa$=0.35). However, for ldCT, agreement was moderate ($\kappa$=0.49).

There was no significant pattern regarding distribution of lesions in the different locations for either sclerosis (p=0.72) or erosions (p=0.88), that is, no single region was affected more frequently than others.

Analysing *joint space* changes, we found moderate correlation for reader 1 (Spearman correlation coefficient of 0.47) and for reader 2 (0.54) between radiography and ldCT, and very strong correlation between ldCT and MRI for reader 1 (0.83) and strong correlation for reader 2 (0.73). Inter-rater agreement of a joint space score >1 was fair for radiography ($\kappa$=0.32), substantial for ldCT ($\kappa$=0.7) and moderate for MRI ($\kappa$=0.55).

Comparison of the sum scores for structural lesions on the patient level found MRI and ldCT to be equivalent for erosions when tolerating a 15% mismatch. Reader 2, but not reader 1, scored joint space alterations significantly different in MRI and ldCT. Nonetheless, both readers assigned lower scores to MRI for sclerosis detection (see online supplementary figure S1).

All healthy controls were negative for erosions, sclerosis and joint space, as defined for our study.

## DISCUSSION

In comparison with low-dose CT as the gold standard, conventional radiography missed more than half of the patients and two-thirds of the joints with structural changes indicating (radiographic) axSpA on low-dose CT. Especially erosions—considered the most characteristic structural damage[21 22]—were not detected reliably on radiographs, confirming earlier studies demonstrating higher sensitivity of CT.[14] Interestingly, only a few cases of false-positive results were obtained with radiography using ldCT as the gold standard. This is the first study comparing conventional radiographs with ldCT performed with a radiation exposure comparable with that of radiography. Previous studies report good diagnostic accuracy for ldCT of the bones in the detection of fractures and malignant infiltration.[23–25] However, for ethical reasons (radiation exposure), a comparison with conventional CT was not performed by us and—to our knowledge—has not been performed by other investigators.

Most importantly, however, our results show that the sensitivity of T1-weighted MRI for the detection of structural lesions, especially erosions and joint space changes (including ankylosis), is similar to that of ldCT and better than that of radiography. Inter-rater agreement about imaging findings was also significantly inferior for radiography compared with both ldCT and MRI, again confirming earlier reports.[3] Not surprisingly, T1-weighted MRI was inferior to radiography in the detection of sclerosis. In terms of our definition of positive sacroiliitis (structural damage) on the patient level, T1-weighted MRI again performed quite well with a sensitivity of approximately 85% vs 48% for radiography, while specificity was approximately 90% for both MRI and radiographs in comparison with ldCT. Thus, based on our study, the major problem with radiography, besides high inter-reader variability, seems to be underdiagnosis rather than overdiagnosis of structural lesions in the SI joints (figure 3). Also, when T1-weighted MRI is compared with ldCT on the individual patient level (see figure 5), false-positive MRI findings seem to be less of a concern than false-negative findings. But, our results also show that some patients with radiographic axSpA might be missed when T1-weighted MRI is used alone. Therefore, in patients with inconclusive T1-weighted MRI findings, ldCT of the SI joints seems to be a suitable supplementary option for the detection of structural lesions.

Eighteen age-matched and sex-matched healthy subjects who underwent MRI were included as controls. Applying our definition of positivity for structural lesions of the SI joints, none of these controls were positive on MRI. Nonetheless, the

increasing evidence showing that T1-weighted MRI might be an alternative (or supplement) to radiography warrants agreement about a generally accepted definition of MRI positivity for structural lesions. Other definitions have been proposed and investigated in patients and controls using the clinical diagnosis as gold standard such as either ≥3 erosions, ≥3 fatty lesions, and/or ≥5 fatty lesions and/or erosions,[26] or just ≥2 erosions.[10] We also analysed our data in relation to the clinical diagnosis of the expert rheumatologist and, here again, T1-weighted MRI performed better than radiography (data not shown). However, this result of our analysis should be treated with caution, because the rheumatologist was aware of the imaging results when making the final diagnosis and therefore might have been biased.

Some earlier studies comparing the performance of MRI, CT and radiography in the detection of sacroiliitis in smaller patient populations already indicate that MRI comes close to CT, especially in the detection of erosions, and is superior to radiographs.[27] While our results are in line with these studies, they also show that cross-sectional techniques are superior in patients with advanced SpA and in identifying structural lesion patterns in patients diagnosed with axial SpA for the first time.

Thus, the next step should probably be to test the sensitivity and specificity of the different definitions of MRI positivity for structural lesions in patients from previous and future studies and to find a generally accepted definition. It is unlikely that T1-weighted MRI will fully replace radiography in the diagnosis of radiographic sacroiliitis in the near future because of the wide availability of this test and the still relatively high costs of MRI. However, patients in whom an MRI is available might not require an additional radiographic examination.

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**Contributors** TD: conception and design of the study, design of scoring system, image scoring, data evaluation, statistical calculations, article draft, critical revision of the manuscript for important intellectual content. K-GAH: conception and design of the study, design of scoring system, image scoring, data evaluation, with critical revision of the manuscript for important intellectual content and final approval of the version to be published. JG: patient acquisition, data management, image scoring, critical revision of the manuscript for important intellectual content. CS: analysis and interpretation of data, statistical calculations. DP: patient acquisition, critical revision of the manuscript for important intellectual content. BH: conception of the study, critical revision of the manuscript for important intellectual content. JS: patient acquisition, conception and design of the study with critical revision of the manuscript for important intellectual content.

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OPEN ACCESS

EXTENDED REPORT

# Quantifying the hepatotoxic risk of alcohol consumption in patients with rheumatoid arthritis taking methotrexate

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## ABSTRACT

**Background** Patients with rheumatoid arthritis (RA) who take methotrexate (MTX) are advised to limit their alcohol intake due to potential combined hepatotoxicity. However, data are limited to support this. The aim of this study was to quantify the risk of developing abnormal liver blood tests at different levels of alcohol consumption, using routinely collected data from primary care.

**Methods** Patients with RA in the Clinical Practice Research Datalink starting MTX between 1987 and 2016 were included. Hepatotoxicity was defined as transaminitis: alanine transaminase or aspartate aminotransferase more than three times the upper limit of normal. Crude rates of transaminitis were calculated per 1000 person-years, categorised by weekly alcohol consumption in units. Cox proportional hazard models tested the association between alcohol consumption and transaminitis univariately, then age and gender adjusted.

**Results** 11 839 patients were included, with 530 episodes of transaminitis occurring in 47 090 person-years follow-up. Increased weekly alcohol consumption as a continuous variable was associated with increased risk of transaminitis, adjusted HR (95% CI) per unit consumed 1.01 (1.00 to 1.02); consuming between 15 and 21 units was associated with a possible increased risk of hepatotoxicity, while drinking >21 units per week significantly increased rates of transaminitis, adjusted HR (95% CI) 1.85 (1.17 to 2.93).

**Conclusions** Weekly alcohol consumption of <14 units per week does not appear to be associated with an increased risk of transaminitis.

## BACKGROUND

Methotrexate (MTX) is the first-line disease-modifying antirheumatic drug in patients with rheumatoid arthritis (RA).[1] It is clinically effective and well tolerated;[2] however, the potential hepatotoxicity of MTX remains a concern,[3] and regular blood monitoring is mandated. Alcohol consumption is also well known to have an adverse effect on the liver, particularly in excess.[4 5] Given these two associations, patients taking MTX have traditionally been advised to limit or even abstain from alcohol consumption. The American College of Rheumatology (ACR) guidelines, published in 1994, recommend abstinence from alcohol with only occasional exceptions.[6] In contrast, more recent guidance from the British Society for Rheumatology, published in 2008, suggests that patients taking MTX should limit their alcohol intake to 'well within the UK national recommendations',[7] without further specification.

While the relationship between MTX and hepatotoxicity has been extensively reviewed,[3] there is a lack of evidence to quantify the potential additional effect of alcohol on liver toxicity while taking MTX. Indeed, the ACR guidance comments that regular alcohol consumption should not occur since there are 'no data about the quantity of alcohol that can safely be consumed with MTX'.[6] The majority of studies which have examined this question have focused on histopathological changes in serial liver biopsies,[8 9] and frequently date back to the 1970s. By contrast, current monitoring guidelines advocate measuring of serum liver function tests (LFTs).[7] Importantly, previous studies have not consistently demonstrated an association between increased alcohol consumption and hepatotoxicity or liver damage;[9–14] yet, it is clearly biologically plausible that there may be an additive. Many patients would like to drink modestly; in the absence of evidence, such patients may be inclined or advised to either abstain from alcohol altogether or avoid MTX, a potentially beneficial drug. If patients do drink alcohol alongside MTX, even in moderation, they anecdotally describe feeling anxious or ill at ease. Understanding whether there is a safe amount of alcohol that can be consumed alongside MTX, and what that amount is, would significantly aid informed decision-making.

The aim of this study, therefore, was to quantify the risk of alcohol consumption on hepatotoxicity in a contemporary group of MTX users with RA, in a large national primary care database.

## METHODS

### Patients and setting

Patients with RA within the Clinical Practice Research Datalink (CPRD) were identified using a previously validated algorithm.[15] CPRD is a large electronic database of routinely collected primary care electronic medical records, beginning in 1987, which includes approximately 8% of the total UK population and is considered broadly representative of the UK population in terms of age, gender and ethnicity.[16] In the UK, MTX therapy is typically initiated in a secondary care setting by a rheumatologist, but subsequent prescriptions and blood monitoring are performed in primary care, and are therefore recorded within their primary care

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electronic records. All patients with RA starting MTX after 1987 were included once a practice had met data quality standards required for participation in CPRD. Follow-up was commenced from the date of the first MTX prescription and continued until February 2016, unless patients were censored earlier (see below).

### Exposures and outcome

The outcome of interest was an episode of transaminitis, defined as alanine transaminase (ALT) or aspartate aminotransferase (AST) levels of three times the upper limit of normal (ULN) or higher, according to local laboratory standards. Patients were included in the analysis if they had ALT and AST measured on average at least six times per 12 months to indicate compliance with regular blood monitoring and avoid introducing surveillance bias. Prior studies have identified persistently raised LFTs as being predictive of progression to cirrhosis;[17] hence we had a secondary definition of transaminitis as three sequential ALT or AST measurements above the ULN. Alcohol consumption was identified first as yes/no, then by units of alcohol consumed per week. A unit of alcohol represents 10 mL or 8 g of pure alcohol,[18] and is used in the UK to make comparisons of alcohol consumption across different beverages. It is also used by the UK government to set national guidelines; currently, the guidance is to drink no more than 14 units of alcohol per week for both men and women.[19] Prior to January 2016, the limit for men was higher at 21 units per week. For patients who had alcohol status recorded more than once within CPRD, the value used was the earliest recorded alcohol consumption data following first MTX prescription. If this was not available, then the nearest alcohol consumption data recorded prior to the first MTX prescription were used. If the only data available on alcohol consumption was yes/no, patients who did not drink were recorded as drinking zero alcohol units, to increase the power of the study. As patients sometimes undertake pauses in their MTX treatment, either through their own choice or through clinician recommendation, person-time was included in the analysis only while patients were actively receiving MTX. Thus, person-time and events of transaminitis occurring while the patient was not taking the drug were excluded. Patients were censored at the time of the first episode of transaminitis, death or 29 February 2016.

### Statistical analysis

Crude rates of transaminitis were calculated per 1000 person-years first for all patients, then in drinkers versus non-drinkers and finally by dividing alcohol units into categories of increasing consumption (0/1–7 (mild)/8–14 (moderate)/15–21 (moderate–high) and >21 (high)). Cox proportional hazard models were used to investigate the association between alcohol consumption and time to first episode of transaminitis, both univariately and age and gender adjusted. As for the crude rates, a number of different models were constructed. First, the risk of transaminitis was identified in drinkers versus non-drinkers, then in the four alcohol unit categories and finally treating alcohol units consumed as a continuous variable. Posterior probability graphs were drawn to assess the probability of the HR exceeding a clinically significant increase, set a priori at a 50% increase in rates of transaminitis, in each of the four categories of alcohol consumption compared with no alcohol consumption. All analyses were carried out for both primary and secondary definitions of transaminitis.

## RESULTS

A total of 44 586 patients with RA were identified, of whom 11 839 were included in the study (figure 1, flow chart); 8401

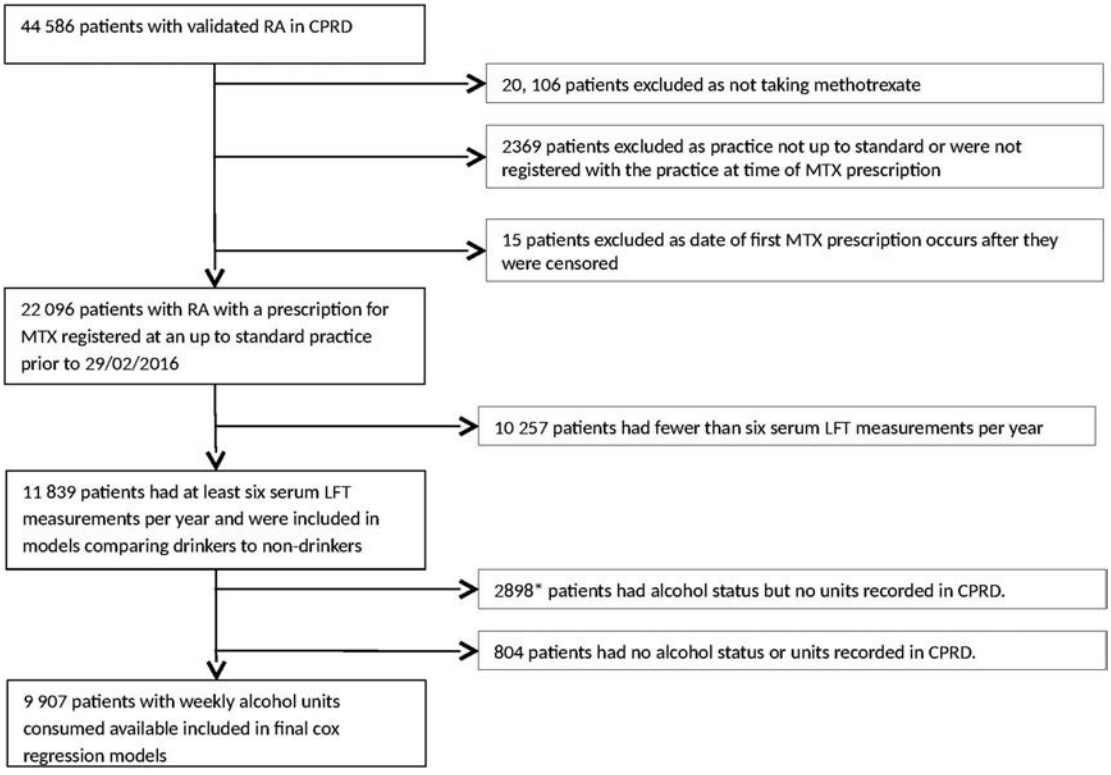


**Figure 1** Flow chart of patients included and excluded from the study of the patients with only alcohol status recorded and no weekly units; those who reported drinking no alcohol (1770) were subsequently recorded as drinking zero units per week and were then included in the final model. CPRD, Clinical Practice Research Datalink; LFT, liver function test; MTX, methotrexate; RA, rheumatoid arthritis.

(71%) were female, and mean age (SD) was 61 (13.9) years. Baseline demographic information is shown in table 1; further details of demographics at each stage of exclusion are available in online supplementary table S1. Excluded patients were slightly younger than patients included in the final study. If only information on whether they drank alcohol at all (rather than weekly units) was available, they were more likely to be female, and a much higher percentage drank no alcohol (60% vs 33%). This is likely because these patients would automatically be assumed to drink zero units of alcohol per week, but this may not be recorded separately. As shown in table 1, the vast majority of patients (7764/9907, 78%) were mild drinkers (≤7 units per week) or drank no alcohol; only 799 (8%) consumed more than the UK recommended limit of 14 units per week. Using the primary definition of transaminitis, there were 530 first episodes of transaminitis in 47 090 person-years follow-up, giving a crude event rate of 11.26 per 1000 person-years. Crude rates of transaminitis were similar between patients who consumed any amount of alcohol and non-drinkers, at 10.08 and 10.64 per 1000 person-years, and in the age-adjusted and gender-adjusted Cox model, there was no increased risk in the occurrence of transaminitis in drinkers compared with non-drinkers; HR (95% CI) 1.06 (0.86 to 1.30).

Crude rates of transaminitis appeared to increase with increasing levels of alcohol consumption (table 2). In the adjusted Cox model, mild-to-moderate alcohol consumption (both 1–7 and 8–14 units per week) was not associated with a statistically significant risk of developing transaminitis compared with non-drinkers (table 2), with HRs (95% CIs) of 1.02 (0.82 to 1.28) and 0.98 (0.71 to 1.35), respectively. There was a trend to higher HR with higher levels of alcohol consumption (table 2) and a statistically significant increase in rates of transaminitis for those patients consuming over 21 units per week compared with non-drinkers, both univariately and in the adjusted model; adjusted HR (95% CI) 1.85 (1.17 to 2.93). Finally, when treated as a continuous variable, each increased unit of alcohol consumed was associated with a higher risk of transaminitis; adjusted HR (95% CI) 1.01 (1.00 to 1.02).

Posterior probability graphs (figure 2) demonstrated that alcohol consumption below 14 units per week was associated with a very low probability (0.93%) of having a clinically important (≥50%) increased risk of transaminitis. For alcohol consumption exceeding 14 units per week, the probability of having a clinically important increased risk of transaminitis was higher, specifically 33% and 81% for moderate–high (15–21 units) and high (>21 units) alcohol consumption, respectively.

Using the secondary definition of transaminitis, there was again no increased risk with 1–7 or 8–14 units of alcohol per week (adjusted HR (95% CI) 0.98 (0.83 to 1.16) and 0.95 (0.75 to 1.21), respectively). There was a non-significant increased risk seen in those consuming 15–21 or >21 units of alcohol per week (HRs 1.18 (0.84 to 1.65) and 1.26 (0.87 to 1.81), respectively). The posterior probabilities were lower for all alcohol consumption categories: below 14 units of alcohol per week, the probability of having a clinically important (≥50%) risk of transaminitis was 0.01%, and for moderate–high (15–21 units) and high (>21 units) alcohol consumption, 8% and 17%, respectively (see online supplementary table S2 and figure S1).

**Table 1** Baseline demographics

| | n=11 839 | Missing n (%) |
|---|---|---|
| Age median (IQR) | 61 (51–70) | 0 |
| Female n (%) | 8401 (71) | 0 |
| No alcohol consumed n (%) | 3259 (28) | 804 (7%) |
| Alcohol (units per week) median (IQR) | 3 (1–8) | 1932 (16%) |
| Weekly alcohol consumption (units) n (%) | | 1932 (16%) |
| 0 | 3259/9907 (33) | |
| 1–7 (mild) | 4505/9907 (45) | |
| 8–14 (moderate) | 1344/9907 (14) | |
| 15–21 (moderate–high) | 429/9907 (4) | |
| >21 (high) | 370/9907 (4) | |

## DISCUSSION

In this study, we have demonstrated that the risk of transaminitis in patients with RA taking MTX does increase with increasing levels of alcohol consumption. However, the risk in those patients who consume ≤14 units of alcohol per week is no greater than those who do not drink alcohol. This is the first study to provide quantifiable estimates of the risk of different levels of alcohol consumption while taking MTX, in a large group of patients who take long-term MTX. The study has important clinical implications. At present, there is uncertainty about the acceptable levels of alcohol consumption while taking MTX, and different rheumatologists and healthcare practitioners may give different advice on what is safe. This can lead to patients avoiding MTX altogether in favour of modest (and perhaps safe) alcohol consumption and thus missing its potential benefits; avoiding any alcohol and potentially affecting their quality of life; or worrying about the potential consequences of any alcohol they consume.

In the literature, there are a small number of studies which have provided a quantitative measure of the risk of alcohol

**Table 2** Associations between weekly alcohol consumption and occurrence of transaminitis

| Units of alcohol per week | Number of events*‡ | Person-years (1000) | Crude rate (95% CI) per 1000 person-years | HR (95% CI), univariate | HR (95% CI), age and gender adjusted |
|---|---|---|---|---|---|
| 0 | 131 | 12.99 | 10.08 | Ref | Ref |
| 1–7 | 193 | 18.83 | 10.25 | 1.02 (0.82 to 1.28) | 1.03 (0.82 to 1.28) |
| 8–14 | 53 | 5.33 | 9.94 | 0.98 (0.71 to 1.35) | 1.01 (0.73 to 1.40) |
| 15–21 | 22 | 1.73 | 12.75 | 1.26 (0.80 to 1.97) | 1.35 (0.85 to 2.14) |
| >21 | 23 | 1.36 | 16.96 | 1.63† (1.05 to 2.54) | 1.85† (1.17 to 2.93) |
| Total | 530 | 47.09 | 11.26 (10.3 to 12.3) | 1.03 (0.87 to 1.21) | 1.02 (0.87 to 1.21) |

†p<0.01.
*Event=transaminitis, defined as alanine transaminase or aspartate aminotransferase more than three times the upper limit of normal.
‡Not all patients who were defined as drinkers/non-drinkers had alcohol consumption defined in units.

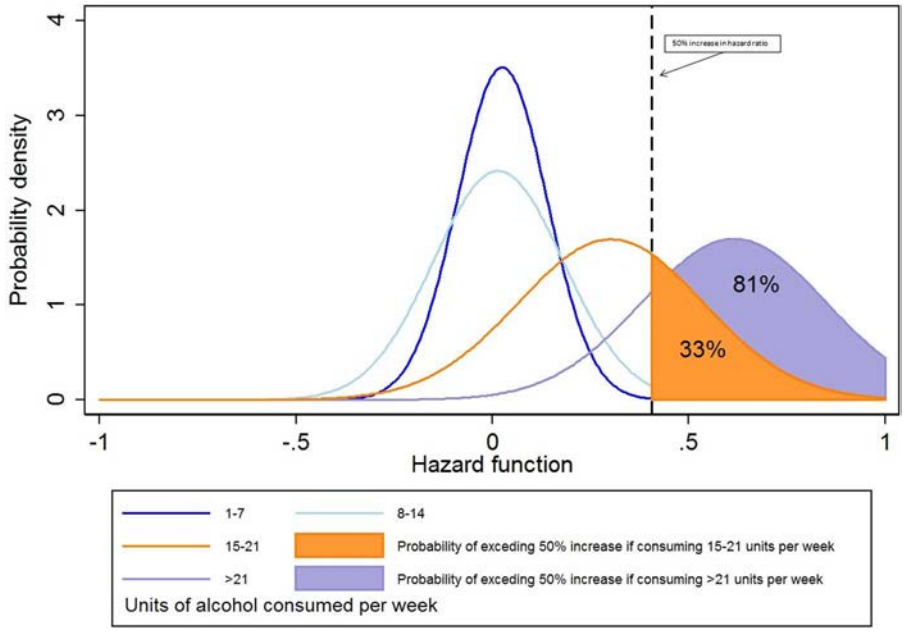


**Figure 2** Posterior probabilities of the hazard function. The area under each curve (AUC) represents the probability of the hazard function at that rate of alcohol consumption. The dotted line denotes an arbitrary clinically significant increase in risk of transaminitis of 50% (which would represent an increase in the crude rate from 12 to 18 per 1000 person-years). The AUC to the right of the dotted line is the probability that the hazard function is greater than the clinically significant margin.

consumption and hepatotoxicity in MTX users.[9 13] In a study recruiting patients between 1979 and 1990, Malatjalian *et al*[9] examined biopsy before and after MTX therapy retrospectively in a cohort of patients with psoriasis starting MTX. They found no significant difference in progression of liver biopsy grades between patients drinking more or less than 14 units of alcohol per week. Laharie *et al*[13] used a fibroscan technique to investigate the presence of fibrosis in a non-invasive manner in 518 patients taking MTX for a variety of indications, including Crohn's disease and psoriasis as well as RA. They showed that alcohol consumption of >14 units per week was associated with increased fibrosis. However, neither the total dose of MTX nor duration of use was associated with higher fibrosis scores, suggesting the association between alcohol consumption and liver fibrosis is the same in MTX users as in the general population. This study was limited by its cross-sectional nature; in addition, this technique has not yet been adopted widely in clinical practice, and certainly not in the context of monitoring MTX therapy.

A key difficulty with the literature is that studies were often conducted in the 1970s and 1980s, and almost exclusively in patients being treated with MTX for psoriasis, with fewer data on patients with RA.[10 11 20–22] They were frequently retrospective with small numbers, and some included pretreatment biopsies which demonstrate abnormalities pre-existent to MTX therapy.[10] Most importantly, however, MTX prescribing and monitoring practices differ markedly now from the time at which the studies were conducted. Critically, liver biopsies are now rarely performed as part of routine monitoring, as there is considerable morbidity and mortality associated with the procedure.[23] Our data therefore provide more useful insight into the consequences of consuming alcohol while undergoing standard MTX monitoring practice in this era.

Some studies have looked at rates of liver enzyme abnormalities. Curtis *et al*[24] studied patients with both RA and psoriatic arthritis, taking leflunomide and MTX. Their results suggested that LFT derangement in patients taking MTX/leflunomide is significantly more likely in patients who drink one to two alcoholic drinks per day, compared with non-MTX/leflunomide users. However this is not useful if we are attempting to provide information to patients beginning MTX therapy; for them, we need to know the risk of alcohol consumption while taking MTX. Kent *et al* studied[25] risk factors for the occurrence of abnormal LFTs in a cohort of patients with RA taking MTX from 1991 to 2002. They found no significant association between current alcohol use and abnormal LFTs. However, there are issues with this study. They included any elevation of AST above the ULN as an event of interest, which may capture a large number of false positives of no clinical significance. In addition, they used standard linear regression as opposed to Cox models, which would not take into account the fact that once a person has had an elevated AST, they are more likely to have that blood test repeated. This could lead to counting elevated AST measure more than once, when in fact they are part of the same clinical incidence.

As there is evidence that persistently raised LFTs may be predictive of hepatotoxicity,[17] we used a secondary transaminitis definition of three consecutive LFTs above the ULN. Using this definition, the results were generally similar with reassurance that up to 14 units of alcohol per week did not increase the risk of hepatotoxicity and a suggestion that higher alcohol consumption did increase the risk. One limitation with this approach is that the outcome definition requires three sequential raised values. If a clinician sees a clinically meaningful rise in LFTs, they would be inclined to stop the MTX therapy following which the transaminases may return to normal and thus not fulfil the outcome definition of three sequential abnormal results. Nonetheless, it is reassuring that both analyses give similar confidence in the safety of modest alcohol consumption.

There are a number of other limitations within our study. The setting within primary care database means that we have to rely on existing general practitioner (GP) codes to identify cases of RA. We used previously validated algorithms[15]; however, it is possible that some misclassification remains. Given that the study design constrained the RA population to MTX users, misclassification is likely to be less than that for an unselected RA cohort. Alcohol use was self-reported, and thus is also prone to misclassification. Patients may be more inclined to underestimate their alcohol consumption, although this would not explain the apparent safety of modest alcohol consumption: were drinkers

reporting lower consumption, we would expect hepatotoxicity in these lower alcohol groups to be higher. Validity of self-reported alcohol consumptions in routinely collected clinical data such as CPRD is not well described. However, although response bias has been reported in survey data literature,[26] there are other data to suggest self-reported alcohol consumption largely valid and reliable, particularly in women.[27 28] It is possible that alcohol use changed through time following commencement of MTX. Unfortunately, there was not sufficient alcohol data recorded to allow us to consider changing use through time.

Patients were included only if they had six or more LFTs measured per year, as those with fewer blood tests would automatically have a lower chance of abnormal LFTs due to observation bias. That said, patients with high levels of alcohol consumption might be less likely to attend for regular blood test and could have been excluded from the study. However, the baseline characteristics of patients who remained in the study were similar to those who were excluded. Despite the large dataset, the number of events identified was relatively small, particularly in groups consuming high levels of alcohol. Knowing that we might therefore generate results that were not statistically significant, yet still potentially clinically meaningful, we chose to also present results as the probability of the HR exceeding a clinically significant increase of 50%. We demonstrated that this was much higher in patients consuming more alcohol. As with all observational data, there may have been unmeasured confounding that we were not able to adjust for, for example, disease severity. There may have been other comorbidities that could explain the raised LFTs that were not measured. We did not consider the dose of MTX as it was only available in patients included in the study before 2011, as this would have further limited the study power to detect differences between different levels of alcohol consumption. It is possible that hepatotoxicity may be higher in patients with higher MTX dosage, although dose is typically titrated upward while monitoring LFTs. A bias may be possible if clinicians give differing doses to patients who drink different levels of alcohol. At higher levels of alcohol consumption, lower dosage would be more likely, and thus the proven increased risk in the high alcohol groups may well be an underestimate of the true risk. Finally, it should be noted that while we have identified no increased risk of transaminitis when consuming <14 units of alcohol per week, this may not capture all hepatotoxicity. It has been suggested that monitoring LFTs is insufficient to assess long-term damage to the liver from MTX, as patients may progress to fibrosis without ever having episodes of transaminitis.[29] Nevertheless, serum LFT measurement remains current best practice for monitoring MTX therapy;[7] therefore, our findings are relevant.

In conclusion, in the largest study of its kind to date, we have shown no increase in the risk of transaminitis in patients who consume <14 units alcohol per week while taking MTX. This may provide the practical and useful information that drinking alcohol within nationally recommended levels in the UK is safe, in terms of risk of transaminitis, for patients commencing MTX therapy for RA. Our study was conducted only in patients with RA and thus cannot be automatically generalisable to other populations. Previous data have suggested that patients with psoriasis may have higher incidence of liver disease in general compared with patients with RA,[13] and therefore confirmatory studies would be required in these patient groups. Inclusion of acceptable alcohol levels into clinical guidelines and patient information leaflets may well improve informed decision-making, clinical outcomes, reduce decision conflict and improve overall quality of life.

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**Contributors** WGD conceived the idea; WGD, AW and JHH were responsible for the design of the study; JHH and RC conducted the analysis; JHH drafted the manuscript; all authors interpreted the results, critically revised the manuscript for important intellectual content and approved the final manuscript.

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**Competing interests** None declared.

**Ethics approval** The protocol for this study has been approved by Independent Scientific Advisory Committee for Medicines and Healthcare Regulatory Agency database research (Protocol number: 12_004Mn).

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**Data sharing statement** Clinical Practice Research Datalink (CPRD) data can be accessed with an appropriate licence from the CPRD and with approval from the Independent Scientific Advisory Committee. Licences are available from CPRD: The Clinical Practice Research Datalink Group, The Medicines and Healthcare Products Regulatory Agency, 5th Floor, 151 Buckingham Palace Road, Victoria, London SW1W 9SZ, England or http://www.cprd.com.



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EXTENDED REPORT

# Tumour necrosis factor inhibitor treatment and occurrence of anterior uveitis in ankylosing spondylitis: results from the Swedish biologics register

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## ABSTRACT

**Objectives** Tumour necrosis factor-α inhibitor (TNFi) treatment has been shown to reduce the rates of anterior uveitis (AU) in patients with ankylosing spondylitis (AS). Our objective was to compare the effect of adalimumab (ADA), etanercept (ETN) and infliximab (IFX) on AU occurrence in AS, using real-world data.

**Methods** Patients with AS starting ADA, ETN or IFX as their first TNFi from January 2003 to December 2010 were extracted from the Swedish Rheumatology Quality Register. AU rates, based on visits to an ophthalmologist with International Classification of Diseases 10 codes for AU, were obtained by linkage to the Swedish National Patient Register. For each TNFi, AU rates 2 years before TNFi start and for the first 2 years on TNFi treatment were compared. In the subgroup of patients who were AU-free during the 2 years before TNFi start, we also compared the risk of a first AU event.

**Results** 1365 patients with AS were included (406 ADA, 354 ETN, 605 IFX). Compared with pretreatment rates, we noted a reduction in overall AU rates for ADA and IFX, and an increase for ETN. The adjusted HRs for AU in 1127 patients who were free of AU in the last 2 years before TNFi start were significantly higher for ETN versus ADA (HR: 3.86 95% CI 1.85 to 8.06) and ETN versus IFX (HR: 1.99, 95% CI 1.23 to 3.22), while the HR for IFX versus ADA was not statistically significant.

**Conclusions** The results suggest differences in effect on AU risk between ADA, ETN and IFX, with a clear advantage for ADA/IFX over ETN.

## INTRODUCTION

Anterior uveitis (AU) is the most common extra-articular manifestation in ankylosing spondylitis (AS), with a recent meta-analysis describing a cumulative incidence of around one in four patients.[1] While AU sometimes precede the onset of axial symptoms,[2] a recent study also showed that the cumulative incidence of acute AU continues to increase for many years after the time-point of AS diagnosis.[3]

In AS, the visual prognosis after acute AU is excellent with adequate treatment, which usually includes topical (and at times oral and locally injected) corticosteroids.[4] Observational studies have suggested that sulfasalazine (SSZ) may prevent recurrence of AU in patients with AS[5 6] and some positive data also exist for methotrexate (MTX)[7] and non-steroidal anti-inflammatory drugs (NSAID).[8] Treatment with tumour necrosis factor-α inhibitors (TNFi) has also repeatedly been shown to reduce the occurrence of AU in patients with AS,[9–11] with reports including both reduced rates compared with placebo-treated patients,[10 12 13] and compared with rates pre-TNFi-treatment.[11 14] One randomised controlled trial has also demonstrated effectiveness of adalimumab (ADA) against a variety of different types of intermediate and posterior uveitis.[15]

Observational studies have suggested that the soluble TNF receptor fusion protein etanercept (ETN) may be less effective in preventing AU, compared with the monoclonal TNF antibodies infliximab (IFX) and ADA.[16–18] However, the previous studies have been heterogeneous in both design and in reporting of data, and two meta-analyses incorporating observational studies and randomised controlled trials (RCT) have reported conflicting results.[10 19] Additional data are thus needed to elucidate whether TNFi have differential effects with regard to preventing AU.

The aim of this study was therefore to compare AU rates in patients with AS during ADA, ETN or IFX treatment.

## METHODS

### Data sources and patients

Our study is based on data from the Swedish Rheumatology Quality Register (SRQ), the Swedish National Patient Register (NPR) and the Swedish Population Register.

The SRQ was established in 1995 and is integrated into clinical practice.[20] The patients are registered in the SRQ with their clinical diagnoses, as determined by the treating rheumatologist, and disease activity and treatment is registered at initiation of biological disease-modifying antirheumatic drugs (DMARDs) and at regular follow-up visits. The SRQ coverage for patients with spondyloarthritis treated with TNFi has recently been estimated to be 86%.[21]

The NPR is kept by the National Board of Health and Welfare, and was started in 1964 as a

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hospitalisation register. Complete national coverage for inpatient care was reached in 1987, and since 2001 the register also includes specialised outpatient care.[22] The Swedish Population Register contains demographic and socioeconomic data on all residents in Sweden.[23]

We included patients registered with a diagnosis of AS in SRQ and who started treatment with ADA, ETN or IFX as their first TNFi from January 2003 through December 2010. January 2003 was chosen as the starting point to allow for at least 2 years of pretreatment outpatient care data in the NPR.

From SRQ covariate data were retrieved on age, sex, start year for TNFi, disease duration at initiation of TNFi and baseline data (at the time-point of starting the first TNFi) on C reactive protein (CRP) and comedication with conventional synthetic DMARD (csDMARD), prednisolone and NSAID. Follow-up data through December 2011 were available at the time of analysis. Covariate data on a history of inflammatory bowel disease (IBD) and psoriasis were retrieved from the NPR and data on level of education was provided by the Population Register.

AU events were based on outpatient visits in ophthalmological specialist care with associated International Classification of Diseases (ICD-10) codes for AU (ICD-10: H20 and H22.1) extracted from the NPR. AU codes associated with outpatient visits to other specialists (eg, rheumatology, internal medicine) were disregarded. AU data were available from 1 January 2001 (start of the outpatient register). End of follow-up was set to 31 December 2011. All patients had at least 2 years (range: 2–10 years) of aggregated data in the registers before start of TNFi treatment.

### Baseline characteristics and descriptive data

Demographics and baseline characteristics were compared across TNFi type (ADA vs ETN vs IFX). The proportion of patients in each TNFi group contributing AU events for different periods of time before TNFi start, and during TNFi treatment, were also described, primarily in order to assess possible channelling effects. Furthermore, the number of subjects in whom AU visits occurred before TNFi start was cross-tabulated with the number of subjects with AU visits during TNFi treatment, in order to assess to what extent the AU events after treatment initiation were new-onset AU, or occurred in subjects with previous AU.

### Outcome, follow-up and main analyses

Three definitions of AU flare were analysed: (a) the total number of AU visits before and on TNFi treatment, (b) AU flare defined by a 60-day penalty from the index visit of one AU flare for a new flare to be counted (flare definition 1) and (c) AU flare defined by a >90-day gap between visits (flare definition 2). See online supplementary figure S1 for an illustration of the flare definitions.

Two main analytical approaches were applied to compare the three TNFi. First, we compared AU rates before TNFi start and during TNFi treatment, for each TNFi. In this analysis, the AU rates per 100 patient-years during the 2 last years prior to TNFi start were compared with the AU rates per 100 patient-years during the first 2 years on TNFi treatment. Only cases with at least 2 years of possible on-treatment observation time were included, thus only including patients starting with TNFi up until 31 December 2009. The on-treatment rates were based on observation time at risk, hence censoring patients either at the time-point of 2 years on-treatment, discontinuation of treatment, death or migration, whichever occurred first.

Second, we compared the hazard of the first on-treatment AU after start of treatment, within the subgroup of patients who had no diagnosis of AU during the 2 years before TNFi start. The rationale for the 2-year AU-free interval was: (1) to minimise channelling bias due to a lower pretreatment rate of AU in any of the three TNFi groups, (2) this was the minimal available pretreatment observation period, if calendar years for the three TNFi compared were to overlap (ADA was approved in 2003, 2 years after our period of data collection started).

### Sensitivity analyses and stratification

Two sets of sensitivity analyses were performed regarding the AU rates. First, we calculated AU rates (as described in the previous section) using all available person time before and after treatment start (and thus not restricting it to a 2-year time frame before and after treatment start). In this analysis, cases were also censored at discontinuation of treatment, death or migration or 31 December 2011. Second, we calculated incidence rates for AU as described in the main analysis, but stratified by the presence or absence of AU during the 2 years before TNFi start. A third sensitivity analysis was performed regarding the hazard of first on-treatment AU, only including patients without csDMARD therapy at baseline.

### Statistics

Baseline data are presented as frequencies with percentages, means with SDs and/or medians with 25 and 75 percentiles, depending on the type of data and the distribution. Demographics and baseline characteristics were compared across TNFi type by $\chi^2$ test, analysis of variance and Kruskal-Wallis test as appropriate; 95% CIs for AU rates were determined through Poisson regression.

HRs with 95% CI, for a first AU flare, were determined through Cox proportional hazard regression analyses, both unadjusted and adjusted for age, sex, start year for TNFi, disease duration at initiation of TNFi, history of IBD and baseline CRP, csDMARD comedication, prednisolone and NSAID. Due to a high proportion of missing data for CRP (22.7%), this variable was categorised as <10 mg/L, ≥10 mg/L and 'missing'.

We used IBM SPSS Statistics V.21 and SAS V.9.3 for the analyses.

## RESULTS

### Patients

The study included 1365 patients with AS starting treatment with ADA (N=406), ETN (N=354) or IFX (N=605) as their first TNFi. At baseline for TNFi start, the proportion using csDMARD was significantly higher in the IFX group (55.4% vs 28.1% and 30.5% for ADA and ETN, respectively) (table 1). The most frequently used csDMARD was MTX (ADA 13.5%, ETN 19.2% and IFX 44.0%), but SSZ was also slightly more frequently used in ADA (11.1%) compared with ETN (7.3%) and IFX (6.1%), the difference only being of statistical significance for ADA compared with IFX (p=0.04). Baseline erythrocyte sedimentation rate and CRP were lower in patients treated with ADA, and ADA was more commonly used in the second half of the period, that is, 2007–2010 (ADA was approved in Sweden 2003). The level of education was also lower for the IFX-treated patients (table 1).

### Proportions of patients with AU before and during TNFi treatment

The proportions of patients with a registered AU visit before TNFi start were remarkably stable, regardless of having >2, >4

**Table 1** Demographics and baseline characteristics across TNFi

| Total N=1365 | Adalimumab N=406 | Etanercept N=354 | Infliximab N=605 | p Value |
|---|---|---|---|---|
| Age years, mean (SD) | 43.5 (12.0) | 44.4 (12.2) | 43.6 (12.5) | 0.569 |
| Male sex, n (%) | 290 (71.4) | 264 (74.6) | 441 (72.9) | 0.622 |
| Disease duration years, median (Q1, Q3) | 12.9 (4.8, 23.6) | 15.5 (6.5, 24.7) | 14.3 (7.4, 25.1) | 0.093 |
| Missing (%) | 6 (1.5) | 14 (4.0) | 18 (3.0) | |
| Previous csDMARD, n (%) | 99 (24.4) | 118 (33.3) | 189 (31.2) | 0.015 |
| History of IBD, n (%) | 33 (8.1) | 24 (6.8) | 63 (10.4) | 0.136 |
| History of psoriasis, n (%) | 13 (3.2) | 22 (6.2) | 32 (5.3) | 0.134 |
| Education | | | | 0.047 |
| 9 years or less, n (%) | 59 (14.5) | 58 (16.4) | 112 (18.5) | |
| 10–12 years, n (%) | 202 (49.8) | 169 (47.7) | 315 (52.1) | |
| >12 years, n (%) | 140 (34.5) | 122 (34.5) | 162 (26.8) | |
| Missing, n (%) | 5 (1.2) | 5 (1.4) | 16 (2.6) | |
| TNFi start year | | | | <0.001 |
| 2003–2006, n (% of total N) | 65 (14.2) | 149 (32.5) | 245 (53.4) | |
| 2007–2010, n (% of total N) | 341 (37.6) | 205 (22.6) | 360 (39.7) | |
| csDMARD comedication, n (%) | 114 (28.1) | 108 (30.5) | 335 (55.4) | <0.001 |
| Prednisolone, n (%) | 48 (11.8) | 53 (15.0) | 84 (13.9) | 0.427 |
| NSAID, n (%) | 230 (56.7) | 197 (55.6) | 308 (50.9) | 0.146 |
| ESR, mm/hour, median (Q1, Q3) | 16 (6.75, 32.25) | 21 (11, 38) | 23 (10, 41) | <0.001 |
| Missing | 72 (17.7) | 47 (13.3) | 142 (23.5) | |
| CRP, mg/L, median (Q1, Q3) | 10 (4, 23) | 15 (6, 30) | 17 (7.75, 36) | <0.001 |
| Missing, n (%) | 84 (20.7) | 59 (16.7) | 167 (27.6) | |
| 28-SJC ≥1, n (%) | 70 (24.6) | 79 (32.1) | 111 (29.7) | 0.146 |
| Missing | 122 (30.0) | 108 (30.5) | 231 (38.2) | |
| Patient global VAS, mm mean (SD) | 58.0 (24.3) | 56.4 (24.3) | 57.9 (23.6) | 0.665 |
| Missing | 115 (28.3) | 99 (28.0) | 226 (37.4) | |

28-SJC, swollen joint count based on 28 joints; CRP, C reactive protein; csDMARD, conventional synthetic disease-modifying antirheumatic drug; ESR, erythrocyte sedimentation rate; IBD, inflammatory bowel disease; NSAID, non-steroidal anti-inflammatory drug; Q1 and Q3, 25 and 75 percentiles; TNFi, tumour necrosis factor-α inhibitor; VAS, visual analogue scale.

**Table 2** Number and proportion of patients contributing with AU visits, before start of TNFi, during the first 2 years of follow-up and during the total follow-up

| | Patients with ≥1 AU visit prior to first TNFi, stratified on the length of the available pretreatment observation time* | | | Patients with ≥1 AU visit during the last 2 years prior to first TNFi 0–2 years | Patients with ≥1 AU visit during TNFi treatment | |
|---|---|---|---|---|---|---|
| | >2 years | >4 years | >6 years | | Total | First 2 years† |
| Adalimumab uveitis/total (%) | 114/406 (28.1) | 107/389 (27.5) | 94/341 (27.6) | 78/406 (19.2) | 31/406 (7.6) | 22/303 (7.3) |
| Etanercept uveitis/total (%) | 84/354 (23.7) | 67/293 (22.9) | 50/205 (24.4) | 58/354 (16.4) | 81/354 (22.9) | 58/320 (18.1) |
| Infliximab uveitis/total (%) | 151/605 (25.0) | 129/481 (26.8) | 95/359 (26.5) | 102/605 (16.9) | 79/605 (13.1) | 56/499 (11.2) |

Values are counts (%).
*The observation time before the start of treatment equals the time from start of the outpatient register 1 January 2001 to the initiation of the first TNFi.
†Only including patients with at least 2 years of observation time after treatment start.
AU visit, visit to an ophthalmologist with an International Classification of Diseases code for AU; AU, anterior uveitis; TNFi, tumour necrosis factor-α inhibitor.

or >6 years of before-treatment observation time (22.9%–28.1%), but as expected lower when only including the last 2 years prior to TNFi start (16.4%–19.2%) (table 2). However, the relative proportion of patients with a history of AU before TNFi start, in the three TNFi groups, remained the same irrespective of the chosen time window, justifying the use of a 2-year pretreatment observation time period in the main analyses. Furthermore, the proportion contributing an AU visit prior to treatment was consistently the highest in the ADA group, followed by IFX and then ETN, suggesting channelling of treatment initiation towards ADA and IFX in patients with a history of AU.

On-treatment, the proportion with AU was the lowest among patients treated with ADA and the highest among patients treated with ETN (table 2), and the pattern was similar for the first 2 years on treatment and the overall observed data.

Table 3 shows the relationship between AU before TNFi start versus on-treatment. Among patients treated with ADA, ETN and IFX, who had an AU visit after treatment start, 80.6%, 54.3% and 69.6%, respectively, also had an AU visit registered prior to TNFi start. Conversely, from table 3 it is also evident that the proportion with possible 'de novo' AU (ie, no AU events registered pretreatment in the available data from 1 January 2001) varied considerably between groups: 13.7% (37 of 270) for ETN, 5.3% (24 of 454) for IFX and 2.1% (6 of 286) for ADA.

### AU rates during the 2 years prior to TNFi start and the first 2 years on treatment

The number of events per 100 patient-years during the last 2 years prior to TNFi start was similar for ADA, IFX and ETN,

with overlapping 95% CI (figure 1). However, the AU rates during the first 2 years on-treatment differed considerably, with the lowest rates for ADA and the highest for ETN. The two alternative flare definitions (definitions 1 and 2) as expected resulted in lower rates, compared with the total AU visit rates, but with an otherwise similar trend of increased rates of AU after initiating ETN, and decreased rates for ADA and IFX (figure 1), compared with the pretreatment rates.

### Hazard of AU during TNFi treatment

In the adjusted Cox regression analysis, among patients who were uveitis-free during the 2 years before TNFi start, ETN was associated with higher hazard than ADA (HR 3.86, 95% CI 1.85 to 8.06) and IFX (HR 1.99, 95% CI 1.23 to 3.22), while there was no statistically significant difference between ADA and IFX (table 4). The HRs were similar in the unadjusted analysis (table 4).

### Sensitivity analyses and stratification

In the sensitivity analysis analysing the AU rates based on all available observed person time at risk, before and on TNFi treatment (table 5a), the rates were generally lower during the pretreatment period, compared with the main analyses restricted to ±2 years, (range 23.1–31.7 vs 36.8–45.5), but similar or slightly higher on-treatment (range 15.7–55.2 vs 13.6–60.3).

In the analysis stratifying the main analysis on cases *with* AU and cases *without* AU in the 2 years prior to TNF, the on-treatment AU rates were, as expected, higher in the former group (table 5b) compared with the latter (table 5c).

Among those treated with ETN, *without* an AU event in the 2 years prior to treatment start (table 5c), the on-treatment rates were similar to the pretreatment rates overall for the ETN group (table 5a), possibly suggesting a lack of protective effect of ETN on AU flares. Furthermore, the CIs for the AU rates, in the group *without* a prior AU event in the 2 years prior to treatment start (table 5c), were non-overlapping between ADA versus ETN and IFX versus ETN, but not for ADA versus IFX, also supporting a significant difference between ETN and the monoclonal TNFi.

In the sensitivity analysis regarding hazard of first on-treatment AU, including only those without csDMARD at baseline, the HRs were slightly lower, but comparable to the HR in the whole population: HR (95% CI) ETN versus ADA 2.82 (1.28 to 6.26); IFX versus ADA 1.66 (0.70–3.93) and ETN versus IFX 1.70 (0.90–3.19).

## DISCUSSION

We found a fourfold increase in the risk for AU, during the first 2 years after treatment start, for patients with AS starting treatment with ETN compared with ADA, and a twofold increase for ETN compared with IFX, but no statistical difference

**Table 3** Relationship between AU occurrence before TNFi start and on drug

| Adalimumab (N=406) | | AU on drug: Yes | AU on drug: No | Etanercept (N=354) | | AU on drug: Yes | AU on drug: No | Infliximab (N=605) | | AU on drug: Yes | AU on drug: No |
|---|---|---|---|---|---|---|---|---|---|---|---|
| AU before* TNFi | Yes | 25 | 89 | AU before* TNFi | Yes | 44 | 40 | AU before* TNFi | Yes | 55 | 96 |
| | No | *6* | 286 | | No | *37* | 233 | | No | *24* | 430 |

The number of patients contributing with an AU visit before start of TNFi, and on drug, regardless of the available observation time prior to treatment and on-treatment. Numbers in italics are possible cases of 'de novo' AU.
*Only registered since the start of the outpatient register in 2001.
AU, anterior uveitis; TNFi, tumour necrosis factor-α inhibitor.

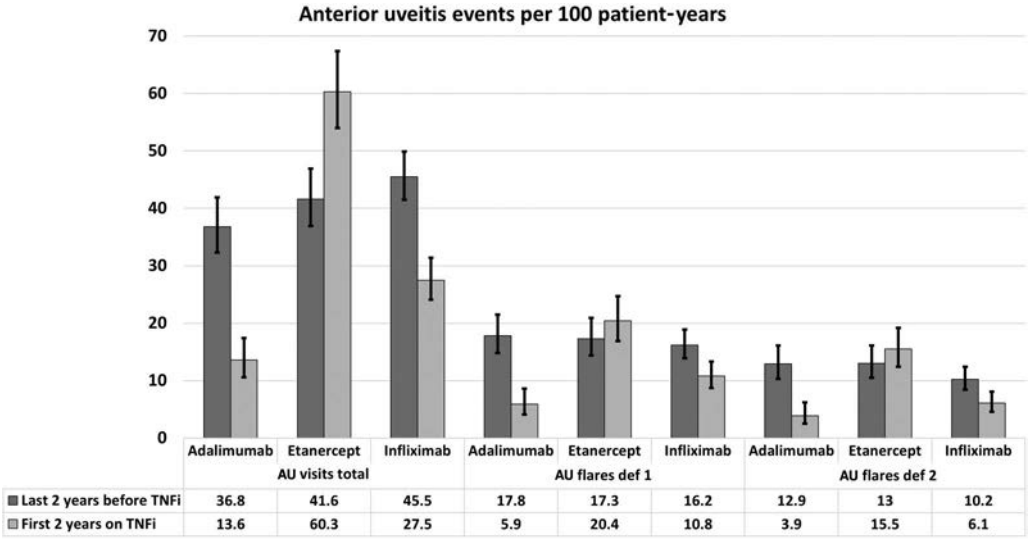


**Figure 1** Anterior uveitis (AU) event rates during the 2 years prior to treatment with tumour necrosis factor-α inhibitors (TNFi) and during the first 2 years on treatment, according to number of visits with a diagnosis of AU and two definitions of AU flare, for adalimumab, etanercept and infliximab. The on-treatment rates are based on observation time 'at risk', censoring patients at either 2 years on treatment, death, discontinuation of treatment or migration, whichever occurred first.

between ADA and IFX. Compared with the rates pretreatment, the AU rates increased when initiating treatment with ETN, but decreased when starting ADA or IFX.

That monoclonal TNF antibodies may protect against AU flares more effectively than the soluble TNF receptor ETN, has been reported previously in one meta-analysis,[10] two retrospective observational studies of different designs[16 18] and one observational study based on a US claims database.[17] In addition, data derived from adverse drugs events reporting (not including cases with AS) have supported this finding.[24] In contrast, a recent meta-analysis, based on eight RCT of TNFi in AS, reported a contradictory finding, where ETN appeared to be more effective against uveitis than the monoclonal therapies.[19] However, in this latter meta-analysis, none of the RCTs included was designed to specifically investigate the effect of TNFi on AU, and the numbers of AU events were small, which may explain the discrepant results. The findings of our study are in line with the study based on US claims data,[17] although we found an even larger difference between ETN and ADA than the US claims study (HR 3.86 vs 1.91).[17] The biological explanation for the differences in AU rates between monoclonal TNF antibodies and ETN is unclear, and suggested mechanisms include both a differential protective effect and the possibility of a paradoxical AU induction by ETN,[17] but it should also be noted that previous studies have indicated that ETN still reduces the number of AU flares more effectively than placebo.[12]

In this study, the decrease in AU rates was in favour of ADA compared with IFX, but the CIs for the HR did not indicate a statistically significant difference. Similar trends have been observed previously,[17] but it should be stressed that the IFX doses in the current study tended to be lower than the labelled dose in AS, which is 5 mg/kg. The comparative effect of ADA versus IFX should therefore be interpreted with caution. At baseline 50% used ≤200 mg IFX, and at the last registration 62% used ≤200 mg. Furthermore, changes in the doses of IFX, ADA and ETN, or changes in csDMARD during follow-up were not adjusted for in this study. In a previous publication of the same patient group, we have shown that changes in csDMARD therapy occurred in only 16% during follow-up.[25] Nevertheless, since concomitant csDMARD therapy is often used as comedication in conjunction with TNFi treatment of AS in Sweden, it cannot be ruled out that the observed differences in baseline csDMARD therapy between the TNFi groups could affect the results. However, the sensitivity analysis excluding those on csDMARD at baseline resulted in similar HR as the main results, which further support the validity of the results.

A number of limitations of this study must be recognised. First, the AU flares are identified based on registered diagnoses

**Table 4** Cox regression analyses of time to first AU flare after start of TNFi during the first 2 years of therapy among those without any AU visit (to ophthalmologist) within 2 years prior to start

| | ADA N=328 HR (95% CI) | ETN N=296 HR (95% CI) | IFX N=503 HR (95% CI) |
|---|---|---|---|
| Cox regression unadjusted | Ref. | 4.12 (2.00 to 8.46) | 2.00 (0.96 to 4.17) |
| | 0.50 (0.24 to 1.04) | 2.06 (1.32 to 3.22) | Ref. |
| Cox regression adjusted* | Ref. | 3.86 (1.85 to 8.06) | 1.94 (0.91 to 4.16) |
| | 0.52 (0.24 to 1.11) | 1.99 (1.23 to 3.22) | Ref. |

*Adjusted for age, sex, TNFi start year, disease duration at initiation of TNFi, history of IBD, BL, CRP and use of DMARD comedication and NSAIDs at baseline.
ADA, adalimumab; AU, anterior uveitis; CRP, C reactive protein; DMARD, disease-modifying antirheumatic drugs; ETN, etanercept; IBD, inflammatory bowel disease; IFX, infliximab; TNFi, tumour necrosis factor-α inhibitors.

**Table 5** Sensitivity analyses showing rates for visits and flares of AU: (a) including all observed person time at risk prior and after start of first TNFi and thus without restriction to a 2-year period, (b) among those having a recorded visit with AU within 2 years prior to start of follow-up and (c) among those without having a recorded visit with AU within 2 years prior to start of follow-up

| Sensitivity analysis (a): all cases, without a restriction regarding observed time prior to TNFi or follow-up time | | | | | | |
|---|---|---|---|---|---|---|
| | Adalimumab N=406 Events per 100 pt-yrs (95% CI) | | Etanercept N=354 Events per 100 pt-yrs (95% CI) | | Infliximab N=605 Events per 100 pt-yrs (95% CI) | |
| (a) All cases | Before TNFi | On drug | Before TNFi | On drug | Before TNFi | On drug |
| AU visits total | 29.9 (28.1 to 31.9) | 15.7 (13.3 to 18.6) | 23.1 (21.2 to 25.2) | 55.2 (51.0 to 59.8) | 31.7 (30.0 to 33.5) | 25.9 (23.7 to 28.4) |
| AU flares definition 1 | 12.9 (11.7 to 14.3) | 7.7 (6.1 to 9.8) | 9.7 (8.4 to 11.0) | 20.2 (17.7 to 23.0) | 12.7 (11.6 to 13.8) | 11.7 (10.2 to 13.4) |
| AU flares definition 2 | 9.5 (8.4 to 10.6) | 6.0 (4.6 to 7.9) | 7.7 (6.6 to 8.9) | 15.0 (12.9 to 17.5) | 9.1 (8.2 to 10.1) | 8.0 (6.8 to 9.4) |
| Stratification (b and c): requiring and only including 2 years of observation time before and after treatment start with TNFi | | | | | | |
| (b) Cases with AU event 2 years prior to TNFi | N=51 | | N=56 | | N=82 | |
| | Last 2 years before TNFi | First 2 years on TNFi | Last 2 years before TNFi | First 2 years on drug | Last 2 years before TNFi | First 2 years on TNFi |
| AU visits total | 218.6 (191.7 to 249.3) | 63.8 (48.4 to 84.2) | 237.5 (210.6 to 267.8) | 238.5 (209.3 to 271.7) | 276.8 (252.5 to 303.5) | 127.2 (109.6 to 147.7) |
| AU flares definition 1 | 105.9 (87.7 to 127.9) | 30.6 (20.5 to 45.7) | 99.1 (82.3 to 119.4) | 77.0 (61.2 to 96.9) | 98.8 (84.7 to 115.2) | 46.3 (36.2 to 59.3) |
| AU flares definition 2 | 78.0 (62.5 to 97.4) | 19.6 (11.8 to 32.5) | 76.8 (62.0 to 95.3) | 52.9 (39.8 to 70.2) | 63.0 (51.9 to 76.4) | 19.4 (13.2 to 28.5) |
| (c) Cases without AU event 2 years prior to TNFi | N=252 | | N=264 | | N=417 | |
| | Last 2 years before TNFi | First 2 years on TNFi | Last 2 years before TNFi | First 2 years on TNFi | Last 2 years before TNFi | First 2 years on TNFi |
| AU visits total | – | 3.2 (1.8 to 5.6) | – | 21.0 (17.1 to 25.8) | – | 6.7 (5.0 to 9.0) |
| AU flares definition 1 | – | 0.8 (0.3 to 2.5) | – | 7.9 (5.7 to 11.1) | – | 3.4 (2.2 to 5.1) |
| AU flares definition 2 | – | 0.8 (0.3 to 2.4) | – | 7.6 (5.4 to 10.7) | – | 3.4 (2.2 to 5.1) |

(b) and (c): the on-treatment rates are based on observation time 'at risk', censoring patients at either 2 years on treatment, death, discontinuation of treatment or migration, whichever occurred first. Flare definition 1: AU flare defined by a 60-day penalty from the index visit of one AU flare for a new flare to be counted. Flare definition 2: AU flare defined by a >90-day gap between visits.
AU, anterior uveitis; pt-yrs, patient-years, TNFi, tumour necrosis factor-α inhibitor.

at a visit to an ophthalmologist, with no information on whether each visit was for a new AU flare, a follow-up visit linked to a previous flare, or to AU of more chronic nature. According to uveitis nomenclature, AU flares are categorised as limited if the duration is <3 months and persistent if >3 months.[26] Two alternative flare definitions were included, but these definitions are imperfect, since for example a persistent AU in one eye cannot be distinguished from a new flare in the contralateral eye, or unilateral versus bilateral AU. However, this limitation, as well as other misclassification and missing data in the registers, ought to be non-differential for the three TNFi, and therefore rather reduce the chances to detect differences between the TNFi. Second, there is an obvious risk for channelling bias, if ETN is less likely to be initiated in a patient with a history of AU (as supported by table 2), but it is also unlikely that this bias would alter the direction of the results. However, it cannot be determined from the register data to what extent the indication for TNFi treatment was in fact AU, or other manifestations of AS disease activity. Third, only including patients without AU 2 years prior to TNFi in the Cox regression, will result in a group that is less prone to develop AU flares. The effect of this restriction should also be non-differential between TNFi and thus again reduce the possibility of detecting differences between the different TNFi. Fourth, in determining the adjusted HR, we adjusted for a number of potential confounders, but there may still, as in all observational studies, be unobserved or residual confounders, such as human leukocyte antigen (HLA)-B27 status, which may affect the results.

The major strength of this study is that it is a large, nationwide study, based on an unselected population of TNFi-treated patients with AS. An additional strength is that the data are collected from several independent sources, providing pretreatment data on AU flares, and the possibility to adjust for a number of potential confounders.

In conclusion, a reduction in AU rates was observed when initiating ADA and IFX, and an increase when initiating ETN. Based on HR, there was a fourfold increase in risk for AU when starting ETN compared with ADA, and a twofold increase for ETN compared with IFX. These results, in addition to previously published data on this topic, support the choice of another TNFi than ETN in patients with AS with a history of AU.

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EXTENDED REPORT

# A randomised controlled trial of the efficacy and safety of allopurinol dose escalation to achieve target serum urate in people with gout

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## ABSTRACT

**Objectives** To determine the efficacy and safety of allopurinol dose escalation using a treat-to-target serum urate (SU) approach.

**Methods** A randomised, controlled, parallel-group, comparative clinical trial was undertaken. People with gout receiving at least creatinine clearance (CrCL)-based allopurinol dose for ≥1 month and SU ≥6 mg/dL were recruited. Participants were randomised to continue current dose (control) or allopurinol dose escalation for 12 months. In the dose escalation group, allopurinol was increased monthly until SU was <6 mg/dL. The primary endpoints were reduction in SU and adverse events (AEs).

**Results** 183 participants (93 control, 90 dose escalation) were recruited. At baseline, mean (SD) urate was 7.15 (1.6) mg/dL and allopurinol dose 269 mg/day. 52% had CrCL<60 mL/min. Mean changes in SU at the final visit were −0.34 mg/dL in the control group and −1.5 mg/dL in the dose escalation group (p<0.001) with a mean difference of 1.2 mg/dL (95% CI 0.67 to 1.5, p<0.001). At month 12, 32% of controls and 69% in the dose escalation had SU <6 mg/dL. There were 43 serious AEs in 25 controls and 35 events in 22 dose escalation participants. Only one was considered probably related to allopurinol. Five control and five dose escalation participants died; none was considered allopurinol related. Mild elevations in LFTs were common in both groups, a few moderate increases in gamma glutamyl transferase (GGT) were noted. There was no difference in renal function changes between randomised groups.

**Conclusions** Higher than CrCL-based doses of allopurinol can effectively lower SU to treatment target in most people with gout. Allopurinol dose escalation is well tolerated.

**Trial registration number:**
ANZCTR12611000845932; Results.

## INTRODUCTION

Serum urate (SU) lowering is critical in the management of gout with international guidelines recommending SU <6 mg/dL (or <5 mg/dL in the presence of tophi).[1 2] Over time, achieving target SU leads to dissolution of monosodium urate crystals, suppression of gout flares and regression of tophi.

Allopurinol is the most commonly used urate-lowering therapy (ULT). Although allopurinol is US Food and Drug Administration-approved to 800 mg daily, doses > 300 mg daily are used infrequently.[3] Reluctance to increase allopurinol dose is due to physician inertia and concerns about adverse events (AEs), including the rare allopurinol hypersensitivity syndrome (AHS). AHS typically occurs in the first eight weeks after commencing allopurinol and risk factors include higher starting dose and chronic kidney disease (CKD).[4] The relationship between AHS and CKD led to recommendations that the maximum dose of allopurinol should be adjusted according to creatinine clearance (CrCL).[5] These recommendations have been followed widely, but frequently result in failure to achieve target SU.[6]

Uncertainty about the role of CrCL-based allopurinol dosing is reflected in recommendations from the major rheumatology societies. The European League Against Rheumatism 2016 recommendations advocate restricting allopurinol to CrCL-based doses[2] while the American College of Rheumatology recommendations advocate gradual escalation of allopurinol above CrCL-based doses to achieve target SU.[1] The aim of this study was to determine the efficacy and safety of allopurinol dose escalation (DE) in a real-life clinical practice setting.

## METHODS

### Study design

This paper reports a 12-month, open, randomised, controlled, parallel-group, comparative clinical trial (ANZCTR12611000845932). The study was conducted at two sites in New Zealand with participants enrolled between March 2012 and March 2014. An independent data safety monitoring committee provided oversight.

### Participants

People with gout defined by the American Rheumatism Association 1977 preliminary classification criteria for gout[7] receiving at least CrCL-based dose of allopurinol for ≥1 month and with SU ≥6 mg/dL at screening were recruited. People with a history of intolerance to allopurinol and those receiving azathioprine were excluded. CKD was not an exclusion criterion. Participants were recruited from primary and secondary care.

### Randomisation and masking

The randomisation sequence was generated electronically by an independent statistician. The

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randomisation sequence was stratified by study site and arranged in permuted blocks of size 10. Participants were randomised on a 1:1 ratio to continue the current dose of allopurinol (control) or DE. Randomisation codes were provided to study coordinators in sealed opaque envelopes, which were opened after the participant had consented.

### Study treatment and procedures

In the DE group, allopurinol was increased monthly until SU was <6 mg/dL on three consecutive visits or there were AEs. For example, if SU was <6 mg/dL allopurinol was not escalated but if at the following month urate was >6 mg/dL allopurinol was increased unless there was evidence of poor adherence. The dose was increased by 50 mg/d for those with CrCL <60 mL/min and 100 mg/d in those with CrCL ≥60 mL/min. In the control group, participants continued on the same allopurinol dose throughout the study period. Anti-inflammatory prophylaxis and treatment of gout flares were at the discretion of the investigator.

Participants were seen 3-monthly by study coordinators with intervening monthly telephone assessment. At each assessment, concomitant medications, self-reported gout flares and AEs were recorded. Blood was obtained monthly for SU and creatinine and 3-monthly for full blood count and liver function tests. The health assessment questionnaire (HAQ), pain visual analogue scale (pain VAS) and swollen joint count (SJC) and tender joint count (TJC) were completed 3 monthly. Target tophi were identified and the longest axis was measured using Vernier callipers 3 monthly.[8]

### Adverse and serious advent event reporting

AEs and serious adverse events (SAEs) were coded according to Common Terminology Criteria for Adverse Events (CTCAE V.4.0). Participants were asked about occurrence of any AEs as well as specific allopurinol-related AEs (abdominal pain, nausea, vomiting, rash and AHS). Laboratory-based allopurinol-related AEs included abnormal liver function, deterioration in creatinine or CrCL and eosinophilia. Treatment-emergent AEs were defined as any AE occurring after entry into the study until the end of month 12. Worsening laboratory AEs were defined as those where there was an increase in CTCAE grade between baseline and month 12. SAEs were defined as an event that was life-threatening, required hospital admission or resulted in death. AEs and SAEs were classified as not related, possibly, probably or definitely related to allopurinol. Management of AEs was at the discretion of the treating physician.

### Study outcomes

The primary efficacy outcome was absolute reduction in SU at the final visit (12 months or the final visit for those deceased or lost to follow-up). Secondary efficacy outcomes included (i) the proportion of participants reaching and maintaining target SU levels, defined as the last 3-monthly visits with SU<6 mg/dL, (ii) the percentage reduction in SU at final visit, (iii) the proportion of individuals with any gout flare in the first and last months of randomised treatment and in 3 monthly intervals, (iv) functional status (HAQ), pain VAS, SJC, and TJC changes from baseline to month 12 visit, and (v) index tophus size change from baseline

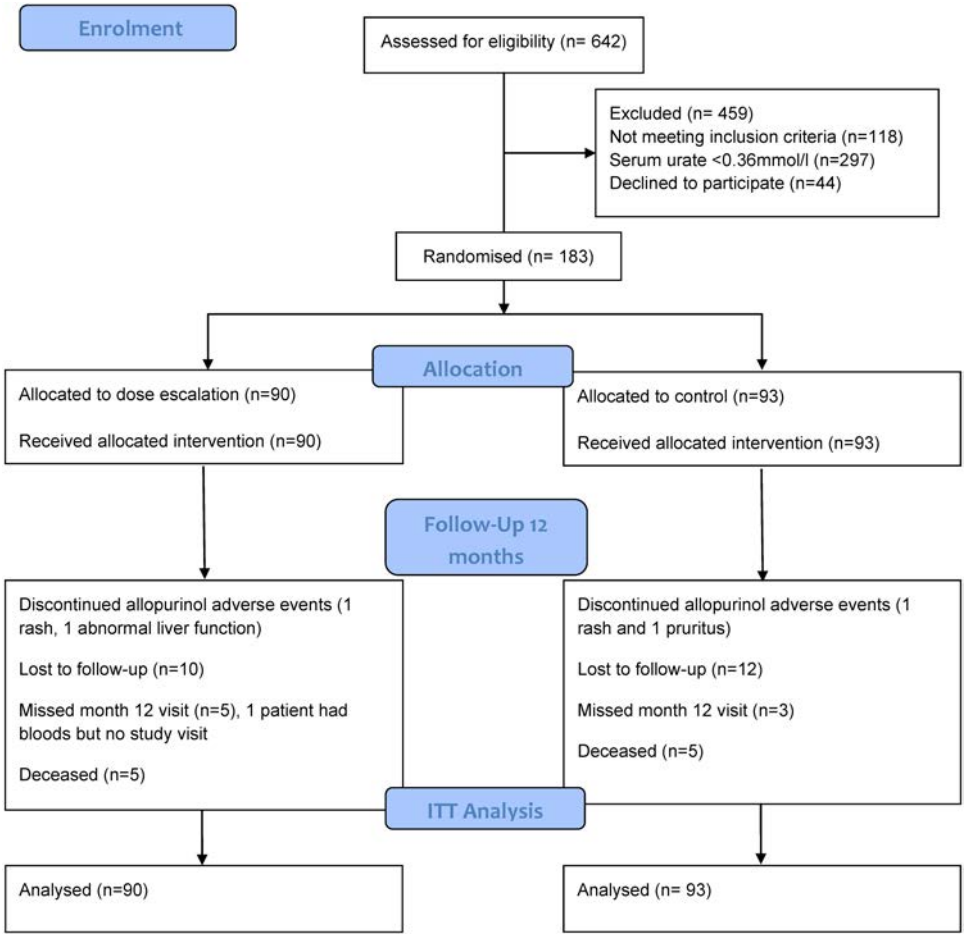


**Figure 1** Consolidated Standards of Reporting Trials (CONSORT) flow of participants. ITT, intention-to-treat.

to 12 months. The SU decremental time-adjusted area under the curve ($AUC_{adj-t}$) was calculated as a measure of the average improvement in SU for each participant over the study period. The primary safety outcome was treatment emergent or worsening AEs, serious and non-serious.

### Sample size and power

A planned sample size of 200 participants (~100/group) was calculated to enable a difference in the decline in SU over 12 months greater than 0.67 mg/dL to be detected as statistically significant (2 tailed $\alpha=0.05$) with 80% power based on data from the previous pilot study.[9] This allowed for up to 10% attrition over the period of the study. Further, we estimated that a difference of 35% or more in the percentage achieving the target SU between the DE and control groups would be detected as statistically significant (2 tailed $\alpha=0.05$) with power >90%.

### Statistical analysis

Baseline demographics and clinical features were summarised using standard descriptive statistics including means, SD, median, range, number and percent as appropriate. All randomised participants were included within the intention-to-treat analysis population and analysed within their randomised group. The primary efficacy outcome, absolute reduction in SU, was compared between randomised groups using a general linear model which included randomised group and study site as fixed factors and baseline SU as a covariate. The proportions of participants achieving and maintaining target SU levels, the proportion experiencing a gout flare and the proportion with a tophus were compared between randomised groups using logistic regression models with site as the stratification variable. The change in HAQ, pain VAS, SJC, TJC, and index tophus size from baseline to month 12 and SU decremental $AUC_{adj-t}$ were compared between randomised groups using a general linear model which included site and randomised group as fixed factors and baseline SU as a covariate. The statistical analysis plan is available as online supplementary material.

## RESULTS

### Participant characteristics

Of the 642 participants screened, 183 were randomly assigned to control (n=93) or DE (n=90) (figure 1). All randomised participants received at least one dose of allopurinol. Two participants in the control group and two in the DE group discontinued allopurinol (figure 1). In the control group, protocol violations were recorded for seven participants who had the dose of allopurinol increased (by healthcare practitioners outside the study setting). Five participants in the DE group were not dose escalated as SU post-screening was <6 mg/dL. For the remainder of the participants allopurinol was increased as per the protocol.

The baseline demographic and clinical features were well matched between randomised groups (table 1). Mean (SD) SU was 7.2 mg/dL (1.6), in 51.9% of participants CrCL was <60 mL/min and in 13.1% CrCL was <30 mL/min. A number of participants had baseline laboratory abnormalities; the majority of these were mild with exception of creatinine (see online supplementary table S1).

### Efficacy

#### Primary endpoint

The mean change in SU at the final visit was −0.34 mg/dL in the control group and −1.5 mg/dL in the DE group (p<0.001) with a mean difference of 1.2 mg/dL (95% CI 0.67 to 1.5), p<0.001). In the control group mean (SD) SU was 7.13 (1.6) mg/dL at baseline and 6.9 (1.5) mg/dL at final visit, compared with 7.18 (1.6) mg/dL and 5.7 (1.2) mg/dL in the DE group (figure 2A).

#### Secondary endpoints

SU was <6 mg/dL at the final visit in 32% of the control group and 69% in the DE group (p<0.001); OR 4.3 (95% CI 2.4 to 7.9). The mean allopurinol dose of those at target was 390 (50–

**Table 1** Participant baseline demographics and clinical features

| Variable | Control (n=93) | Dose escalation (n=90) | All participants (n=183) |
|---|---|---|---|
| Age years* | 60.9 (12.8) | 59.5 (12.1) | 60.2 (12.5) |
| Male, n (%) | 78 (84%) | 82 (91%) | 160 (87.4%) |
| Ethnicity, n (%) | | | |
| NZ European | 39 (42%) | 37 (41%) | 76 (41.5%) |
| Maori | 22 (24%) | 29 (32%) | 51 (27.9%) |
| Pacific Island | 27 (29%) | 19 (21%) | 46 (25.1%) |
| Asian | 4 (4%) | 5 (6%) | 9 (4.9%) |
| Other | 1 (1%) | 0 (0%) | 1 (1.1%) |
| Duration of gout (years) | 17.9 (13.2) | 16.5 (11.3) | 17.2 (12.3) |
| Baseline serum urate mg/dL* | 7.13 (1.6) | 7.18 (1.6) | 7.15 (1.6) |
| Creatinine (mg/dL)* | 1.47 (1.02) | 1.58 (0.11) | 1.58 (1.02) |
| CrCL (mL/min) | 60.3 (27.7) | 60.1 (27.3) | 60.2 (27.4) |
| Body mass index (kg/m$^2$)* | 35.2 (7.4) | 35.2 (7.9) | 35.2 (7.7) |
| Flare frequency in the preceding year (median, IQR) | 4 (1.3–11.8) | 3 (1.0–5.3) | 3 (1–8) |
| Baseline allopurinol dose mg/day† | 275.8 (100–600) | 261.9 (100–600) | 269.0 (100–600) |
| Allopurinol dose mg/day n (%) | | | |
| 100–200 | 31 (33.3%) | 37 (41.1%) | 68 (37.2%) |
| >200–300 | 50 (53.4%) | 47 (52.2%) | 97 (53%) |
| >300 | 12 (12.9%) | 7 (7.7%) | 19 (10.4%) |
| Presence of palpable tophi n (%) | 46 (49%) | 35 (39%) | 81 (44.2%) |
| Coexisting conditions n (%) | | | |
| Obesity‡ | 70 (75%) | 64 (71%) | 134 (73.2%) |
| CrCL <60 mL/min | 45 (48%) | 50 (56%) | 95 (51.9%) |
| CrCL <30 mL/min | 14 (15%) | 10 (11%) | 24 (13.1%) |
| Kidney stones | 3 (10%) | 5 (14%) | 8 (12.3%) |
| Cardiovascular disease§ | 38 (41%) | 41 (46%) | 79 (43.2%) |
| Diabetes | 33 (36%) | 29 (32%) | 62 (33.9%) |
| Hypertension | 65 (70%) | 67 (74%) | 132 (72.1%) |
| Hyperlipidaemia | 58 (62%) | 47 (52%) | 105 (57.4%) |
| Concurrent medications n (%) | | | |
| Diuretic | 43 (46%) | 38 (42%) | 81 (44.3%) |
| Aspirin | 41 (44%) | 40 (44%) | 81 (44.3%) |
| Any anti-inflammatory prophylaxis | 45 (48%) | 51 (57%) | 96 (52.5%) |
| Colchicine | 35 (38%) | 34 (38%) | 69 (37.7%) |
| Non-steroidal anti-inflammatory drugs | 9 (10%) | 15 (17%) | 24 (13.1%) |
| Prednisone | 12 (13%) | 12 (13%) | 24 (13.1%) |

*Mean (SD).
†Mean (range).
‡Obesity defined as body mass index ≥30 kg/m$^2$.
§Cardiovascular disease defined as ischaemic heart disease, heart failure or peripheral vascular disease.
CrCL, creatinine clearance.

900) mg daily compared with 290 (0–700) mg daily in those not at target (p<0.001).Time course of achieving target SU is shown in figure 2B. SU <6 mg/dL at each of the last 3 monthly visits was achieved by 14% of the control group and 59% of the DE group (p<0.001); OR 8.0 (95% CI 3.6 to 17.7). The mean percentage change in SU from baseline to final visit was −3.3% in the control group compared with −17.8% in the DE group (p<0.001) with a mean difference of 14.5% (95% CI 8.4 to 20.6%) (figure 2C). There was a significantly higher $AUC_{adj\text{-}t}$ in the DE group compared with the control group (0.99 vs 0.29 mg/dL; p<0.001) with a mean difference of 0.69 mg/dL (95% CI 0.42 to 0.96). The mean final dose of allopurinol was 288 mg/day (0–600 mg/day) in the control group and 413 mg/day (0–900 mg/day) in the DE group (figure 2D).

### Gout flares and other activity measures

During the study period, 59% of the control group and 54% of the DE group experienced at ≥one self-reported gout flare (p=0.58) (see online supplementary figure S1A). By the end of the study period there had been a reduction in use of prophylaxis in both groups (see online supplementary figure S1B). There was no significant difference in the mean change in index tophus size over the study period between randomised groups (see online supplementary figure S1C). Of those with measurable tophi, complete resolution of tophi occurred in 8/43 (19%) of the control group and 6/32 (19%) of the DE group. There was no significant difference in the mean change from baseline to 12 months between randomised groups for HAQ, pain VAS, SJC or TJC (see online supplementary table S2).

## Safety

### Serious adverse events

There were 43 SAEs in 25 control participants and 35 in 22 DE participants (tables 2 and 3). Five participants in each group died. None of the deaths was attributed to allopurinol. In the control group, deaths were attributed to sepsis (n=2), heart failure (n=1), respiratory failure (n=1) and long-standing CKD refusing dialysis (n=1). In the DE group, deaths were attributed to heart failure (n=2), myocardial infarction (n=2) and aortic dissection (n=1). One SAE was considered probably related to allopurinol, increase in international normalised ratio (INR) in a DE participant who commenced warfarin after elective mitral valve replacement.

### Non-laboratory AEs

There were 336 non-laboratory AEs in 80 control participants and 339 in 73 DE participants (table 2 and online supplementary table S3). The number of participants experiencing at least one non-laboratory AE in each CTCAE category is shown in table 2. In the control group, 11 participants developed rash; one was considered probably related to allopurinol and allopurinol was discontinued. Five participants in the control group developed pruritus, one was considered probably related to allopurinol. In the DE group, eight participants developed rash, two were considered possibly related but resolved despite continuing allopurinol and one was probably related and allopurinol was discontinued. Ten participants in the DE group developed pruritus, of which one was considered possibly related to allopurinol.

Of the other non-laboratory AEs, one was definitely related; a DE participant who accidentally took 2–3 times the prescribed dose of allopurinol for 2 days after confusing medication bottles. There were no clinical sequelae. Two DE participants had malaise possibly related to allopurinol; one of these participants also had a headache possibly related to allopurinol. One DE participant had vertigo, nausea and abdominal pain probably related to allopurinol. No other non-laboratory AEs were thought to be related to allopurinol.

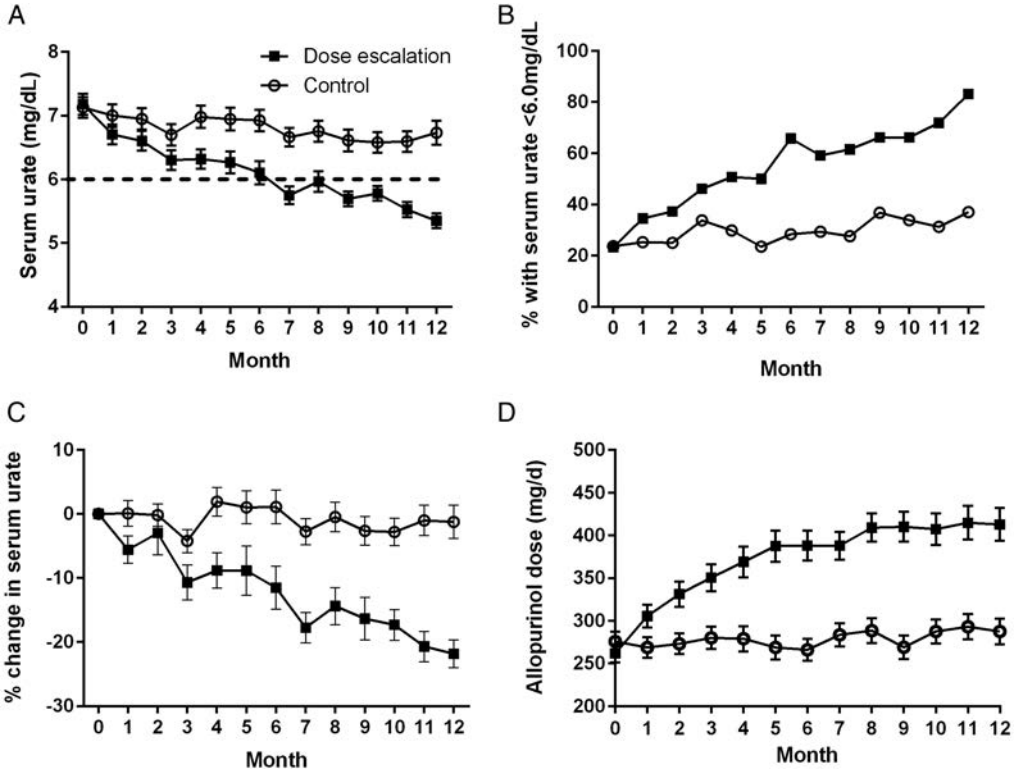


**Figure 2** Mean serum urate (A), time course for achieving target serum urate (B), mean percentage change in serum urate (C) over the 12-month study period and (D) mean allopurinol dose in control and dose escalation groups.

### Laboratory AEs

For the 3-monthly visits, there were 56 liver function treatment-emergent or worsening AEs in 28 control participants and 77 in 32 DE participants. For aspartate aminotransferase (AST), alanine transferase (ALT) and alkaline phosphatase (ALP), the majority were CTCAE grade 1 (figure 3A–D). For gamma glutamyl transferase (GGT), there were 19 abnormalities in 12 control participants and 36 in 19 DE participants, of which 3 participants in the DE group increased by two CTCAE grades.

For creatinine, an increase from baseline value was used to determine CTCAE grade. From the monthly visits, there were 465 events in 82 control participants and 452 events in 81 DE participants; >96% were grade 1 (>1–1.5× above baseline) (figure 3E). There were 28 control participants and 34 DE participants who experienced more than a 20% decrease in CrCL at any stage over the study (figure 3F). A similar proportion of participants had an increase in CrCL (figure 3F).

Haematological treatment-emergent or worsening AEs included eosinophilia, anaemia, thrombocytopenia, neutropenia and lymphopenia. From the 3-monthly visits, there were 45 events in 25 control participants and 65 events in 30 DE participants (see online supplementary table S4 and figure S2). Eosinophilia occurred in 15 control participants and 14 DE participants at some stage during the 12-month period (figure 3G).

### Improvement in laboratory variables

A number of laboratory variables improved during the study. A 20% improvement in CrCL at some point during the 12 months was observed in 24 control participants and 18 DE participants (figure 3F). Of those with abnormal GGT at baseline, 16/33 participants in the control group and 13/39 participants in the DE group improved by at least one CTCAE grade during the study.

## DISCUSSION

We have shown that DE of allopurinol is effective in people with gout, including in those with CKD, with 69% achieving target SU at final visit and 59% achieving and maintaining SU <6 mg/dL at the last 3-monthly visits. A number of DE participants failed to achieve target SU; this may reflect poor adherence or true resistance to allopurinol. A small number of control participants achieved target urate, most likely reflecting improved compliance or simply variation in SU around the target.

**Table 2** Number (%) of participants with at least one serious adverse event and the number (%) of individuals in each category and non-laboratory adverse events summary; number of participants (%) with at least one event during the study period

| | Serious adverse event | | Non-laboratory treatment emergent adverse event | |
|---|---|---|---|---|
| | Control (n=93) | Dose escalation (n=90) | Control (n=93) | Dose escalation (n=90) |
| Number of participants with at least one adverse event | 25 (27%) | 22 (24%) | 80 (86%) | 73 (81%) |
| Cardiac disorders | 8 (9%) | 11 (12%) | 9 (10%) | 5 (6%) |
| Gastrointestinal disorders | 6 (7%) | 3 (3%) | 21 (23%) | 18 (20%) |
| General disorders | 1 (1%) | 1 (1%) | 47 (51%) | 48 (53%) |
| Hepatobiliary disorders | 0 | 1 (1%) | 0 | 2 (2%) |
| Infections and infestations | 8 (9%) | 3 (3%) | 18 (19%) | 14 (16%) |
| Injury, poisoning and procedural complications | 2 (2%) | 1 (1%) | 15 (16%) | 24 (27%) |
| Investigations | 0 | 1 (1%) | | |
| Metabolism and nutrition | 0 | 2 (2%) | 4 (4%) | 1 (1%) |
| Musculoskeletal | 1 (1%) | 1 (1%) | 27 (29%) | 24 (27%) |
| Nervous system disorders | 3 (3%) | 1 (1%) | 10 (11%) | 11 (12%) |
| Renal and urinary disorders | 5 (5%) | 2 (2%) | 0 | 2 (2%) |
| Respiratory, thoracic and mediastinal disorders | 2 (2%) | 2 (2%) | 16 (17%) | 15 (17%) |
| Skin and subcutaneous tissue disorders | 1 (1%) | 1 (1%) | 20 (22%) | 23 (26%) |
| Blood and lymphatic system | | | 0 | 1 (1%) |
| Ear and labyrinth | | | 3 (3%) | 3 (3%) |
| Endocrine | | | 0 | 1 (1%) |
| Eye | | | 4 (4%) | 3 (3%) |
| Immune system | | | 1 (1%) | 1 (1%) |
| Neoplasms benign, malignant and unspecified | | | 4 (4%) | 4 (4%) |
| Psychiatric disorders | | | 5 (5%) | 4 (4%) |
| Reproductive and breast disorders | | | 2 (2%) | 0 |
| Surgical and medical procedures | | | 2 (2%) | 2 (2%) |
| Vascular disorders | | | 8 (9%) | 10 (11%) |
| Venous disorders | | | 1 (1%) | 0 |
| Allopurinol-specific adverse events | | | | |
| Allopurinol hypersensitivity syndrome | | | 0 | 0 |
| Rash | | | 11 (12%) | 8 (9%) |
| Pruritus | | | 5 (5%) | 10 (11%) |
| Nausea/vomiting | | | 9 (10%) | 6 (7%) |
| Abdominal pain | | | 5 (5%) | 6 (7%) |

**Table 3** Number of serious adverse events in each Common Terminology Criteria for Adverse Events category

| | Control (n=93) | Dose escalation (n=90) |
|---|---|---|
| Cardiac disorders | 14 | 14 |
| Gastrointestinal disorders | 6 | 3 |
| General disorders | 1 | 1 |
| Hepatobiliary disorders | 0 | 1 |
| Infections and infestations | 8 | 4 |
| Injury, poisoning and procedural complications | 2 | 1 |
| Investigations | 0 | 1 |
| Metabolism and nutrition | 0 | 2 |
| Musculoskeletal | 1 | 1 |
| Nervous system disorders | 3 | 1 |
| Renal and urinary disorders | 5 | 2 |
| Respiratory, thoracic and mediastinal disorders | 2 | 2 |
| Skin and subcutaneous tissue disorders | 1 | 2 |

As with all ULT clinical trials,[10–13] the primary efficacy endpoint in this study was SU lowering. Although the majority of the DE group achieved target SU, there was no significant reduction in gout flares during the study period. Importantly, there was no difference between the DE and control groups with regard to flares. Previous studies have shown an increase in flare rate after staring ULT[10 11] and that flares can persist for several years after SU target is achieved.[14] Likewise, there was no difference in tophus regression, HAQ and joint counts between groups. These results are similar to other clinical trials of oral ULT,[10 13 14] which show it takes longer than 12 months for changes in these outcomes to occur. As about half the participants had CrCL <60 mL/min, the escalation of allopurinol was slow (50 mg per month) so mean SU <6 mg/dL was not reached until month 7 which may have affected outcomes. A longer observation period is likely to be necessary to see any difference in flare rates. An open-label extension phase of this study will further address these endpoints.

There were a number of SAEs in both groups, although only one was related to allopurinol. No new safety signal was identified. There were no cases of AHS; however, the study was not powered to detect AHS, which is rare (<0.1%) and usually occurs within 8 weeks after starting allopurinol.[4] Participants in this study had been on allopurinol for ≥1 month prior to enrolment. Given the rarity of AHS, it is unlikely that any allopurinol DE study will be undertaken that is sufficiently powered to detect this specific SAE. There were a number of participants in both groups who developed rashes and pruritus. However, only two participants discontinued allopurinol, highlighting how common and non-specific rash and itch are. It is important to note that management of these AEs was at the discretion of the treating physician; in most cases, allopurinol was reduced with subsequent rechallenge to be sure symptoms were not allopurinol related.

A number of treatment-emergent liver function abnormalities occurred. For AST, ALT and ALP, the majority of these were mild and similar between randomised groups. There were more elevations in GGT and a small number of higher-grade abnormalities in the DE group compared with controls. An increase in GGT was also noted in the LASSO study.[15] The clinical significance of these elevated GGTs remains unclear particularly in this group of patients with multiple comorbidities which might contribute to increases. GGT is an inducible enzyme, and allopurinol at higher doses may contribute to this induction.

There were a large number of creatinine AEs during the study using the definition of change from baseline. This definition results in some individuals with creatinine levels well within the laboratory normal reference range having an 'adverse event'. Approximately 10% of individuals had a decrease in CrCL

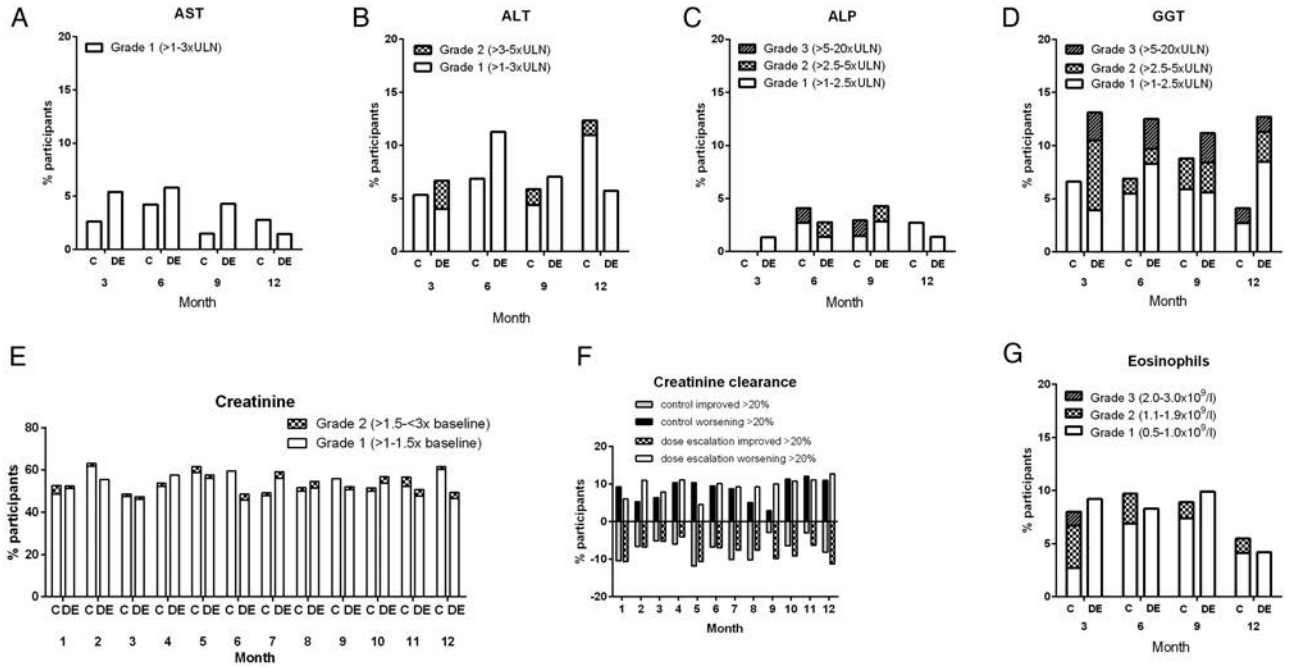


**Figure 3** Treatment-emergent or worsening laboratory adverse events: (A–D) liver function over the 12-month study period by Common Terminology Criteria for Adverse Events grade in control and dose escalation (DE) groups. (E) Percentage of participants with increase in creatinine over baseline and (F) percentage of participants with more than a 20% decrease (worsening) or increase (improvement) in creatinine clearance from baseline and (G) percentage of participants with eosinophilia. C, control.

≥20% with no obvious difference between groups. Importantly, similar numbers of individuals had an improvement in CrCL.

There are a number of limitations of this study. The study was not blinded and thus carries the inherent risks of bias in an open-label study. However, the primary endpoint was a laboratory value which is not open to bias. The main source of bias was around attribution of AEs to allopurinol. It is possible that AEs were more likely to be attributed to allopurinol in the DE group than the control group.

One of the key strengths of this study is the population which has a high prevalence of comorbid conditions, particularly CKD (52% having CrCL<60 mL/min and at least some of these having CKD stage 3 or higher) as well as severe gout (44% with tophi). Data from NHANES 2007–2008 showed that of the individuals with gout 74% had hypertension, 71% had ≥stage 2 CKD, 53% were obese, 26% had diabetes, 14% had a history of myocardial infarction and 10% had a history of stroke.[16] Thus our population is representative of people with gout, represents real-life clinical practice and the results are generalisable to other gout populations.

In conclusion, in people with gout, including those with kidney impairment, who tolerate CrCL-based doses of allopurinol but fail to reach target urate, gradual DE to achieve target urate is effective and well-tolerated.

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**Contributors** LKS and ND: literature search, study design, data collection, data analysis, data interpretation and manuscript preparation. PTC and JD: study design, data collection, data analysis, data interpretation and manuscript preparation. MLB and CF: study design, data analysis, data interpretation and manuscript preparation. AH and PT: data collection, data analysis, data interpretation and manuscript preparation.

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OPEN ACCESS

EXTENDED REPORT

# Anticollagen type II antibodies are associated with an acute onset rheumatoid arthritis phenotype and prognosticate lower degree of inflammation during 5 years follow-up

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## ABSTRACT

**Objective** Antifibrillar collagen type II (anti-CII) antibody-positive patients with rheumatoid arthritis (RA) have early but not late signs of increased inflammation and joint erosions. We wanted to replicate this in a large RA cohort, and to relate to human leukocyte antigen (HLA)-DRB1* alleles.

**Methods** Anti-CII and anti-cyclic citrullinated peptide (CCP)2 were measured at baseline in 773 patients with RA from the Swedish Epidemiological Investigation in Rheumatoid Arthritis (EIRA) study with clinical follow-up data from the Swedish Rheumatology Quality Register (SRQ) registry, and 1476 with HLA-DRB1* information. Comparisons were done concerning C reactive protein (CRP), erythrocyte sedimentation rate (ESR), tender joint count (TJC), swollen joint count (SJC), Disease Activity Score encompassing 28 joints based on ESR (DAS28), DAS28CRP, pain-Visual Analogue Scale (VAS), global-VAS and Health Assessment Questionnaire Score (HAQ) at eight occasions during 5 years, and association with HLA-DRB1* alleles.

**Results** Anti-CII associated with elevated CRP, ESR, SJC, DAS28 and DAS28CRP at diagnosis and up to 6 months, whereas anti-CCP2 associated with SJC and DAS28 from 6 months to 5 years, but not earlier. The anti-CII-associated phenotype was strong, and predominated in anti-CII/anti-CCP2 double-positive patients. Anti-CII was associated with improvements in CRP, ESR, SJC, TJC and DAS28, whereas anti-CCP2 was associated with deteriorations in SJC and DAS28 over time. Anti-CII-positive patients achieved European League Against Rheumatism good or moderate response more often than negative patients. Anti-CII was positively associated with HLA-DRB1*01 and HLA-DRB1*03, with significant interaction, and double-positive individuals had >14 times higher mean anti-CII levels than HLA double negatives. Whereas smoking was associated with elevated anti-CCP2 levels, smokers had lower anti-CII levels.

**Conclusions** Anti-CII seropositive RA represents a distinct phenotype, in many respects representing the converse to the clinical, genetic and smoking associations described for anticitrullinated protein peptide autoantibodies. Although not diagnostically useful, early anti-CII determinations predict favourable inflammatory outcome in RA.

## INTRODUCTION

Rheumatoid arthritis (RA) is a multifactorial disease. RA can be classified as seropositive by the presence of rheumatoid factor and/or anticitrullinated protein peptide autoantibodies (ACPA).[1] ACPA-positive RA represents a distinct phenotype associated with genetic and environmental factors, notably the HLA-DRB1* shared epitope (SE) and smoking.[2 3] The fibrillar collagen type II (CII) is essentially restricted to hyaline cartilage, where it is the major protein.[4] A subgroup of patients with RA (3%–27%) have elevated levels of antibodies against CII (anti-CII), especially around the time of RA diagnosis, whereafter levels decline.[5–7] We have described that anti-CII bound to CII in surface-bound immune complexes (IC) can induce pro-inflammatory cytokines and chemokines from mononuclear cells (MNC) and polymorphonuclear granulocytes (PMN).[8–10] Anti-CII are thus functionally active, and we have previously shown that changes in anti-CII levels temporally associate with in vitro function of anti-CII-containing IC and to C reactive protein (CRP) and erythrocyte sedimentation rate (ESR) in corresponding serum samples. Anti-CII thus represent a RA phenotype with early but not late signs of inflammation.[9 11] This is in contrast to ACPA, associated with late occurrence of signs and symptoms of inflammation in the same RA cohort.[12]

This previous comparison of the anti-CII-dependent and ACPA-dependent RA phenotypes was performed in a small group of patients (n=274). By linking the Swedish Epidemiological Investigation in Rheumatoid Arthritis (EIRA) study to the Swedish Rheumatology Quality Register (SRQ), we have obtained clinical follow-up data in a larger RA cohort. Here, we validate and extend the characterisation of the anti-CII-dependent acute onset RA phenotype, and show that it also represents the contrariety to the ACPA-associated phenotype concerning association with HLA-DRB1* and smoking.

## PATIENTS AND METHODS

### Study subjects

EIRA patients (n=2000) and controls (n=960) were included between 1996 and 2005. All patients fulfilled the 1987 American College of Rheumatology

classification criteria.[13] Controls were selected from the Swedish population register and matched for age, locality and sex. Detailed description of EIRA and the clinical follow-up data acquired through linkage to SRQ has been described previously.[14–16] All participants consented to join the study that was approved by the ethical committee of Karolinska Institutet.

SRQ data included CRP, ESR, swollen joint count (SJC), tender joint count (TJC), Disease Activity Score encompassing 28 joints based on ESR (DAS28) or CRP (DAS28CRP), Visual Analogue Scale data for pain (pain-VAS) and global disease activity (global-VAS) and Health Assessment Questionnaire Score (HAQ). Exclusion was made of patients lacking ACPA data (n=18), disease duration >365 days at diagnosis (n=170), patients lacking linked SRQ data (n=650), >10 days between clinical diagnosis and inclusion in EIRA (n=226) and non-specific anti-CII reactivity (n=163). Of the remaining 773 patients, SRQ data were available for 768 (99.4%) at baseline, 663 (85.8%) at 3 months, 627 (81.1%) at 6 months, 725 (93.8%) at 1 year, 669 (86.6%) at 2 years, 426 (55.1%) at 3 years, 265 (34.3%) at 4 years and 480 (62.1%) at 5 years.

HLA association studies were performed in 1476 patients, after exclusion of patients lacking information on anti-CCP2 (n=18) or HLA-DRB1* (n=23), disease duration >365 days (n=163) or non-specific reactivity (n=316).

### Detection of anti-CII antibodies

Anti-CII antibodies were measured as previously described by ELISA using human native collagen type II (Chondrex, Redmond, Washington, USA) as antigen. Levels >95th percentile of blood donors (29 AU/mL) were considered positive.[11] Serum samples yielding higher optical density (OD) in blocked wells without the CII antigen were regarded as non-specific, and were treated separately.

### Anti-CCP2 measurements, genotyping and smoking data

Anti-CCP2 was measured by ELISA (Immunoscan CCPlus, Euro-Diagnostica, Malmö, Sweden) with a cut-off of 25 U/mL. Genotyping was done by PCR using sequence-specific primers. HLA-DRB1* alleles 0101/0401/0404/0405/0408/10 were defined as SE as described previously.[17–19] Patients were classified as ever or never smokers.

### Statistical analysis

Associations between anti-CII and anti-CCP2 and clinical and laboratory measures were performed with the Mann-Whitney U test. Two-way analysis of variance (ANOVA) was used to study the association between anti-CII and anti-CCP2 status and clinical and laboratory measures, as well as between anti-CII levels and HLA-DRB1*01 and HLA-DRB1*03 alleles. ORs between HLA-DRB1 alleles and anti-CII and anti-CCP2 status and attainment of European League Against Rheumatism (EULAR) response (good and moderate compared with no response) were calculated with 95% CIs. The impact of age, sex and smoking status were investigated with logistic regression, but had only minor impact and were left out in the final calculations. In some comparisons with HLA status, a higher anti-CII cut-off corresponding to tumour necrosis factor induction by the corresponding IC in vitro were used.[9 20] HLA associations were investigated for all individuals and after excluding of

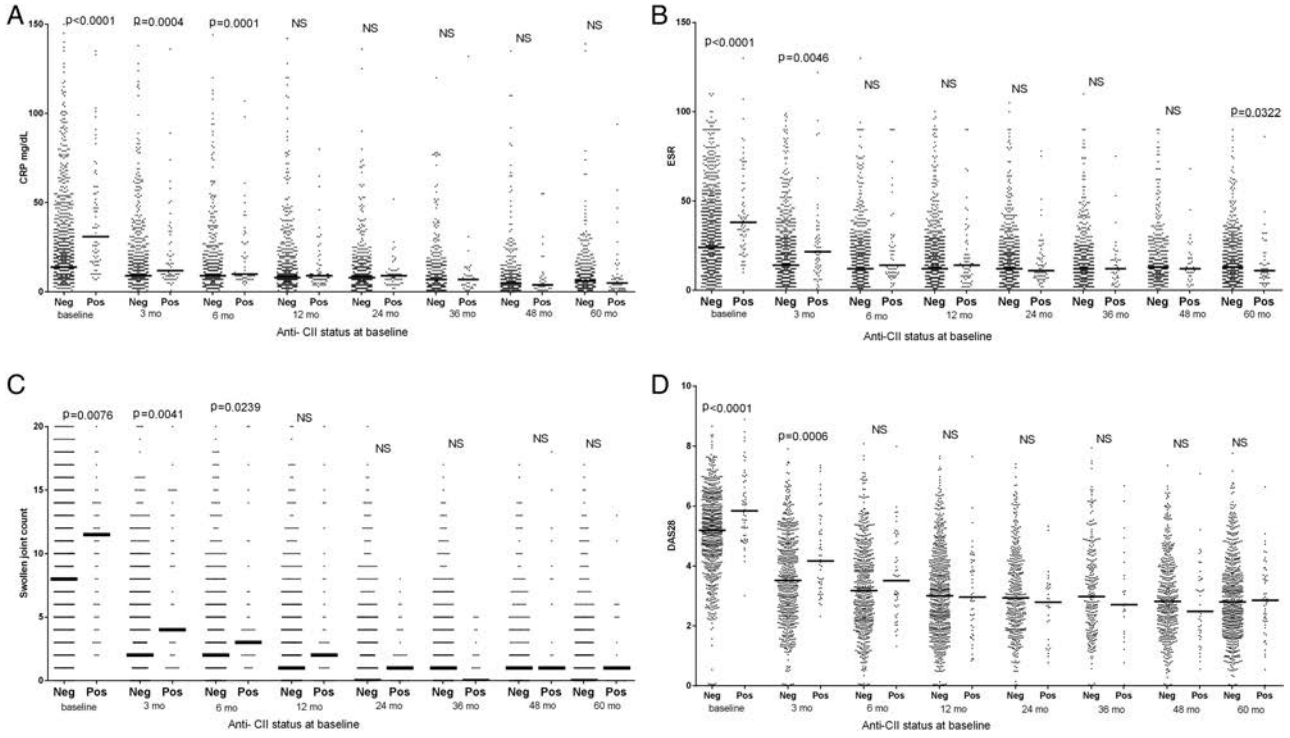


**Figure 1** Association between baseline antifibrillar collagen type II (anti-CII) levels and (A) C reactive protein (CRP), (B) erythrocyte sedimentation rate (ESR), (C) swollen joint count (SJC) and (D) Disease Activity Score encompassing 28 joints based on ESR (DAS28) during 5 years follow-up in 773 newly diagnosed patients with rheumatoid arthritis (RA). Figures show significance between anti-CII-positive and anti-CII-negative patients at the different time points; only significant (p<0.05) differences are shown. The underlined p value for ESR after 5 years indicate lower median levels in the initially anti-CII-positive group. Data on the same patients dichotomised according to anti-CCP status are shown in figure 2. Data on (A) 29 and (C) 57 individuals with very high values were not depicted in the graphs, but were included in the statistical calculations. mo, months; neg, negative; NS, not significant; pos, positive.

SE-positive patients, as ACPA are strongly linked to SE. All statistical analyses were done using JMP11.

## RESULTS

### Anti-CII and anti-CCP2 antibodies in EIRA

Among 1476 patients, 97 (6.6%) were anti-CII positive and 855 (57.9%) were anti-CCP2 positive. Thirty-nine patients (2.6%) had only anti-CII, 797 (54%) had only anti-CCP2, 58 (3.9%) were double positive and 582 (39.4%) lacked both antibodies. Among the EIRA controls, 15/926 (1.6%) were anti-CII positive (34 showed non-specific binding) and 16/958 (1.7%) were anti-CCP2 positive. Anti-CII levels were significantly higher among patients than among controls (median (mean) 13.3 (38.4) vs 9.3 (21.6) AU/mL, p<0.0001). There was no association between the occurrence of anti-CII and anti-CCP2 among patients (p=0.7), nor between anti-CII levels and age. The baseline disease-modifying antirheumatic drug (DMARD) usage did not differ between patients with and without anti-CII and anti-CCP2, respectively.

### Anti-CII and anti-CCP2 associations with clinical and laboratory measures

As illustrated in figures 1 and 2 and online supplementary figures S1 and S2, the occurrence of anti-CII was associated with higher CRP values at baseline and the first follow-up visits; and the same was evident for ESR, SJC, DAS28 and DAS28CRP. Anti-CCP2 on the other hand was associated with higher disease activity later during the 5-year follow-up, and with CRP and ESR during the full period.

In early time points when anti-CII-positive patients showed elevated activity measures, non-specific samples showed values between negative and positive samples, usually significantly lower than anti-CII-positive individuals (see online supplementary table S1). The temporal association between anti-CII levels and early inflammation was strong: we originally chose patients with up to 40 days between clinical phenotype (SRQ data) and antibody measurement (EIRA inclusion), but every significant association with early inflammation became stronger when we restricted the time difference to 10 days (data not shown).

The 773 patients were divided into patients expressing only anti-CII (n=20), only anti-CCP2 (n=432) or both (n=36) and each group was compared with double-negative patients (n=285). Patient expressing only anti-CII or only anti-CCP mirrored the phenotypes described above, anti-CII was associated with high measures for CRP, ESR, SJC, DAS28 and DAS28CRP and HAQ early, whereas anti-CCP2 was associated with high measures for SJC, TJC, DAS28, DAS28CRP late, and with CRP and ESR during the whole follow-up period. Patients in the anti-CII and anti-CCP2 double-positive group mainly followed the anti-CII pattern, with early but not late increased values for CRP, ESR, DAS28 and DAS28CRP, but with a mixed pattern for SJC (see table 1 and online supplementary table S2).

The ANOVA analyses confirmed that anti-CII was associated with CRP, ESR, SJC, DAS28, DAS28CRP and HAQ early, whereas anti-CCP2 was associated with late elevations CRP, SJC, DAS28 and DAS28CRP, and with ESR during all time points except 48 months. Except for concerning ESR, anti-CII and anti-CCP2 showed marginal interactions (see online supplementary table S3).

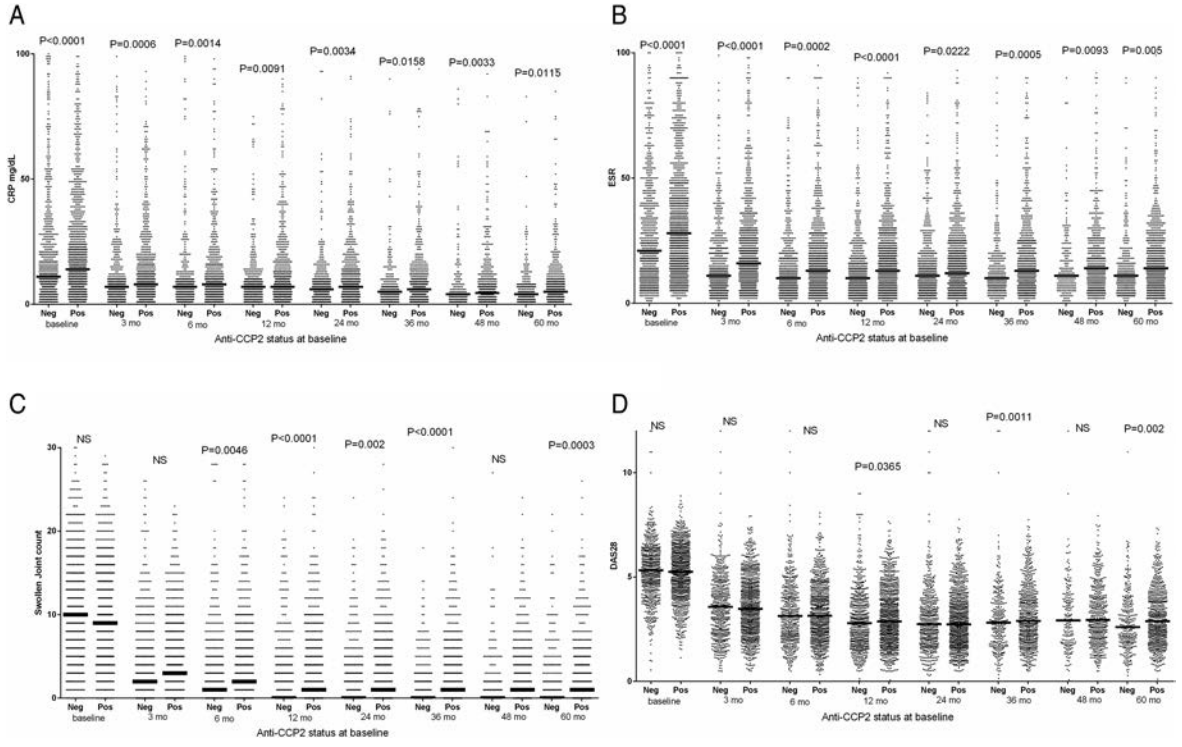


**Figure 2** Association between baseline anti-CCP levels and (A) C reactive protein (CRP), (B) erythrocyte sedimentation rate (ESR), (C) swollen joint count (SJC) and (D) Disease Activity Score encompassing 28 joints based on ESR (DAS28) during 5 years follow-up in 773 newly diagnosed patients with rheumatoid arthritis (RA). Figures show significance between anti-CCP-positive and anti-CCP-negative patients at the different time points; only significant (p<0.05) differences are shown. Data on the same patients dichotomised according to antifibrillar collagen type II status are shown in figure 1. Data on (A) 122, (B) 21, (C) 52 and (D) 58 individuals with very high values were not depicted in the graphs, but were included in the statistical calculations. mo, months; neg, negative; NS, not significant; pos, positive.

**Table 1** Associations between the occurrence of anti-CII and anti-CCP, individually or in combination and clinical symptoms during 5-year follow-up after RA diagnosis

| Variable | Median anti-CII−/ anti-CCP− | Median anti-CCP+ | Median anti-CII+ | Median anti-CII+/ anti-CCP+ | p Value (anti-CCP) | p Value (anti-CII) | p Value (anti-CII +/anti-CCP+) |
|---|---|---|---|---|---|---|---|
| CRP 0 mo | 11.5 | 16 | 31.5 | 31 | **0.0003** | **0.0002** | **<0.0001** |
| CRP 3 mo | 8 | 9 | 12 | 15 | **0.0023** | **0.0028** | **0.0356** |
| CRP 6 mo | 8 | 9 | 10 | 18 | **0.0098** | **0.0217** | **<0.0001** |
| CRP 12 mo | 8 | 8 | 7 | 10 | **0.0187** | 0.4074 | **0.0242** |
| CRP 24 mo | 8.54 | 8 | 7 | 11.31 | **0.0069** | 0.7158 | **0.0214** |
| CRP 36 mo | 7 | 7 | 4.5 | 9 | **0.0201** | 0.0689 | 0.1553 |
| CRP 48 mo | 4 | 7 | 4 | 5 | **0.003** | 0.993 | 0.2067 |
| CRP 60 mo | 5 | 6 | 4 | 8.5 | **0.0285** | 0.1562 | **0.0042** |
| ESR 0 mo | 20 | 27 | 38 | 41 | **<0.0001** | **0.0057** | **<0.0001** |
| ESR 3 mo | 10 | 16 | 13 | 24.5 | **<0.0001** | 0.627 | **<0.0001** |
| ESR 6 mo | 10 | 13 | 10 | 20 | **0.0011** | 0.8962 | **0.0002** |
| ESR 12 mo | 10 | 14 | 8 | 16 | **<0.0001** | 0.2334 | **0.0007** |
| ESR 24 mo | 11 | 12 | 8 | 15 | **0.0351** | 0.2046 | 0.0716 |
| ESR 36 mo | 10 | 14 | 9 | 17 | **0.0006** | 0.1641 | 0.1191 |
| ESR 48 mo | 11 | 16.5 | 10 | 14 | **0.008** | 0.6684 | 0.3414 |
| ESR 60 mo | 12 | 15 | 8 | 15 | **0.0039** | **0.0107** | 0.4832 |
| SJC 0 mo | 9 | 8 | 12.5 | 10 | 0.3044 | **0.0094** | 0.4849 |
| SJC 3 mo | 2 | 3 | 4 | 4 | 0.1803 | **0.0286** | **0.0137** |
| SJC 6 mo | 1 | 2 | 2 | 3.5 | **0.0133** | **0.0438** | **0.005** |
| SJC 12 mo | 0 | 1 | 2 | 2 | **<0.0001** | 0.2162 | **0.0079** |
| SJC 24 mo | 0 | 1 | 0 | 1 | **0.004** | 0.8665 | **0.0193** |
| SJC 36 mo | 0 | 1 | 0 | 0 | **<0.0001** | 0.7398 | 0.5592 |
| SJC 48 mo | 0 | 1 | 1 | 1 | 0.1558 | 0.4832 | 0.1326 |
| SJC 60 mo | 0 | 1 | 0 | 1 | **0.0005** | 0.6476 | **0.0255** |
| DAS28 0 mo | 5.19 | 5.21 | 5.87 | 5.78 | 0.6974 | **0.0124** | **0.0012** |
| DAS28 3 mo | 3.6 | 3.52 | 3.58 | 4.24 | 0.6942 | 0.2602 | **0.0107** |
| DAS28 6 mo | 3.17 | 3.27 | 2.85 | 3.77 | 0.4274 | 0.609 | **0.0135** |
| DAS28 12 mo | 2.92 | 3.06 | 2.19 | 3.08 | **0.0451** | 0.3007 | 0.2391 |
| DAS28 24 mo | 2.73 | 2.81 | 2.38 | 3.07 | 0.2976 | 0.4362 | 0.1166 |
| DAS28 36 mo | 2.56 | 3.07 | 2.27 | 2.89 | **0.0008** | 0.1776 | 0.5288 |
| DAS28 48 mo | 2.6 | 3.09 | 2.72 | 2.99 | 0.0691 | 0.913 | 0.4416 |
| DAS28 60 mo | 2.42 | 2.95 | 2.07 | 3.16 | **0.0018** | 0.1548 | 0.354 |

Median levels are shown for anti-CII/anti-CCP double-negative patients (n=285), anti-CII-positive and anti-CCP-negative patients (n=20), anti-CII-negative and anti-CCP-positive patients (n=432) and anti-CII-positive and anti-CCP2-positive patients (n=36). p Values refer to comparisons with the anti-CII-negative/anti-CCP2 double-negative group. Significant differences are depicted in bold, and also underlined if the median level for the corresponding antibody is lower than for the double-negative group.
Corresponding data for tender joint count, DAS28CRP, pain-VAS, global-VAS and Health Assessment Questionnaire are shown in online supplementary table S2.
CII, collagen type II; CRP, C reactive protein; DAS28, Disease Activity Score encompassing 28 joints based on ESR; ESR, erythrocyte sedimentation rate; mo, months; RA, rheumatoid arthritis; SJC, swollen joint count; VAS, Visual Analogue Scale.

### Anti-CII and anti-CCP associations with changes in clinical and laboratory measures

When the occurrence of anti-CII and anti-CCP2 were associated with changes as compared with corresponding baseline measures, anti-CII was associated with most, especially late changes in CRP, ESR, SJC, TJC, DAS28, DAS29CRP, but also with changes in pain-VAS, global-VAS and HAQ. Anti-CCP2 was associated with fewer changes: in ESR until 48 months, SJC until 12, 24, 36 and 60 months, TJC until 36 months, DAS28 until 36 months, DAS28CRP until 12, 24 and 36 months and HAQ until 36 months. In all cases, significant changes for anti-CII were associated with larger improvements than for antibody double-negative subjects, and all significant changes for anti-CCP2 except ESR at 48 months and HAQ at 36 months were associated with smaller improvements as compared with antibody double-negative subjects (see table 2 and online supplementary table S4).

Anti-CII was associated with EULAR response at 12, 24 36 and 60 months, whereas anti-CCP2 was associated negatively to EULAR response at 36 and 60 months. Anti-CII-positive patients achieved EULAR response to a larger extent than initially DMARD-treated patients (see figure 3 and online supplementary table S5).

Anti-CII was thus associated with a favourable prognosis and anti-CCP2 with an unfavourable prognosis, compared with patients without any of the antibodies.

### Association of anti-CII and anti-CCP with HLA-DRB1* alleles

Anti-CII was positively associated with HLA-DRB1*03 (OR 1.92, 95% CI 1.23 to 2.97), and negatively with HLA-DRB1*04 (OR 0.6, 95% CI 0.39 to 0.9). After exclusion of SE-positive individuals, the HLA-DRB1*03 association remained (OR 2.82, 95% CI 1.27 to 6.28). When a higher cut-off (200 AU/mL) was employed, the positive associations

**Table 2** Association between changes in inflammatory markers as compared with baseline values and the occurrence of anti-CII and anti-CCP2 at the time of RA diagnosis

| Symptom changes | Mean CCP−CII− | Mean CCP+CII− | Mean CCP−CII+ | Mean CCP+CII+ | ANOVA total p value | Anti-CCP p value | Anti-CII p value | Interaction |
|---|---|---|---|---|---|---|---|---|
| CRP Δ 3 mo | −9.47 | −12.72 | −24.35 | −22.36 | **0.045** | 0.8945 | **0.01** | 0.5803 |
| CRP Δ 6 mo | −10.39 | −15.3 | −29.17 | −25.45 | **0.016** | 0.9089 | **0.0053** | 0.4046 |
| CRP Δ 12 mo | −10.11 | −14.96 | −34.72 | −23.84 | **0.008** | 0.5792 | **0.0021** | 0.1479 |
| CRP Δ 24 mo | −14.32 | −18.18 | −32.5 | −30.91 | **0.0177** | 0.8305 | **0.0036** | 0.6061 |
| CRP Δ 36 mo | −12.45 | −16.07 | −43.7 | −32.42 | **0.0079** | 0.5847 | **0.0007** | 0.2879 |
| CRP Δ 48 mo | −14.78 | −23.26 | −28 | −42.72 | **0.072** | 0.2027 | 0.0731 | 0.7311 |
| CRP Δ 60 mo | −15.51 | −22.49 | −38.93 | −29.54 | 0.0668 | 0.8596 | 0.0257 | 0.2301 |
| ESR Δ 3 mo | −8.65 | −11.74 | −17.87 | −18.56 | **0.0183** | 0.5493 | **0.0113** | 0.7035 |
| ESR Δ 6 mo | −10.6 | −14.53 | −22.76 | −21.82 | **0.0055** | 0.6539 | **0.0037** | 0.465 |
| ESR Δ 12 mo | −11.11 | −14.21 | −26 | −23.48 | **0.0012** | 0.93 | **0.0003** | 0.3982 |
| ESR Δ 24 mo | −11.16 | −15.71 | −23.35 | −30.06 | **<0.0001** | 0.1098 | **0.0002** | 0.7588 |
| ESR Δ 36 mo | −9.37 | −12.47 | −30.55 | −29.94 | **0.0002** | 0.782 | **<0.0001** | 0.6819 |
| ESR Δ 48 mo | −10.35 | −15.8 | −16.71 | −36.28 | **0.0044** | **0.0455** | **0.0319** | 0.2578 |
| ESR Δ 60 mo | −11.92 | −14.52 | −22.29 | −32.42 | **0.0018** | 0.1444 | **0.0013** | 0.3875 |
| SJC Δ 3 mo | −5.89 | −5.17 | −6.71 | −4.52 | 0.2651 | 0.1054 | 0.9271 | 0.4165 |
| SJC Δ 6 mo | −7.11 | −6.04 | −8.61 | −6.3 | 0.0825 | 0.0689 | 0.3453 | 0.5041 |
| SJC Δ 12 mo | −8.05 | −6.43 | −11.05 | −7.32 | **0.0003** | **0.0031** | **0.0305** | 0.242 |
| SJC Δ 24 mo | −8.49 | −7.16 | −13.56 | −8.19 | **<0.0001** | **0.0003** | **0.001** | 0.0296 |
| SJC Δ 36 mo | −9.02 | −6.7 | −14.45 | −8.5 | **<0.0001** | **0.0007** | **0.0029** | 0.1329 |
| SJC Δ 48 mo | −8.28 | −7.49 | −10.63 | −8.58 | 0.5046 | 0.3442 | 0.2547 | 0.6784 |
| SJC Δ 60 mo | −8.86 | −7.17 | −13.79 | −8.15 | **0.0006** | **0.0015** | **0.0106** | 0.0875 |
| DAS28 Δ 3 mo | −1.5 | −1.48 | −1.89 | −1.46 | 0.7689 | 0.3588 | 0.4541 | 0.3938 |
| DAS28 Δ 6 mo | −1.82 | −1.77 | −2.77 | −2.16 | 0.0514 | 0.1903 | 0.008 | 0.2601 |
| DAS28 Δ 12 mo | −2.2 | −1.92 | −3.28 | −2.72 | **0.0002** | 0.0912 | **0.0002** | 0.5795 |
| DAS28 Δ 24 mo | −2.29 | −2.19 | −3.54 | −2.78 | **0.0012** | 0.0749 | **0.0002** | 0.1778 |
| DAS28 Δ 36 mo | −2.36 | −1.89 | −4.09 | −2.99 | **<0.0001** | **0.018** | **<0.001** | 0.3422 |
| DAS28 Δ 48 mo | −2.38 | −2.08 | −3.19 | −3.12 | 0.0639 | 0.6651 | 0.0336 | 0.7835 |
| DAS28 Δ 60 mo | −2.45 | −2.07 | −3.71 | −3 | **0.0004** | 0.0769 | **0.0005** | 0.5868 |

Analysis was performed with two-way ANOVA, and changes in clinical and laboratory measures were expressed as differences between values at different time points and corresponding baseline values. Mean levels are shown for anti-CII/anti-CCP double-negative subjects (n=285), anti-CII-positive and anti-CCP-negative subjects (n=20), anti-CII-negative and anti-CCP-positive patients (n=432) and anti-CII-positive anti-CCP2-positive patients (n=36). p Values for the total ANOVA, anti-CCP2, anti-CII and the interaction between anti-CII and anti-CCP2 are given in individual columns. Significant p values for the individual antibodies are depicted in bold, and also underlined if the mean level for the corresponding antibody is lower than for the double-negative group. Corresponding data for tender joint count, DAS28CRP, pain-VAS, global-VAS and Health Assessment Questionnaire are shown in online supplementary table S2.
ANOVA, analysis of variance; CII, collagen type II; CRP, C reactive protein; DAS28, Disease Activity Score encompassing 28 joints based on ESR; ESR, erythrocyte sedimentation rate; mo, months; RA, rheumatoid arthritis; SJC, swollen joint count; VAS, Visual Analogue Scale.

with HLA-DRB1*03 (OR 3.45, 95% CI 1.73 to 6.92) and the negative association with HLA-DRB*04 (OR 0.15, 95% CI 0.06 to 0.39) were further increased, together with the appearance of positive associations with HLA-DRB1*01 (OR 2.37, 95% CI 1.18 to 4.76) and HLA-DRB1*08 (OR 2.54, 95% CI 1.02 to 6.28). After exclusion of SE-positive individuals, only the positive association with HLA-DRB1*03 remained (OR 3.4, 95% CI 1.03 to 11.25). Anti-CCP2 showed numerous HLA-DRB1* associations, all disappearing after exclusion of SE-positive patients (see online supplementary table S6).

One thousand four hundred and seventy-six patients where then divided into patients expressing only anti-CII (n=39), only anti-CCP2 (n=797) or both (n=58) and each group was individually compared with double-negative patients (n=582). Whereas patients expressing only anti-CII or anti-CCP2 showed the same HLA-DRB1* associations as described above, patients with both antibodies showed a positive association with HLA-DRB1*04 (OR=2.66, 95% CI 1.53 to 4.61) using the conventional anti-CII cut-off (see online supplementary table S7). With increased anti-CII cut-off, double antibody positivity associated with HLA-DRB1*01 (OR 3.51, 95% CI 1.25 to 9.85), whereas association with HLA-DRB1*04 was lost (not shown).

After stepwise regression with backward elimination or forward selection including HLA-DRB1*01–16, only HLA-DRB1*01 and HLA-DRB1*03 remained associated with anti-CII. Two-way ANOVA showed that both HLA-DRB1*01 and HLA-DRB1*03 were associated with anti-CII levels ($p<0.0001$ for both), with a highly significant interaction ($p<0.0001$). Whereas mean anti-CII level in HLA-DRB1*01/*03 double-negative patients (n=818) was 21.1 AU/mL, it was 77.7 AU/mL for individuals only positive for HLA-DRB1*01 (n=338) and 38.6 AU/mL for individuals only positive for HLA-DRB1*03 (n=268). The statistic interaction was manifested as strikingly increased anti-CII levels in patients with both HLA-DRB1*01 and HLA-DRB1*03 (n=52); mean 330.1 AU/mL (figure 4).

Three hundred and sixteen patients with non-specific reactivity showed no association with any HLA-DRB1* allele except a weak association with HLA-DRB1*14 (see online supplementary table S8).

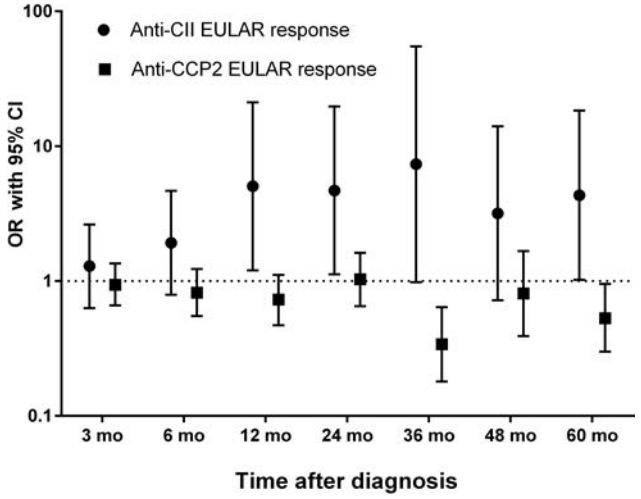


**Figure 3** Patients with rheumatoid arthritis (RA) attaining European League Against Rheumatism(EULAR) response at the different time points, in relation to anti-CCP2 and antifibrillar collagen type II (anti-CII) autoantibody status. EULAR responses were calculated according to van Gestel *et al*, but using the EULAR recommended Disease Activity Score encompassing 28 joints (DAS28) based on erythrocyte sedimentation rate (ESR) limits as described by Jerram *et al*.[21 22] Patients achieving moderate and good EULAR response were pooled, and data are expressed as ORs with 95% CIs for attaining EULAR response. The proportion of patients receiving disease-modifying antirheumatic drug treatment at baseline did not differ between patients with and without anti-CII and anti-CCP2, respectively. Out of 773 patients, those with required clinical follow-up data were included. Full data on DAS28 components both at baseline and at the respective time point were available for 587, 559, 634, 586, 380, 229 and 435 patients at 3, 6, 12, 24, 36, 48 and 60 months, respectively. The corresponding data are shown in detail in online supplementary table S3. EULAR, European League Against Rheumatism; mo, months.

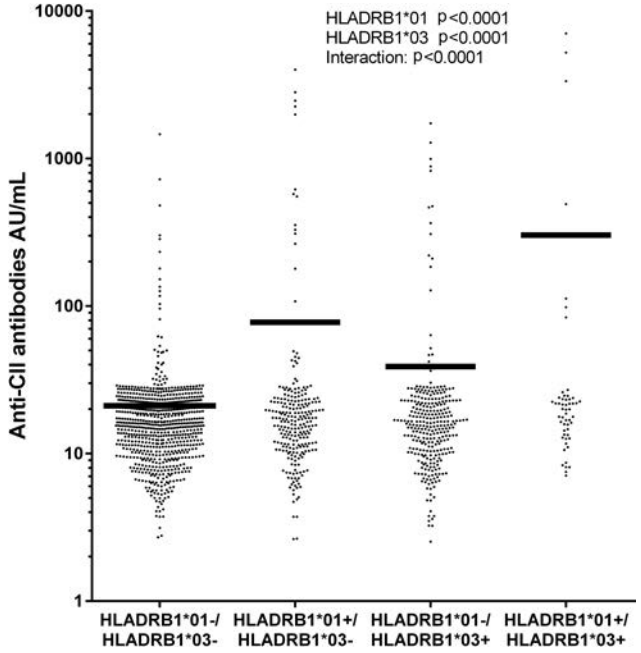


**Figure 4** Mean levels of antibodies against native antifibrillar collagen type II (anti-CII) in relation to the occurrence of HLA-DRB1*01 and/or HLA-DRB1*03 alleles. Statistical results were obtained with two-way analysis of variance with occurrence of HLA-DRB1*01 and HLA-DRB1*03 and their interaction as independent variables and anti-CII levels as the dependent variable. Data on 103 patients with very low anti-CII levels were not depicted in the graph, but are included in the statistical calculations.

### Anti-CII associated negatively with smoking

Anti-CII levels were negatively associated with smoking as ever smokers had lower anti-CII levels (median 15.2 AU/mL) compared with never smokers (median 16.5 AU/mL; p=0.0264). After exclusion of SE-positive patients, the negative association with smoking was still present (median ever smokers 14.8 AU/mL vs never smokers 17.1 AU/mL; p=0.0294). There was no difference in anti-CII levels between ever and never smoking controls (p=0.2).

Anti-CCP2 associated with smoking (median ever smokers 159 AU/mL vs never smokers 18.71 AU/mL; p=<0.0001). After exclusion of SE-positive patients, the association disappeared (7.98 vs 6.8 AU/mL; p=0.12).

## DISCUSSION

We have shown the association of anti-CII with a distinct RA phenotype characterised by acute but transient inflammation around the time of diagnosis. Whereas we previously described increased CRP and ESR at diagnosis in anti-CII-positive patients, this acute onset phenotype has now been extended to encompass clinically relevant markers like SJC, DAS28 and EULAR response.[11] This phenotype is in many respect the opposite to the ACPA phenotype associated with late increases in inflammatory markers and signs of disease activity; anti-CII also shows opposite associations with HLA-DRB1*03, HLA-DRB1*04 and smoking as compared with ACPA. The anti-CII-associated phenotype is strong, and predominates over the ACPA phenotype in patients with both antibodies. Most interestingly, when analysing future changes in inflammatory markers in newly diagnosed patients with RA, anti-CII is associated with a favourable outcome and anti-CCP2 with a more severe outcome both measured as changes in individual clinical and laboratory measures and attainment of EULAR response. This indicates that detection of ACPA at RA diagnosis could argue for more aggressive treatment, and the detection of anti-CII could predict a less aggressive disease course as compared with antibody-negative patients.

In a number of studies we have previously shown that anti-CII antibodies are functionally active, and that anti-CII-containing IC can stimulate MNC and PMN to cytokine and chemokine production, and probably contribute to joint erosions.[8–11 20] There is also a strong temporal association between serum anti-CII levels, in vitro function of the corresponding IC and ESR, CRP and radiological destruction.[11 20] In the previous study, as well as in the present study, antibody analyses were performed on serum samples obtained before institution of DMARD therapy. An obvious question is whether different DMARDs have divergent effects on future anti-CII levels, thus affecting anti-CII IC-driven inflammation to different degrees.

We found anti-CII in 6.6% of the patients with RA; a figure close to the 8.8% we reported previously.[11] Although anti-CII-positive RA represent a small group as compared with ACPA, the anti-CII-associated phenotype is so profound that also the small group of patients single positive for anti-CII (2.2%; 20/773) show a strong and statistically highly significant phenotype as compared with antibody-negative patients (table 1). We believe that anti-CII measurement will contribute to the prognostic armamentarium in newly diagnosed patients with RA.

There was a positive association between anti-CII and HLA-DRB1*03 and HLA-DRB1*01, and a strong negative

association with HLA-DRB1*04 and with the subtypes HLA-DRB1*0401 and HLA-DRB1*0404. The negative associations were probably second to the strong association between ACPA and SE, and disappeared after stepwise regression. Only HLA-DRB1*03 and HLA-DRB1*01 remained, and interacted, as double-positive patients had more than 14 times higher anti-CII levels than patients without HLA-DRB1*03 and HLA-DRB1*01. Three early studies on smaller groups of patients with RA (n=3160 and 166, respectively) have reported an association between HLA-DR3 and HLA-DR7 and antinative CII, but no previous studies have noted an association with HLA-DR1.[23–25] Others have reported a negative association between ACPA and HLA-DRB1*03, although this has not been replicated in EIRA.[26 27] However, when we restrict the 797 anti-CCP2 single positive patients in online supplementary table S3 to those not expressing SE (n=116), we find a negative association with HLA-DRB1*03 (OR 0.61, 95% CI 0.38 to 0.97, data not shown), arguing that a positive association between anti-CII and HLA-DRB1*03 might mask a negative association with ACPA.

Although both the clinical phenotype and the HLA-DRB1*03 and HLA-DRB1*04 associations with anti-CII represent the counterpart to ACPA, the two antibodies were not statistically inversely related in this study as we have previously described.[11] Anti-CII and anti-CCP2 showed very little of interaction when evaluated in two-way ANOVA against clinical measures. We interpret this that the clinical phenotypes associated with anti-CII and ACPA, respectively, exist and are regulated independent of each other.

A significant number of samples showed non-specific reactions, as, although they yielded OD levels compatible with high anti-CII levels in the ELISA, they reacted even stronger with wells that had only been blocked but did not contain the CII antigen. We believe that this reactivity is due to general 'stickiness', possibly related to inflammation, as we have recently described in highly inflamed *Leishmania*-infected patients.[28] In our previous RA studies, such reactivities were treated as anti-CII-negative, but here they were treated separately. The group of non-specifically reacting patients showed none of the HLA associations found for anti-CII-positive subjects. Patients with non-specific reactivity showed early inflammatory measures in between the anti-CII-positive and anti-CII-negative patients. Although these non-specific samples showed the strongest differences when compared with the anti-CII-positive subjects, in many cases they also showed significantly higher levels as compared with anti-CII-negative patients. A plausible conclusion is that although most of these subjects lack anti-CII, they contain a group of true anti-CII-positive subjects which could not be properly identified with the ELISA. We are currently investigating alternative confirmatory techniques to extend the group of correctly identified anti-CII-positive patients.

In mice, anti-CII antibodies have been shown to be pathogenic causing acute arthritis in the non-major histocompatability complex (MHC)-dependent Collagen Antibody Induced Arthritis (CAIA) model.[29 30] We believe that early inflammation associated with anti-CII-positive RA may represent the human counterpart to CAIA. There are many similarities. We have recently shown that PMN reactivity against anti-CII IC is associated with joint destruction in RA, and that PMN+MNC cocultures stimulated with anti-CII IC produce enhanced levels of many chemokines, whereby inflammatory cells can be recruited to inflamed joints in early RA when anti-CII levels are high. The mechanism is dependent on TLR4 and functionally active granulocyte enzymes.[8 10] In rodents anti-CII induce CAIA after injection of lipopolysaccharide (LPS), a TLR4 ligand, and the ensuing polyarthritis is associated with PMN activation and can be ameliorated with a serine protease inhibitor, implying a central pathogenetic role for PMN.[31 32] The central role for TLR4 that we have described in anti-CII IC-induced production of chemokines is intriguing, as it represents an autoantibody-dependent mechanism that probably not is epitope dependent. In agreement with this, we have not been able to block anti-CII IC-stimulated chemokine production with CII peptides.

In conclusion, anti-CII-positive RA represents a distinct RA phenotype that in many ways behaves as the opposite to ACPA-associated RA concerning clinical outcome, HLA-DRB1* association and relation to smoking history. Anti-CII-positive patients with RA have an acute onset, but favourable prognosis as compared with the high disease activity at diagnosis. This opens the possibility that early detection of anti-CII together with concomitant clinical signs of elevated disease activity, might associate with a transient inflammatory phenotype, as anti-CII levels diminish during the first year and as the associated phenotype is associated with the functional activity of anti-CII, probably bound in CII-containing IC in joints.[11] As anti-CCP2 instead is associated with poor prognosis, the combined analysis of anti-CII and ACPA/anti-CCP2 may be a new two-dimensional tool for predicting the prognosis and choosing therapy in newly diagnosed patients with RA.

**Contributors** MM performed antifibrillar collagen type II ELISA measurements; LP performed HLA-DRB1* tissue typing; HW and SS provided clinical data from the Swedish Rheumatology Quality Register registry; LK and LA contributed data and analyses from the Epidemiological Investigation in Rheumatoid Arthritis study. VAM and JR conceived and planned the study, made all statistical calculations and drafted the manuscript. JR is responsible for the merged data file containing serological, genetic and clinical data. All authors have read and approved the final manuscript.

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**Competing interests** None declared.

**Patient consent** Obtained.

**Ethics approval** Ethical board at Karolinkska Institutet.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data sharing statement** All data for the study are available in the manuscript and in the online supplementary material.



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OPEN ACCESS

EXTENDED REPORT

# Pharmaceutical-grade Chondroitin sulfate is as effective as celecoxib and superior to placebo in symptomatic knee osteoarthritis: the ChONdroitin versus CElecoxib versus Placebo Trial (CONCEPT)

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## ABSTRACT

**Objectives** Chondroitin sulfate 800 mg/day (CS) pharmaceutical-grade in the management of symptomatic knee osteoarthritis consistent with the European Medicines Agency guideline.

**Methods** A prospective, randomised, 6-month, 3-arm, double-blind, double-dummy, placebo and celecoxib (200 mg/day)-controlled trial assessing changes in pain on a Visual Analogue Scale (VAS) and in the Lequesne Index (LI) as coprimary endpoints. Minimal-Clinically Important Improvement (MCII), Patient-Acceptable Symptoms State (PASS) were used as secondary endpoints.

**Results** 604 patients (knee osteoarthritis) diagnosed according to American College of Rheumalogy (ACR) criteria, recruited in five European countries and followed for 182 days. CS and celecoxib showed a greater significant reduction in pain and LI than placebo. In the intention-to-treat (ITT) population, pain reduction in VAS at day 182 in the CS group (−42.6 mm) and in celecoxib group (−39.5 mm) was significantly greater than the placebo group (−33.3 mm) (p=0.001 for CS and p=0.009 for celecoxib), while no difference observed between CS and celecoxib. Similar trend for the LI, as reduction in this metric in the CS group (−4.7) and celecoxib group (−4.6) was significantly greater than the placebo group (−3.7) (p=0.023 for CS and p=0.015 for celecoxib), no difference was observed between CS and celecoxib. Both secondary endpoints (MCII and PASS) at day 182 improved significantly in the CS and celecoxib groups. All treatments demonstrated excellent safety profiles.

**Conclusion** A 800 mg/day pharmaceutical-grade CS is superior to placebo and similar to celecoxib in reducing pain and improving function over 6 months in symptomatic knee osteoarthritis (OA) patients. This formulation of CS should be considered a first-line treatment in the medical management of knee OA.

## INTRODUCTION

Osteoarthritis (OA) is the most prevalent musculoskeletal disease affecting humans, an important cause of pain, loss of function, disability and a major public health problem[1 2] that is associated with a substantial and ever increasing burden on society.[3 4] OA of the knee and hip tends to generate the greatest impact on the population, as pain and stiffness in these large weight-bearing joints often lead to the need for medical intervention.[2] Medical management of knee OA includes both pharmacological and non-pharmacological modalities and numerous scientific societies have produced recommendations for the non-surgical management of knee OA.[5–9] Although several differences are observed between these evidence-based guidelines, mostly reflecting heterogeneity of the expert panels involved, geographical differences in the availability of chemical entities [10 11] there was, until recently, a general consensus that analgesics, including paracetamol and non-steroidal anti-inflammatory drugs (NSAIDs) have demonstrated a positive benefit-risk profile when used to treat symptoms of knee OA.[5–11] However, recent publications have aggressively challenged the use of paracetamol for the treatment of symptomatic OA because of a lack of efficacy and a considerable degree of toxicity, especially at the upper end of the standard analgesic dose.[12–14] Similarly, safety profiles of oral NSAIDs remain a concern and caution is recommended before selecting the preparation and dose for a patient.[14] Therefore, recent guidelines recommend maintenance therapy to be conducted with symptomatic slow-acting drugs for OA (SYSADOAs), a class of drugs that is recognised to offer a high degree of safety and tolerability.[5] Although discrepancies can be found in the literature regarding recommendations on SYSADOAs in the management of knee OA,[10 11] higher quality evidence seems to be provided for patented, prescription formulations of chondroitin sulfate (CS) and crystalline glucosamine sulfate (GS).[9]

Chondroitin sulfate (CS) is a sulfated glycosaminoglycan composed of chains of alternating D-glucuronic acid and N-acetyl-D-galactosamine.[15] CS is available as pharmaceutical-grade and nutraceutical-grade products, the latter exhibiting striking variations in preparation, composition, purity as well as clinical effects. These differences may explain why, whereas pharmaceutical-grade CS (ie, the 4&6isomer of sodium CS) was shown to improve pain and function and/or delay structural progression of knee OA in several well-conducted studies,[16–18] these results were not confirmed when lower grade formulations were used.[19 20] Indeed, a recent systematic review conducted by the Cochrane Collaborative Group concludes that CS, alone or in combination with GS, is better than placebo in improving pain in participants with OA in short-term studies, with CS having a lower risk of serious adverse events compared with controls.[21]

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Another potential source of inconsistency in the results from previous studies of SYSADOAs in knee OA has been idiosyncratic trial design. For this reason, the European Medicines Agency (EMA) produced a Guideline on Clinical Investigation of Medicinal Products Used in the Treatment of Osteoarthritis (CPMP/EWP/784/97 Rev. 1), guideline which has been recently supported by a European experts consensus.[22] It recommends that efficacy of chemical entities used in the treatment of symptomatic OA be tested according to a standard study design with the following basic parameters: a minimum 6-month study duration; a three-arm study design including a placebo and an active comparator (ie, oral NSAID); and two co-primary endpoints evaluating pain and function, respectively.

Herein, we report results from a study of pharmaceutical-grade CS in patients with symptomatic knee OA, which, to our knowledge, is the first ever to have been conducted in full accordance with the aforementioned EMA guideline.

## MATERIAL AND METHODS

### Study design and selection of patients

The study comprised patients from Belgium, Czech Republic, Italy, Poland and Switzerland, who were enrolled between June 2014 and October 2015. The main inclusion criteria were outpatients status, age above 50 years and primary knee OA of the medial or lateral femorotibial compartment diagnosed according to the clinical and radiographic criteria of the American College of Rheumatology (ACR).[23] The more symptomatic knee (with a pain score of at least 50 mm on a 0–100 mm Visual Analogue Scale (VAS) for at least 3 months before enrolment) was defined as the target knee. The main exclusion criteria were those listed in the last version of the Guideline on Clinical Investigation of Medicinal Products used in the Treatment of Osteoarthritis released by the EMA in 2010 (CPMP/EWP/784/97 Rev. 1) and grade 4 radiographic OA according to the Kellgren-Lawrence (K-L) grading system.[24] Use of any intra-articular injection in the target knee in the last 6 months, SYSADOAs in the last 3 months, NSAIDs in the last 5 days and paracetamol in the 10 hours preceding enrollment was also specifically forbidden by the study protocol. There were two co-primary endpoints, predefined as stipulated by the EMA guidelines: pain and Lequesne Index (LI) assessment. Ethics Committee approval from all participating centres was obtained and all patients gave their written informed consent to participate.

This study has been designed to assess the symptomatic effect of CS. Bone and cartilage markers were not the target in this short study.

## TREATMENT ASSIGNMENT

Patients were randomly assigned to one of the following three groups: (1) Group CS: one tablet of CS 800 mg and one capsule of placebo celecoxib; (2) Group celecoxib: one tablet of placebo CS and one capsule of celecoxib 200 mg (Celebrex Pfizer); (3) Group placebo: one tablet of placebo CS and one capsule of placebo celecoxib. The tablets of Celebrex available on the market were encapsulated to allow for a double-blind, double-dummy design. CS tablets contained highly purified chondroitin 4 & 6 sulfate in a concentration not less than 95% (European patents E 1582214 and EP 1705192) (Condrosulf (other brand name: Chondrosulf, Condral) 800; IBSA Institut Biochimique SA; Pambio-Noranco, Switzerland). All treatments were taken once daily, every evening with a glass of water, for 6 months. For rescue analgesia, patients were allowed to take paracetamol 500 mg tablets (maximum dosage 3 g/day), and they recorded use thereof in a diary. An appropriate washout period of 10 hours was required before symptom assessment at in-clinic visits. No other pharmacological or non-pharmacological interventions for OA were allowed. Compliance with the study treatments was established by asking the patients about missing doses and by counting unused study drugs.

## OUTCOME MEASURES

There were two co-primary endpoints, as stipulated by the EMA guideline, and both were assessed as the change from baseline, that is, the difference between enrollment and study conclusion. One endpoint was the patient's estimate of pain on a 100 mm Visual Analogue Scale (VAS). The other endpoint was the Lequesne Index (LI), which integrates pain and function and results in a score from 0 to 24.[25] Secondary endpoints included the proportion of patients reaching the Minimal-Clinically Important Improvement (MCII), defined as the smallest change in measurement that signifies an important improvement in a patient's symptom,[26] and the Patient Acceptable Symptom State (PASS), defined as the value of symptoms beyond which patients consider themselves well.[27] Patient and investigator global assessment were scored on a 5-point Likert ordinal scale (excellent, good, fair, poor, none). All adverse events and abnormal laboratory test results were recorded.

## STATISTICAL ANALYSIS

All statistical analyses were performed using SAS Software (V. 9.4 and V. 8.2) on a Windows 7 operating system.

We calculated a sample size of 600 patients based on an estimated difference of 9 mm between CS and placebo after 6 months of treatment, with a standard deviation (SD) of 25 mm, a power of 90%, an alpha risk of 5% and a drop-out rate of 15%. The intention-to-treat (ITT) population was defined as all randomised patients who received one dose of the study medication. Safety analyses were conducted on all randomised patients.

VAS (pain in mm) and LI score from D1 to D182 were compared between the three treatment groups by means of a linear mixed model carried out by using the SAS MIXED procedure, with patient as random effect, centre, treatment group, time point, interaction between treatment group and time point as categorical covariates (interaction between treatment group and centre excluded from the final models because not statistically significant. p=0.101 for VAS mixed model, p=0.998 for LI mixed model). No missing values replacement (LOCF, last observation carried forward or BOCF, basal observation carried forward) was performed for this analysis. The proportion of patients reaching the MCII, the PASS and the Outcome Measures in Rheumatology (OMERACT-OARSI) criteria were compared using a Chi-square ($\chi^2$)) test. Patient's and investigator's global assessments were analysed by means of Mantel-Haenszel $\chi^2$ test. Differences between groups in rates of patients with treatment-emergent adverse events (TEAEs), serious adverse events (SAEs), adverse drug reactions (ADRs) and study withdrawals due to AEs were assessed using the $\chi^2$ test.

## RESULTS

Of 656 patients screened, 604 were randomised and 603 considered eligible for ITT analysis (all patients who received the study medication). Of these patients, 199 received CS, 199 received celecoxib and 205 received placebo. The cumulative time distribution of withdrawals was similar in the three groups without significant differences in reasons for withdrawals (figure 1). Patients in the three groups had similar demographic

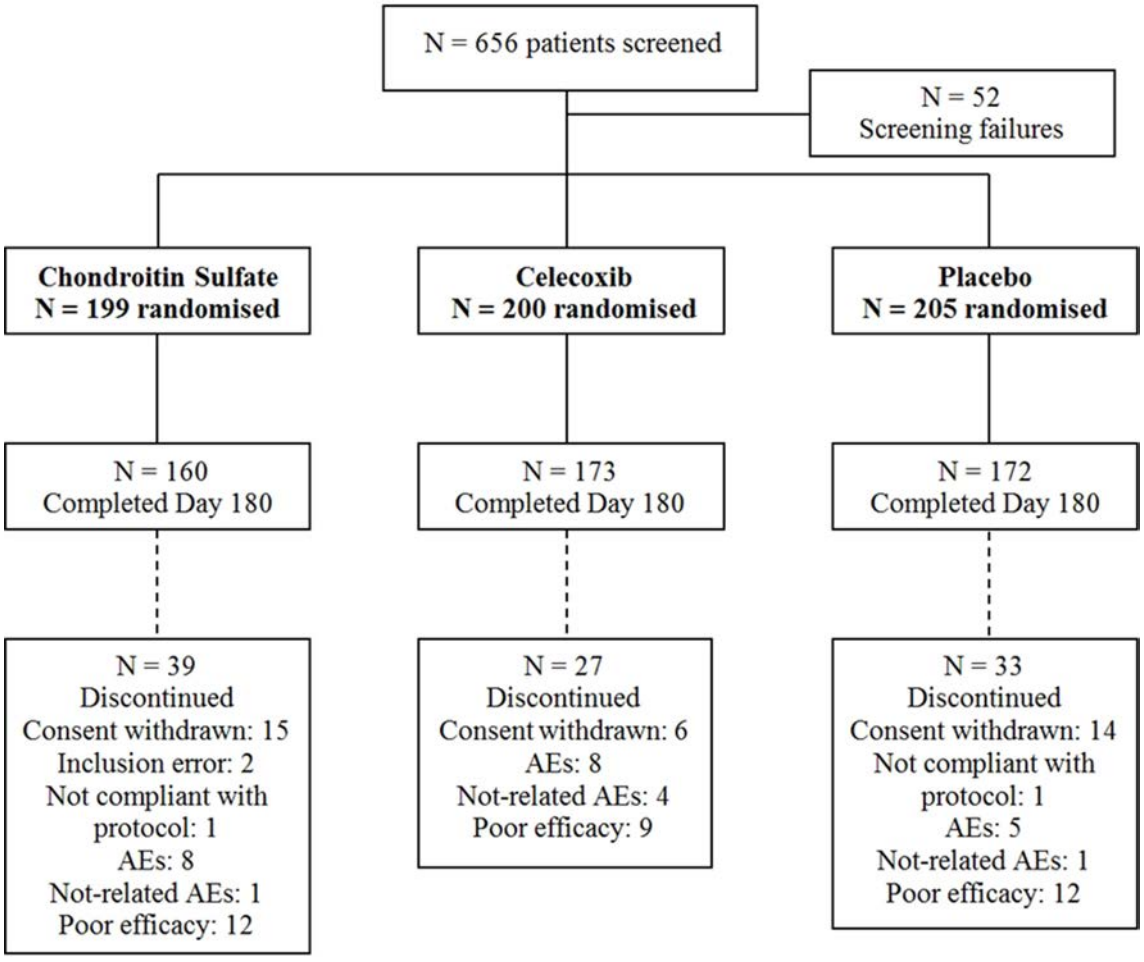


**Figure 1** Disposition of patients. AE, adverse events.

and baseline characteristics (table 1). Grades 1 to 3 of K-L were equally distributed between the three groups, with roughly 50% of the patients presenting a grade 2 OA and 25% corresponding to either a grade 1 or a grade 3 overall.

Analysis of pain scores in ITT revealed a significant improvement in all three groups compared with baseline at day 30, 91 and 182 (all $p<0.001$) (figure 2). Both the CS and the celecoxib group showed a statistically greater reduction in pain compared with the placebo group ($p=0.001$ for CS and $p=0.009$ for celecoxib, table 2) after 6 months without any significant difference between the two active groups ($p=0.446$).

Analysis of LI scores in ITT revealed a significant amelioration in all three groups compared with baseline at day 30, 91 and 182 (all $p<0.001$) (figure 3). At day 91 and 182, both CS and celecoxib induced a significantly greater reduction in LI than placebo ($p=0.050$ for CS and $p=0.027$ for celecoxib at day 91, $p=0.023$ for CS and $p=0.015$ for celecoxib at day 182) while no difference was observed between CS and celecoxib ($p=0.799$ at day 91 and $p=0.890$ at day 182, table 2). The decrease in LI observed in the celecoxib group attained statistical significance in comparison to the placebo group at day 30 ($p=0.045$), while it took the CS group until day 91 ($p=0.050$) (figure 3).

After 6 months, a greater proportion of patients reached the MCII (20 mm of VAS reduction) in the CS (68%) and celecoxib (69%) groups than in the placebo group (61%). This difference was not significant for the CS–placebo comparison ($p=0.122$), for the celecoxib–placebo comparison ($p=0.098$) and not significant for CS–celecoxib comparison ($p=0.914$). Similar results were obtained for the proportion of patients reaching the PASS in the CS (57%), celecoxib (59%) and placebo (49%) groups. The PASS data were significant for the celecoxib–placebo comparison ($p=0.047$), not significant for the CS–placebo comparison ($p=0.130$) and not significant for the CS–celecoxib comparison ($p=0.611$).

Significant results were observed when defining responders patients with at least 40% or 50% of improvement in pain or LI scores, and when patients were classified according to OMERACT-OARSI (scenario F). CS and celecoxib provided significantly higher number of responders than placebo and no difference was observed between CS and celecoxib (table 3).

At study conclusion (day 182), significantly more patients and more investigators scored the global assessment as excellent or good in the CS and celecoxib groups compared with the placebo ($p=0.027$ for CS, $p=0.013$ for celecoxib), while there was no difference between the two active groups ($p=0.774$). Study medication usage was >95% in all groups, demonstrating excellent compliance and the absence of intergroup differences.

Finally, there was no significant difference between CS, celecoxib or placebo usage in the rate of TEAEs, SAEs, ADRs and withdrawal related to TEAEs. Abdominal pain/discomfort was the most frequently reported ADR (2.5% in the CS group, 4.5% in the celecoxib group and 2.9% in the placebo group). Routine laboratory testing identified one case of leukopenia and one case of thrombocytopenia in the placebo group, but no significant abnormalities in the CS or celecoxib groups.

## DISCUSSION

In this report, we provide data from the CONCEPT trial, which, to our knowledge, is the first-ever evidence supporting a durable

**Table 1** Demographic and baseline characteristics of patients

| | CS n=199 | Celecoxib n=199 | Placebo n=205 |
|---|---|---|---|
| Age (years) | | | |
| Mean (SD) | 65.5 (8.0) | 65.5 (7.8) | 64.9 (8.0) |
| Sex, n (%) | | | |
| Female | 156 (78.4) | 160 (80.4) | 152 (74.1) |
| Height (cm) | | | |
| Mean (SD) | 163.3 (8.8) | 162.8 (9.4) | 164.6 (9.5) |
| Weight (kg) | | | |
| Mean (SD) | 80.4 (14.1) | 78.4 (13.9) | 82.9 (14.7) |
| BMI (kg/m$^2$) | | | |
| Mean (SD) | 30.2 (4.7) | 29.5 (4.4) | 30.6 (5.0) |
| Time from diagnosis of knee OA (months) | | | |
| Mean (SD) | 72.3 (69.2) | 64.4 (63.4) | 69.2 (72.5) |
| KL grade, n (%) | | | |
| Grade 1 | 48 (24.1) | 46 (23.1) | 53 (25.9) |
| Grade 2 | 100 (50.3) | 101 (50.8) | 101 (49.3) |
| Grade 3 | 50 (25.1) | 52 (26.1) | 51 (24.9) |
| Grade 4 | 1 (0.5) | 0 (0.0) | 0 (0.0) |
| Duration of regular pain (months) | | | |
| Mean (SD) | 41.7 (60.3) | 39.9 (56.5) | 47.8 (68.1) |
| Target knee (the most symptomatic) | | | |
| Left, n (%) | 85 (42.7) | 95 (47.7) | 92 (44.9) |
| Target knee pain (VAS, mm) | | | |
| Mean (SD) | 70.9 (9.8) | 69.7 (10.2) | 70.0 (10.3) |
| Lequesne's Algo-Functional Index (LI total score) | | | |
| Mean (SD) | 11.8 (2.9) | 11.6 (2.9) | 11.8 (3.1) |

BMI, body mass index; KL, Kellgren-Lawrence; LI, Lequesne Index; OA, osteoarthritis; VAS, Visual Analogue Scale.

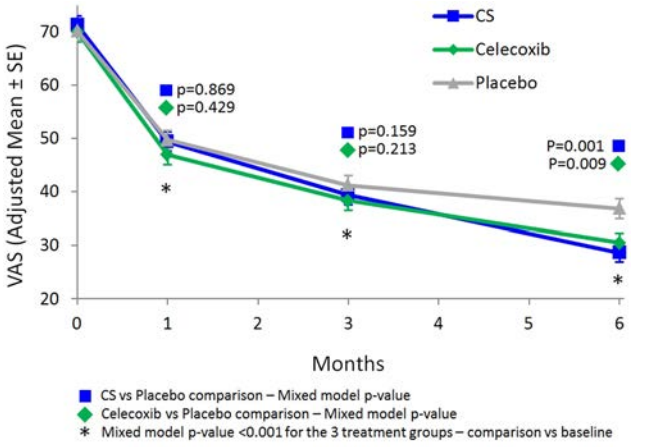


**Figure 2** Visual Analogue Scale (VAS).

therapeutic benefit of SYSADOAs in a knee OA clinical trial that is fully aligned with the current EMA guideline. We demonstrated that CS is superior to placebo and similar to celecoxib across multiple outcome measures, including reduction in pain and LI (co-primary endpoints), as well as in the proportion of patients experiencing MCII (secondary endpoint) and patient/investigator global assessments.

Prior to CONCEPT, the only study that assessed the impact of a SYSADOA on knee OA in a three-arm design was the Glucosamine Unum-in-Die Efficacy (GUIDE) study, a study that demonstrated that patented crystalline glucosamine sulfate (GS) was superior to placebo and equivalent to acetaminophen in reducing LI after 6 months of treatment.[28] However, the present study utilised celecoxib as an active comparator, a NSAID that was recently shown to provide substantially greater clinical effect than acetaminophen in knee OA.[12 14]

All treatments, including placebo, provided a statistically significant improvement from baseline on pain and function as early as day 30, and this effect persisted until the end of the trial. This is not surprising as a substantial placebo effect was previously reported in trials assessing drugs in OA.[29 30] However, both active groups (CS and celecoxib) provided a significantly greater reduction in pain (VAS) and a better improvement in function (LI) than the placebo, after 6 months and 3 months, respectively. With respect to LI, it is interesting to note that celecoxib treatment resulted in a statistically significant change at day 30 compared with placebo, while CS did not. Although impossible to know definitively, this observation may be related to an intrinsic difference in the mechanism of action of the two molecules.

One important consideration in any clinical investigation that uses a pain assessment is how to equate statistical significance with clinical benefit. Indeed, the relevance of statistically significant CS-dependent improvements in OA symptoms in previous trials[16–18] has been challenged.[21] The EMA 2010 guideline document suggests that the improvement in pain observed with the

**Table 2** VAS and LI

| | CS | | | Celecoxib | | | Placebo | | |
|---|---|---|---|---|---|---|---|---|---|
| | n | Mean (SE)* | p Value† | n | Mean (SE)* | p Value† | n | Mean (SE)* | p Value‡ |
| VAS | | | | | | | | | |
| Baseline | 199 | 71.2 (0.8) | | 195 | 70.0 (0.8) | | 205 | 70.2 (0.8) | |
| Day 30 | 195 | 49.4 (1.5) | 0.869 | 195 | 46.9 (1.5) | 0.159 | 204 | 49.7 (1.4) | 0.309 |
| Day 91 | 179 | 39.4 (1.7) | 0.429 | 182 | 38.3 (1.7) | 0.213 | 188 | 41.2 (1.6) | 0.450 |
| Day 182 | 160 | 28.6 (1.8) | 0.001 | 173 | 30.5 (1.7) | 0.009 | 172 | 36.8 (1.7) | 0.002 |
| LI | | | | | | | | | |
| Baseline | 199 | 11.8 (0.2) | | 195 | 11.6 (0.2) | | 205 | 11.8 (0.2) | |
| Day 30 | 195 | 9.6 (0.3) | 0.714 | 195 | 9.1 (0.3) | 0.045 | 204 | 9.8 (0.3) | 0.105 |
| Day 91 | 179 | 8.1 (0.3) | 0.050 | 182 | 8.0 (0.3) | 0.027 | 188 | 8.8 (0.3) | 0.052 |
| Day 182 | 160 | 7.1 (0.3) | 0.023 | 173 | 7.0 (0.3) | 0.015 | 172 | 8.0 (0.3) | 0.024 |

*Estimated mean and SE from a mixed-model analysis.
†Compared with placebo.
‡Comparing three treatment groups.
LI, Lequesne Index; VAS, Visual Analogue Scale.

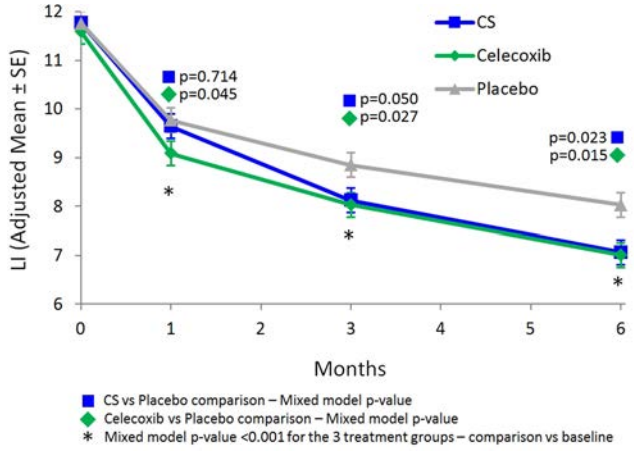


**Figure 3** Lequesne Index (LI).

test article must be clinically relevant, but unfortunately the guideline does not provide an associated numerical threshold. However, a group of European academic scientists and regulators recently published an expert consensus statement in which they suggest that at least a 5 mm difference on a 100 mm VAS between the placebo and active groups constitutes a clinically relevant threshold for a SYSADOAs.[22] These recommendations were partially based on the observation that most clinical trials published with SYSADOAs in knee or hip OA show symptomatic improvements in the 5–6 mm range on a 100 mm VAS.[31–33] In our study, the difference in pain reduction between CS and placebo is 8.2 mm after 6 months in the ITT analysis. This difference exceeds this range and compares favourably with the previous publications reporting beneficial effects of SYSADOAs in knee or hip OA.[31–33]

The improvement in pain and function observed in the CS group corresponds to an effect size (ES) of 0.35 for pain and 0.27 for LI, whereas the ES in the celecoxib group was 0.27 for pain and 0.30 for LI. ES ≤0.2 is usually considered as small while ES between 0.2 and 0.5 is defined as medium. An ES value of 0.27 for pain in the celecoxib group is consistent with previous publications[8 14] and provides thus external validation of CONCEPT trial data. For CS, an ES of 0.35 for pain is consistent with values previously reported for pharmaceutical-grade GS or CS,[8 9] compares well with the reported ES for most NSAIDs[8 14] and is two-fold higher than the ES (0.14) commonly reported for acetaminophen in knee OA.[8 13]

Although both acetaminophen and NSAIDs have been shown to be efficacious in the setting of knee OA, the chronic use of these medicines is known to be associated with frequent and serious adverse events.[13 14] It is notable in this regard, that CS, in addition to a robust efficacy profile that is comparable to NSAIDs, also exhibits a safety profile that was similar to placebo in this study and in others.[5 8 16 17 21] This combination of therapeutic effect and well-documented safety and tolerability explain why recent guidelines[8 9] recommend SYSADOAs, including pharmaceutical-grade CS, as a first-line treatment in the management of knee OA.

**Table 3** OMERACT-OARSI and MCII

| | CS n=199 | Celecoxib n=199 | Placebo n=205 | CS vs placebo $\chi^2$ p Value | Celecoxib vs placebo $\chi^2$ p Value |
|---|---|---|---|---|---|
| VAS–MCII 20 mm, n (%) | | | | | |
| Day 30—Yes (%) | 94 (47) | 99 (50) | 93 (45) | 0.706 | 0.378 |
| Day 91—Yes (%) | 126 (63) | 128 (64) | 125 (61) | 0.628 | 0.487 |
| Day 182—Yes (%) | 136 (68) | 137 (69) | 125 (61) | 0.122 | 0.098 |
| PASS, n (%) | | | | | |
| Day 30—Yes (%) | 62 (31) | 80 (40) | 65 (32) | 0.905 | 0.075 |
| Day 91—Yes (%) | 93 (47) | 108 (54) | 91 (44) | 0.636 | 0.047 |
| Day 182—Yes (%) | 113 (57) | 118 (59) | 101 (49) | 0.130 | 0.043 |
| VAS–MCII 40%, n (%) | | | | | |
| Day 30—Yes (%) | 59 (30) | 78 (39) | 64 (31) | 0.731 | 0.093 |
| Day 91—Yes (%) | 105 (53) | 103 (52) | 102 (50) | 0.545 | 0.687 |
| Day 182—Yes (%) | 127 (64) | 116 (58) | 106 (52) | 0.014 | 0.184 |
| VAS–MCII 50%, n (%) | | | | | |
| Day 30—Yes (%) | 43 (22) | 50 (25) | 49 (24) | 0.582 | 0.775 |
| Day 91—Yes (%) | 86 (43) | 83 (42) | 77 (38) | 0.247 | 0.394 |
| Day 182—Yes (%) | 115 (58) | 103 (52) | 83 (40) | 0.005 | 0.023 |
| LI–MCII 40%, n (%) | | | | | |
| Day 30—Yes (%) | 34 (17) | 45 (23) | 27 (13) | 0.272 | 0.013 |
| Day 91—Yes (%) | 71 (36) | 67 (34) | 56 (27) | 0.070 | 0.165 |
| Day 182—Yes (%) | 94 (47) | 90 (45) | 72 (35) | 0.013 | 0.038 |
| LI–MCII 50%, n (%) | | | | | |
| Day 30—Yes (%) | 18 (9) | 27 (14) | 13 (6) | 0.307 | 0.015 |
| Day 91—Yes (%) | 52 (26) | 44 (22) | 34 (17) | 0.019 | 0.159 |
| Day 182—Yes (%) | 74 (37) | 70 (35) | 56 (27) | 0.034 | 0.088 |
| OMERACT-OARSI—scenario F, n (%) | | | | | |
| Day 30—Yes (%) | 82 (41) | 89 (45) | 82 (40) | 0.805 | 0.337 |
| Day 91—Yes (%) | 118 (59) | 119 (60) | 110 (54) | 0.253 | 0.213 |
| Day 182—Yes (%) | 132 (66) | 133 (67) | 113 (55) | 0.021 | 0.016 |

If we use the ITT2 population the results for MCII (20 mm) reported in the text of the publication are not correct (the comparisons vs placebo are not statistically significant, see table above).

ITT, intention-to-treat; LI, Lequesne Index; MCII, Minimal-Clinically Important Improvement; PASS, Patient-Acceptable Symptoms State; VAS, Visual Analogue Scale.

In addition to the classic efficacy parameters, pain and LI, regulatory and clinical guidelines continue to place additional emphasis on patient's perception of their clinical status, thus requiring the use of additional measures to assess treatment outcome. The significantly higher proportion of patients reaching the self-assessed MCII and the significantly greater number of patients ranking their treatment as good or excellent, compared with the placebo group, further reflects the importance of clinical benefits obtained with CS usage.

In conclusion, the CONCEPT study provided evidence that daily administration of 800 mg of 4 &6 CS in patients with symptomatic knee OA lead to improvement in pain and function superior to placebo and similar to the NSAID celecoxib. In addition, we confirmed the excellent safety profile of CS that has been previously observed by others. This compelling benefit-risk profile, in light of the known clinical risks associated with chronic usage of NSAIDs and paracetamol, underscores the potential importance of pharmaceutical-grade CS in the management of knee OA, especially in this older population requiring long-term treatment. More generally, this study corroborates the need for future clinical guidelines on the pharmacological management of knee OA to consider the study design, as well as the composition and quality of the test product, when assessing the effectiveness of SYSADOAs.

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EXTENDED REPORT

# Stroke in systemic lupus erythematosus: a Swedish population-based cohort study

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## ABSTRACT

**Objective** To study the occurrence of ischaemic and haemorrhagic stroke in systemic lupus erythematosus (SLE) compared with the general population by age, sex and time since SLE diagnosis

**Methods** Adults with incident SLE were identified from the Swedish National Patient Register (NPR, n=3390) and general population comparators from the Total Population Register were matched on age, sex and county (n=16730). Individuals were followed prospectively until first of death, December 2013, emigration or incident stroke (identified from the NPR, Cause of Death Register and the Stroke Register). Incidence rates, rate differences and HR were estimated comparing SLE with non-SLE. Estimates were stratified by sex, age and time since diagnosis.

**Results** We observed 126 strokes in SLE and 304 in the general population. Individuals with SLE had a twofold increased rate of ischaemic stroke compared with the general population (HR 2.2; 95% CI 1.7 to 2.8). The HR for intracerebral haemorrhage was 1.4 (95% CI 0.7 to 2.8). There was effect modification by sex and age, with the highest HRs for females and individuals <50 years old. The HR for ischaemic stroke was highest in the first year of follow-up (3.7; 95% CI 2.1 to 6.5).

**Conclusions** The relative risk of ischaemic stroke in SLE was more than doubled compared with the general population, and importantly, the highest relative risks were observed within the first year after SLE diagnosis. Thus, the first encounter with patients presents an opportunity for rheumatologists to screen for risk factors and intervene.


## INTRODUCTION

Systemic lupus erythematosus (SLE) is a complex autoimmune disease with heterogeneous presentation which is associated with an increased risk of cardiovascular disease.[1 2] Stroke has been investigated in some previous studies as a composite endpoint with cardiovascular disease, but given that it is an important cause of morbidity and mortality in SLE and has a different aetiology, a separate investigation is warranted.[3] Traditional risk factors, such as hypertension which is more common in SLE, likely contribute to the increased risk of cerebrovascular disease. SLE-specific factors including proinflammatory cytokines, prothrombotic antiphospholipid antibodies, impaired renal function and exposure to medications like glucocorticoids may also play important roles.[4–7]

A recent meta-analysis reported a twofold increased risk of ischaemic stroke in SLE compared with the general population.[8] The relative risk of intracerebral haemorrhage was estimated to be almost threefold, but this was based on only three studies.[9–11] Few studies have examined the relative risk of stroke stratified by age, sex or disease duration.[9–11] Absolute rates are seldom reported, which are especially necessary for communicating risk to young individuals for whom the outcome is rare.

In the general population, treatment of hypertension and lifestyle modifications have been shown to substantially reduce the risk of stroke.[12 13] Individuals with SLE who have a higher risk of stroke may greatly benefit from early intervention. Understanding who is at the highest risk of stroke and when the highest risk occurs could help to target groups of people for preventative treatment and provide clues to the underlying drivers of stroke in SLE. Our aim was to study the occurrence of ischaemic and haemorrhagic stroke in SLE compared with the general population by age, sex and time since diagnosis.

## METHODS

We used Swedish national registers and a matched cohort study design to compare the risk of incident stroke in individuals with SLE with those without SLE.

### Study population

A population-based SLE cohort was identified using the National Patient Register (NPR), which contains data on inpatient care 1964–2013 (nationwide since 1987) and outpatient, non-primary care 2001–2013. We included individuals 18 years or older with at least two discharge diagnoses listing an International Classification of Diseases (ICD) code for SLE, one or more registered with a relevant specialist (rheumatology, dermatology, nephology, internal medicine or paediatrics). This definition has been shown to result in an accurate classification of SLE in these data.[14] To best identify newly diagnosed (incident) SLE, we required that individuals have no inpatient or outpatient SLE-coded visits for at least 2 years before they were first identified with an SLE ICD code. Individuals were included if they received their first ever SLE ICD code January 2003 or later thus allowing for at least 2 years of outpatient data.

For each individual with SLE, five comparators were selected from the Total Population Register matched on sex, year of birth and residential county. These general population comparators (non-SLE) were required to be living in Sweden at the time

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their matched SLE case fulfilled the case definition (index date) and were eligible to become cases later during follow-up.

### Covariates

Information from several national registers was retrieved and linked to the study population using each individual's unique personal identification number. Education was obtained from the Education Register and categorised into ≤9 years, 10–12 years, >12 years of education and missing. Country of birth was obtained from the Total Population Register (categorised as Nordic, non-Nordic Europe and outside Europe). Information on comorbidities was collected from the NPR. Indicator variables were created if any visit occurred before start of follow-up listing an ICD code for hypertension, congestive heart disease, diabetes, atrial fibrillation and antiphospholipid syndrome (APS); see online Supplementary table 1 for details.

### Stroke identification

Date and type of stroke (ischaemic stroke, intracerebral haemorrhage, subarachnoid haemorrhage, unspecified) were identified using ICD codes from three sources (see online Supplementary table S1 for ICD codes used):

1. The NPR: both hospitalisations and outpatient non-primary visits listing an ICD code for stroke either as main diagnosis or contributory diagnosis. ICD codes for stroke from the NPR have been shown to be valid (Positive Predictive Value (PPV) 94.0%).[15]
2. The Swedish Stroke Register (Riksstroke): a National quality register that covers all hospitals admitting patients with acute stroke with excellent data on stroke subtype (PPV ≥95%).[16] Riksstroke does not contain information on subarachnoid haemorrhage.
3. The Cause of Death Register: date of death and main and contributory causes for almost 100% of all deaths in Sweden was used to identify fatal strokes (PPV 87.3%).[15]

Some strokes were listed in both the NPR and in Riksstroke (n=259). If the diagnoses from the two sources were different, the Riksstroke diagnosis was considered the gold standard and the stroke was reclassified accordingly (seven unspecified strokes were reclassified as ischaemic and one ischaemic stroke was reclassified as intracerebral haemorrhage).

### Follow-up

Start of follow-up began on the date of the second SLE-coded visit or index date and ended at incident stroke, emigration date (retrieved from the Total Population Register), death or 31 December 2013, whichever came first. Individuals with a history of stroke at start of follow-up were excluded from all analyses. Three thousand five hundred and sixty-two individuals with SLE and 17 062 general population comparators were identified before excluding those with a history of stroke. One hundred and seventy-two (4.8%) individuals with SLE had a history of stroke at start of follow-up compared with 332 (1.9%) in the general population.

### Statistical analysis

Incidence rates (IRs) of stroke per 1000 person-years (py) were calculated. Age and sex-standardised rate differences with 95% CIs comparing SLE and non-SLE were estimated. Age and sex-adjusted Cox models were used to estimate the HR and 95% CI (HR 95% CI) for stroke comparing SLE with non-SLE. Models were additionally adjusted for history of hypertension, congestive heart disease, diabetes, atrial fibrillation and educational level. The proportional hazards assumption was tested by calculating the p value for the interaction term between log of follow-up time and SLE. A p value<0.05 was considered statistically significant.

Models were stratified by sex, age at start of follow-up (<50, 50–59, ≥60 years) and time since start of follow-up (a proxy for SLE disease duration; <1, 1 to <5, 5–11 years). Stratification categories were collapsed if fewer than five events occurred in a stratum and if there still remained strata with fewer than five individuals, numbers were not reported and no IRs or HRs were calculated. Effect modification by sex, age and time since start of follow-up was tested using the likelihood ratio test.

### Sensitivity analyses

Although using two ICD codes for SLE including at least one from a specialist clinic from the NPR has been shown to identify individuals with SLE accurately, some misclassification may still remain.[14] Therefore, we restricted to a subset of the SLE population who were enrolled in one of two clinical SLE cohorts in Sweden (Linköping and Stockholm) and had four or more American College on Rheumatology criteria for SLE fulfilled. In this analysis, start of follow-up began on the date of inclusion in their respective clinical cohort or 1 January 2003, whichever came later, to make the study period comparable to the main analysis. Follow-up ended at incident stroke, emigration, death or end of follow-up (31 December 2011 for the Linköping Cohort and 31 December 2013 for the Stockholm Cohort), whichever came first. Unlike the primary nationwide analysis, these data include individuals with prevalent SLE. More information on these cohorts can be found elsewhere.[17 18]

Because we estimate cause-specific HRs in our primary analyses, which assume that the relative hazards of stroke and death are independent, we also calculated HRs using Fine and Grey competing risk regression models to account for the competing risks of other stroke types and mortality.[19] Lastly, HRs were recalculated restricting the study population to individuals without a history of APS at start of follow-up and censored at APS diagnosis during follow-up to determine whether our results were driven by APS. Because this ICD code has not been validated, we also re-ran analyses restricted to data from the Stockholm clinical cohort excluding those with APS recorded in medical records.

## RESULTS

The study population included 3390 individuals with SLE and 16 730 general population comparators. Eighty-five per cent of the study population was female and the mean age at start of follow-up was 49 years (table 1). A higher proportion of individuals with SLE had a history of hypertension, atrial fibrillation, diabetes and congestive heart disease compared with the general population (table 1). During the study period, 126 incident strokes occurred in SLE, 304 strokes occurred in non-SLE and approximately three-fourths of strokes were ischaemic in both SLE and non-SLE. When excluding subarachnoid haemorrhage and unspecified stroke, 87% of strokes were ischaemic, which is similar to the national average.[20] Mean age at stroke was younger in SLE compared with non-SLE (68.4 vs 73.3; table 2). Fourteen per cent of strokes in SLE occurred before the age of 50 compared with 4% in the general population. A higher proportion of individuals with stroke were female in SLE compared with the general population (79% vs 68%).

**Table 1** Characteristics of the systemic lupus erythematosus and general population groups at start of follow-up

| | General population | Systemic lupus |
|---|---|---|
| n | 16 730 | 3390 |
| Female, % | 85.0 | 85.1 |
| Age at start of follow-up, mean (SD) | 48.9 (17.3) | 49.5 (17.6) |
| Person-years, mean (SD) | 5.1 (3.1) | 4.8 (3.1) |
| Country of birth, % | | |
| Nordic | 89.4 | 85.0 |
| Non-Nordic Europe | 2.6 | 2.5 |
| Non-Europe | 7.9 | 12.5 |
| Education level, % | | |
| <9 years | 19.4 | 22.8 |
| 10–12 years | 40.0 | 38.4 |
| >12 years | 29.7 | 27.0 |
| Missing | 10.9 | 11.9 |
| Comorbidities, % | | |
| Atrial fibrillation | 2.0 | 3.8 |
| Congestive heart disease | 1.2 | 4.0 |
| Hypertension | 6.4 | 15.7 |
| Diabetes | 2.9 | 4.4 |

### Association between SLE and stroke

The IR of any stroke was 7.7 per 1000 py (95% CI 6.5 to 9.2) in SLE and 3.5 per 1000 py (95% CI 3.2 to 4.0) in their age and sex-matched general population comparators. The age and sex-adjusted rate difference was 4.4 strokes per 1000 py (95% CI 3.0 to 5.9). Individuals with SLE had a twofold increased hazard of ischaemic stroke compared with the general population (HR 2.2; 95% CI 1.8 to 2.8; table 3). The test of non-proportionality was statistically significant for analyses of ischaemic stroke; therefore, the HR stratified by follow-up time may better represent the association between SLE and ischaemic stroke. The HRs for intracerebral and subarachnoid haemorrhage were HR 1.4 (95% CI 0.7 to 2.9) and HR 1.4 (95% CI 0.5 to 3.9), respectively. The risk of unspecified stroke was higher in the SLE population than that in the general population (HR 2.7 95% CI 1.3 to 5.6).

### Association between SLE and stroke according to age, sex and time since diagnosis

Stratified analyses were only conducted for the ischaemic stroke and intracerebral haemorrhage outcomes due to limited power for the other types of stroke. There was statistically significant effect modification by sex and age, with the highest HRs for females and individuals <50 years old. Although the relative risk was highest for the youngest age groups, the absolute rate was highest among the oldest (for those aged 60 or older, nine additional ischaemic strokes per 1000 years occurred in the SLE group compared with the non-SLE group). The HR for ischaemic stroke was highest in the first year of follow-up and effect modification by time was observed (table 4).

For intracerebral haemorrhage, there were fewer than five events in males with SLE; therefore, estimates were not calculated for this stratum. Age and follow-up time categories were collapsed due to small numbers. The highest HR was in individuals<60 years old, based on only five events in the SLE group and eight in the non-SLE group. There was no effect modification by time since start of follow-up but there was significant modification by age with the highest HR occurring in the youngest age group.

**Table 2** Characteristics of individuals with systemic lupus erythematosus and their general population comparators who were diagnosed with incident stroke 2003–2013

| | General population | Systemic lupus |
|---|---|---|
| N | 304 | 126 |
| Female, % | 68.1 | 78.6 |
| Age at stroke diagnosis, mean (SD) | 73.3 (12.0) | 68.4 (15.6) |
| Minimum, maximum age at stroke | 32, 96 | 20, 93 |
| Type of stroke, n (%) | | |
| Ischaemic stroke | 228 (75.0) | 99 (78.6) |
| Intracerebral haemorrhage | 37 (12.2) | 11 (8.7) |
| Subarachnoid haemorrhage | 17 (5.6) | 5 (4.0) |
| Unspecified | 22 (7.2) | 11 (8.7) |
| Deceased within 3 months of stroke diagnosis, n (%) | 41 (13.5) | 24 (19.0) |

### Sensitivity analyses

Analyses restricted to clinically confirmed SLE from the Linköping and Stockholm cohorts resulted in similar HR estimates for all stroke and ischaemic stroke (see online Supplementary table S2). Numbers were too small to estimate HRs for other types of stroke in this subset. After excluding individuals with an APS diagnosis code at start of follow-up and censoring on date of APS diagnosis code if it was received during follow-up, the results were similar. Using the higher quality APS diagnosis from the Stockholm cohort to exclude individuals with APS in this subset, the results were also similar to the main analyses (see online Supplementary table S2). HRs from competing risks models were similar to those from cause-specific models, although slightly lower (see online Supplementary table S3).

## DISCUSSION

Individuals with SLE have twice the risk of ischaemic stroke compared with the general population and the relative risk differed by age and sex, with a higher relative risk in females and individuals younger than 50 years of age. The relative risk of ischaemic stroke in SLE was increased within the first year after diagnosis and remained relatively constant over up to 11 years of follow-up. This demonstrates that early in the disease course, patients with SLE are already at increased risk of stroke and the first encounter with patients represents an opportunity for rheumatologists to intervene. The relative risk of intracerebral haemorrhage was also higher at a younger age in the SLE group compared with non-SLE. The more time since SLE diagnosis, the higher the relative risk of intracerebral haemorrhage. This may be due to a cumulative effect of inflammation or medications used more often in SLE such as anticoagulants.

Although young individuals with SLE were at a higher relative risk of stroke, stroke before the age of 60 was not common and the rate of stroke in SLE was low. In fact, the highest rate of stroke was observed in older patients with SLE (≥60 years old at incident SLE; 17 ischaemic strokes per 1000), indicating that prevention of stroke in this vulnerable group would make an important impact on this patient population.

There are several factors which likely contribute to the increased stroke risk associated with SLE. There exists a large inflammatory burden in SLE which causes endothelial activation leading to the development of atherosclerosis and thrombus formation.[7] In this setting, an immunological challenge such as an infection or a lupus flare may result in stroke.[21] Risk factors that predict stroke in the

**Table 3** Number of strokes, person-years, incidence rates and HRs for incident stroke (all strokes, ischaemic stroke, intracerebral haemorrhage, subarachnoid haemorrhage and unspecified) comparing SLE with the general population 2003–2013 in Sweden

| | General population | | SLE | | Age and sex-adjusted rate difference (95% CI) | Model 1 HR (95% CI) | Model 2 HR (95% CI) |
|---|---|---|---|---|---|---|---|
| | Strokes, n | Incidence rate* (95% CI) | Strokes, n | Incidence rate* (95% CI) | | | |
| Any stroke | 304 | 3.5 (3.2 to 4.0) | 126 | 7.7 (6.5 to 9.2) | 4.4 (3.0 to 5.9) | 2.3 (1.8 to 2.8) | 2.1 (1.7 to 2.6) |
| Ischaemic stroke | 228 | 2.7 (2.3 to 3.0) | 99 | 6.0 (5.0 to 7.4) | 3.6 (2.3 to 4.9) | 2.4 (1.9 to 3.0) | 2.2 (1.8 to 2.8) |
| Intracerebral haemorrhage | 37 | 0.4 (0.3 to 0.6) | 11 | 0.7 (0.4 to 1.2) | 0.3 (0.2 to 0.7) | 1.6 (0.8 to 3.2) | 1.4 (0.7 to 2.9) |
| Subarachnoid haemorrhage | 17 | 0.2 (0.1 to 0.3) | 5 | 0.3 (0.1 to 0.7) | 0.1 (–0.2 to 0.4) | 1.6 (0.6 to 4.3) | 1.4 (0.5 to 3.9) |
| Unspecified | 22 | 0.3 (0.2 to 0.4) | 11 | 0.7 (0.4 to 1.2) | 0.5 (0.0 to 0.9) | 2.7 (1.3 to 5.5) | 2.7 (1.3 to 5.6) |

*Incidence rate per 1000 person-years, CI estimated based on the Poisson distribution.
No. of person-years in general population: 85 944. No. of person-years in SLE population: 16 386.
Model 1 Cox regression model adjusted for age and sex. Model 2 adjusted for age, sex, education, history of hypertension, diabetes, congestive heart disease and atrial fibrillation.
SLE, systemic lupus erythematosus.

general population do not appear to accurately predict stroke in SLE.[22 23] This suggests that an SLE-specific risk score, with evidence-based guidelines for stroke prevention, is necessary to decrease the excess risk we observe in these patients. This should be studied in a large cohort of individuals with SLE with prospective follow-up and time-varying measurements of risk factors easily obtained and/or available in most clinics.

Our findings are similar to previous studies of overall stroke and ischaemic stroke, which report between a twofold to threefold increased risk associated with SLE. However, our estimated relative risk is lower for intracerebral haemorrhage and subarachnoid haemorrhage compared with some previous studies.[9–11] One explanation for this difference is that we identified individuals with SLE from both inpatient and outpatient care, whereas previous studies on haemorrhagic stroke have identified SLE solely from hospitalisation data. Previous studies may have included SLE cases with a greater disease severity and/or more comorbidities compared with those seen in outpatient care. If we were to restrict our SLE population to those with at least one hospitalisation for SLE, the HR for haemorrhagic stroke would increase from 1.4 to 2.5 and reach a similar magnitude as previous reports.

Some limitations of our study should be mentioned. The date of second SLE-coded visit was used as date of incident disease, which is likely misclassified if it takes some time to be diagnosed with SLE. When comparing date of diagnosis in the cases included in the Stockholm and Linköping cohorts with first date registered in the NPR, over 90% of individuals were registered within two years of first clinical cohort diagnosis. In our study, the median time between first and second SLE diagnosis (when criteria for

**Table 4** Number of strokes, person-years, incidence rates, rate differences and HRs for incident ischaemic stroke and intracerebral haemorrhage stratified by sex, age and time since diagnosis 2003–2013 in Sweden

| | General population | | SLE | | Age and sex-adjusted rate difference (95% CI) | Model 1 HR (95% CI) | Model 2 HR (95% CI) |
|---|---|---|---|---|---|---|---|
| | Strokes/person-years, n | Incidence rate* (95% CI) | Strokes/person-years, n | Incidence rate* (95% CI) | | | |
| Ischaemic stroke | | | | | | | |
| Males | 79/12 833 | 6.2 (4.9 to 7.7) | 22/2366 | 9.3 (6.1 to 14.1) | 2.9 (–1.1 to 7.0) | 1.5 (0.9 to 2.4) | 1.4 (0.9 to 2.2) |
| Females | 149/73 111 | 2.0 (1.7 to 2.4) | 77/14 021 | 5.5 (4.4 to 6.9) | 3.7 (2.4 to 5.0) | 2.8 (2.1 to 3.7) | 2.8 (2.1 to 3.7) |
| <50 years | 14/46 863 | 0.3 (0.2 to 0.5) | 14/9189 | 1.5 (0.9 to 2.6) | 1.2 (0.4 to 2.1) | 5.1 (2.5 to 10.8) | 4.6 (2.1 to 9.9) |
| 50 to <60 years | 36/16 020 | 2.2 (1.6 to 3.1) | 14/3016 | 4.6 (2.7 to 7.8) | 2.4 (–0.2 to 4.9) | 2.1 (1.1 to 3.9) | 1.7 (0.9 to 3.1) |
| ≥60 years | 178/23 061 | 7.7 (6.7 to 8.9) | 71/4181 | 17.0 (13.5 to 21.4) | 9.2 (5.1 to 13.3) | 2.2 (1.7 to 2.9) | 2.1 (1.6 to 2.8) |
| 0 to <1 years | 27/15 920 | 1.7 (1.2 to 2.5) | 22/3173 | 6.9 (4.6 to 10.5) | 1.1 (0.5 to 1.7) | 3.9 (2.2 to 6.8) | 3.4 (1.9 to 6.1) |
| 1 to <5 years | 125/46 780 | 2.7 (2.2 to 3.2) | 49/8980 | 5.5 (4.1 to 7.2) | 1.7 (0.8 to 2.6) | 2.1 (1.5 to 2.9) | 2.0 (1.4 to 2.8) |
| 5–11 years | 76/23 244 | 3.3 (2.6 to 4.1) | 28/4232 | 6.6 (4.6 to 9.6) | 1.4 (0.4 to 2.3) | 2.3 (1.5 to 3.6) | 2.2 (1.4 to 3.4) |
| Intracerebral haemorrhage | | | | | | | |
| Males | 9/12 833 | 0.7 (0.4 to 1.3) | NA | NA | NA | NA | NA |
| Females | 28/73 111 | 0.4 (0.3 to 0.6) | 10/14 020 | 0.7 (0.3 to 1.3) | 0.4 (–0.1 to 0.8) | 2.0 (1.0 to 4.0) | 1.8 (0.9 to 3.7) |
| <60 years | 8/62 883 | 0.1 (0.1 to 0.3) | 5/12 204 | 0.4 (0.2 to 1.0) | 0.3 (–0.1 to 0.7) | 3.3 (1.1 to 10.0) | 3.2 (1.1 to 9.9) |
| ≥60 years | 29/23 061 | 1.3 (0.9 to 1.8) | 6/4181 | 1.4 (0.6 to 3.2) | 0.2 (–1.1 to 1.4) | 1.2 (0.5 to 2.8) | 1.0 (0.4 to 2.4) |
| <5 years | 24/62 700 | 0.4 (0.3 to 0.6) | 6/12 154 | 0.5 (0.2 to 1.1) | 0.1 (–0.2 to 0.4) | 1.3 (0.5 to 3.2) | 1.1 (0.5 to 2.8) |
| 5–11 years | 13/23 244 | 0.6 (0.3 to 1.0) | 5/4232 | 1.2 (0.5 to 2.8) | 0.2 (–0.2 to 0.6) | 2.3 (0.8 to 6.3) | 2.2 (0.8 to 6.2) |

NA marks cell counts <5, corresponding incidence rates and HRs were therefore not estimated.
Incidence rates per 1000 person-years. CIs calculated using the Poisson distribution.
Model 1 Cox regression model adjusted for age and sex. Model 2 adjusted for age, sex, education, history of hypertension, diabetes, congestive heart disease diagnoses and atrial fibrillation.

inclusion were fulfilled) was 3 months. We do not have data on first encounter in primary care. If a stroke occurred soon after first SLE diagnosis before a second SLE diagnosis in non-primary care was given, these individuals were excluded. This minimises SLE misclassification by requiring specialist visits with SLE, but we may have missed early strokes, which would lead to an underestimation of the risk in the first year after diagnosis.

Some strokes could have been misclassified, especially in the SLE group if a neurological manifestation is diagnosed at first as a stroke. The Patient Register and the Cause of Death Register may have some misclassification of stroke subtype, and the accuracy of subarachnoid haemorrhage codes has not been investigated. We minimised misclassification of stroke through the use of the Riksstroke register, which has high quality data on acute stroke.[15]

We could not take into account smoking or obesity in this study, though it is unlikely that these would entirely explain our findings. How medications and disease activity play a role in stroke occurrence was not addressed, but examining these factors as chronic exposures or triggers is an important next step which requires a different study design. Lastly, we attempted to exclude individuals with a diagnosis of APS but we acknowledge that missing data on APS is likely and the ICD-10 code for APS has not been validated. To address this, we also performed a sensitivity analysis using medical record-confirmed APS diagnoses in the Stockholm Clinical Cohort data and observed no difference in the relative risk for stroke associated with SLE.

The current study benefits from the inclusion of high-quality inpatient and outpatient data as well as stroke information from acute care clinics registered in Riksstroke. Because these registers have nationwide coverage and allowed for follow-up of a relatively large population of individuals diagnosed with SLE for up to 11 years, it was possible to investigate the risk of stroke associated with SLE stratified by patient characteristics. The large sample size also allowed for the examination of stroke subtypes, which have previously been ignored or treated as a composite outcome despite their aetiological differences. Our results are further strengthened by testing our findings in a subgroup of clinically confirmed SLE cases demonstrating the robustness of our register-based estimates. We do not have information on race, but previous studies have shown that more than 90% of patients with SLE treated in Swedish university clinics are Caucasian.[24] Our results are generalisable to other Caucasian SLE populations seen in outpatient care in the last decade.

In conclusion, younger individuals and women with SLE have an especially higher relative risk than the general population, although their absolute risk remains low. The increased relative risk in the first year after diagnosis highlights a time period where preventative measures could be taken. It is recommended to screen for traditional risk factors at SLE diagnosis and at least annually thereafter,[25] but which of the SLE-specific factors are most important should be assessed in future work. Furthermore, what actions should be taken to *modify* stroke risk should be clarified so that the burden of stroke can be reduced in this vulnerable population.

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**Contributors** EVA and JFS designed the study. EVA conducted the analyses and wrote the manuscript. All authors contributed to interpreting the results and revising the manuscript. All authors approved of the final version before submission.

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**Competing interests** None declared.

**Patient consent** No identifiable data was collected on patients and all information was anonymised.

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OPEN ACCESS

EXTENDED REPORT

# Efficacy and safety of abatacept, a T-cell modulator, in a randomised, double-blind, placebo-controlled, phase III study in psoriatic arthritis

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## ABSTRACT

**Objectives** To assess the efficacy and safety of abatacept, a selective T-cell costimulation modulator, in a phase III study in psoriatic arthritis (PsA).

**Methods** This study randomised patients (1:1) with active PsA (~60% with prior exposure to a tumour necrosis factor inhibitor) to blinded weekly subcutaneous abatacept 125 mg (n=213) or placebo (n=211) for 24 weeks, followed by open-label subcutaneous abatacept. Patients without ≥20% improvement in joint counts at week 16 were switched to open-label abatacept. The primary end point was the proportion of patients with ≥20% improvement in the American College of Rheumatology (ACR20) criteria at week 24.

**Results** Abatacept significantly increased ACR20 response versus placebo at week 24 (39.4% vs 22.3%; p<0.001). Although abatacept numerically increased Health Assessment Questionnaire–Disability Index response rates (reduction from baseline ≥0.35) at week 24, this was not statistically significant (31.0% vs 23.7%; p=0.097). The benefits of abatacept were seen in ACR20 responses regardless of tumour necrosis factor inhibitor exposure and in other musculoskeletal manifestations, but significance could not be attributed due to ranking below Health Assessment Questionnaire–Disability Index response in hierarchical testing. However, the benefit on psoriasis lesions was modest. Efficacy was maintained or improved up to week 52. Abatacept was well tolerated with no new safety signals.

**Conclusions** Abatacept treatment of PsA in this phase III study achieved its primary end point, ACR20 response, showed beneficial trends overall in musculoskeletal manifestations and was well tolerated. There was only a modest impact on psoriasis lesions.

**Trial registration number** ClinicalTrials.gov number, NCT01860976 (funded by Bristol-Myers Squibb).

## INTRODUCTION

Psoriatic arthritis (PsA) is an inflammatory arthritis that occurs in up to one-third of patients with psoriasis and is usually diagnosed years after the appearance of psoriatic skin disease.[1 2] Although current treatments for PsA benefit many patients, a substantial proportion do not achieve significant improvement in their disease.[3–5] Consequently, there remains an unmet need for effective and well-tolerated treatments.

PsA is associated with specific major histocompatibility complex class I molecules that are involved in antigen presentation to T cells, which are implicated in disease pathogenesis.[6] Abatacept, a selective T-cell costimulation modulator, is a soluble fusion protein comprising the extracellular domain of human cytotoxic T-lymphocyte-associated antigen-4 linked to the modified Fc (hinge, CH2 and CH3 domains) portion of human immunoglobulin G1.[7 8] By selectively modulating the CD28 costimulatory signal required for full T-cell activation, abatacept blocks the process that triggers the inflammatory cascade and, therefore, is a potential therapy for PsA with a distinct mechanism of action upstream of currently available agents.[4 7] Abatacept is an approved treatment for rheumatoid arthritis (RA) and juvenile idiopathic arthritis, with an established acceptable safety profile.[9–14]

Data have previously been reported from a phase II, dose-ranging study of abatacept in patients with active PsA and prior exposure to disease-modifying antirheumatic drugs (DMARDs), 37% of whom had previously received tumour necrosis factor inhibitor (TNFi). At 6 months, the dose of 10 mg/kg given intravenously every 4 weeks showed the greatest increase in the proportion of patients with ≥20% improvement in the American College of Rheumatology criteria (ACR20) versus placebo (48% vs 19%, respectively; p=0.006).[15] The proportion of patients achieving a Health Assessment Questionnaire–Disability Index (HAQ-DI) response (reduction from baseline score ≥0.3) at 6 months was also increased in the 10 mg/kg group versus placebo (45% vs 19%, respectively). In addition, trends towards improvements over placebo were seen in joint damage, based on MRI. Following these results, the phase III *A*ctive P*S*oria*T*ic Arthritis *RA*ndomiz*E*d Tri*A*l (ASTRAEA) Study was conducted to assess the efficacy and safety of abatacept in patients with active PsA, using a more convenient subcutaneous 125 mg weekly dose that has shown therapeutic equivalence to intravenous dosing with 10 mg/kg every 4 weeks in RA.[16]

## METHODS

### Study design and oversight

This ongoing phase III study (total study duration including long-term extension, 729 days) was initiated in June 2013 and conducted across 76 centres worldwide (ClinicalTrials.gov number, NCT01860976). Clinical and radiographic database locks were in August and October 2015 (24-week analysis) and in March and April 2016 (1-year analysis), respectively. Using a central interactive voice

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response system, patients were randomly assigned (1:1) in a double-blind manner to receive subcutaneous abatacept 125 mg weekly or matched placebo for 24 weeks. Randomisation was stratified globally (rather than at site level) by factors that were considered to potentially impact results, including current methotrexate use, prior TNFi use and whether plaque psoriasis involved ≥3% of body surface area (BSA). Within each stratum, permuted block randomisation was conducted with a block size of two. Patients who had not achieved ≥20% improvement in swollen and tender joint counts from baseline to week 16 were switched to open-label abatacept weekly (early escape (EE)) for 28 weeks (total study time for these patients, 44 weeks). At week 24, all remaining patients transitioned to the open-label period and received subcutaneous abatacept weekly for 28 weeks (total study time, 52 weeks). At the end of the open-label period, patients had the option of entering a 1-year, long-term extension.

The study was conducted in accordance with the Declaration of Helsinki, International Conference on Harmonisation Guidelines for Good Clinical Practice and local regulations. An Institutional Review Board or Independent Ethics Committee approved the protocol, consent form and any other written information provided to patients. Patients were evaluated by the investigators, and the data were collected and analysed by Bristol-Myers Squibb under the direction of the investigators.

### Patients

Enrolled patients were aged ≥18 years, met the classification criteria for PsA[17] and had active arthritis (≥3 swollen and ≥3 tender joints), active plaque psoriasis with ≥1 qualifying target lesion ≥2 cm in diameter and inadequate response or intolerance to ≥1 non-biologic DMARD. Concomitant treatment with methotrexate, leflunomide, sulfasalazine or hydroxychloroquine, non-steroidal anti-inflammatory drugs and oral corticosteroids (<10 mg/day) and use of low-potency topical corticosteroids in sensitive areas were permitted (as detailed in online section 1 in the supplementary appendix). To reflect a typical patient population in clinical practice and that of the phase II study,[15] both TNFi-naïve and TNFi-exposed patients were enrolled. All patients gave written informed consent prior to study entry.

### Assessments

Arthritis was assessed in 66 joints for swelling and 68 joints for tenderness by ACR response criteria for per cent improvement from baseline[18] and post hoc by the Disease Activity Score (DAS)28 (C-reactive protein (CRP)).[19] Enthesitis at six locations was evaluated using the Leeds Enthesitis Index (range 0–6)[20] and dactylitis by the number of tender and swollen digits with a circumference ≥10% greater than the contralateral digit according to the Leeds Dactylitis Index basic score.[21] Physical function was measured using the HAQ-DI (range 0–3).[22] Among patients with plaque psoriasis involving ≥3% BSA at baseline, skin lesions were assessed using the Psoriasis Area and Severity Index (PASI; range 0–72).[23] PsA disease activity was assessed using the minimal disease activity (MDA) criteria,[24] the modified Composite Psoriatic Disease Activity Index,[25] the Psoriatic Arthritis Disease Activity Score[26] and post hoc for the Disease Activity index for Psoriatic Arthritis (DAPSA).[27] Quality of life was evaluated using the Short Form-36 (SF-36)[28] and the Dermatology Life Quality Index.[29]

Plain radiographs of hands and feet were taken at baseline and at weeks 24 and 52 (or weeks 16, 24 and 44 for EE patients). Radiographs were scored independently by two central, trained assessors (and an adjudicator in predefined cases) with experience using the PsA-modified Sharp–van der Heijde (SHS) scoring method (total score 0–528).[30] Assessors were blinded to patient identity, treatment, clinical data and order of radiographs. Initially, baseline and week 24 (week 16 for EE) radiographs were scored; in a second round, all radiographs, including week 52 (week 44 for EE), were scored. For joint erosion, joint space narrowing and total score, and the proportion of non-progressors, the mean of the scores from two assessors was used. If one score was missing, then the available score was used. If required, an adjudicator reviewed the images, and the mean of the adjudicator's total score and the other total score that was closer to the adjudicator's score was used (>0=progressors, ≤0=non-progressors). Safety was evaluated throughout the study by monitoring of adverse events (AEs) and routine laboratory tests.

### Efficacy end points

The primary end point was the proportion of patients with ACR20 responses at week 24. Key secondary end points at week 24, in hierarchical order, were the proportions of patients with an HAQ-DI response (reduction from baseline, ≥0.35), an ACR20 response in the TNFi-naïve and TNFi-exposed subgroups and a radiographic non-progression (change from baseline score, ≤0) according to PsA-modified total SHS score. Other secondary end points at week 24 included the proportion of patients with ≥50% and ≥70% improvement in ACR criteria (ACR50 and ACR70, respectively), the proportion who achieved ≥50% improvement in PASI score from baseline (PASI 50) and the mean change from baseline in SF-36 physical and mental component summary scores. Prespecified exploratory end points and post hoc analyses are described in online section 2 in the supplementary appendix.

### Statistical analysis

A hierarchical testing procedure (ie, testing outcomes in a predefined order) was used for the primary and key secondary end points to ensure preservation of the overall type I error. All estimates used for the sample size determination were based on the results of the phase II study of abatacept in PsA,[15] except for non-progressors using PsA-modified total SHS score. A two-sided continuity corrected $\chi^2$ test at alpha=0.05 was used. To achieve ≥80% power for each of the hierarchical end points and PASI 50 responders, recruitment of 400 patients was required: 152 (38%) and 248 (62%) in the TNFi-naïve and TNFi-exposed subgroups, respectively (see online section 3 in the supplementary appendix).

All efficacy analyses (including those up to week 44 or 52) were conducted using the intent-to-treat (ITT) population, which comprised all randomised patients who received at least one dose of study medication. Comparisons between treatment arms were performed for the primary and key secondary end points, and PASI 50 responders at week 24, using a two-sided Cochran-Mantel-Haenszel $\chi^2$ test, stratified by current methotrexate use, prior TNFi use and plaque psoriasis involving ≥3% of BSA, at a 5% significance level for generating p values. The p values that did not control for overall type I error (nominal p values) were provided for end points that ranked lower in the statistical hierarchy than the first end point that was non-statistically significant at the 5% level, and for PASI 50 response, MDA and DAPSA score at week 24. For other end points, only 95% CIs of differences between abatacept and placebo arms were generated without obtaining p values. For binary responder analyses during the double-blind period, EE

patients at week 16 were imputed as non-responders at weeks 20 and 24 (for radiographic analysis, EE patients were imputed as progressors at week 24). Patients who discontinued treatment were imputed as non-responders/radiographic progressors at all subsequent visits. Continuous variables for the double-blind analysis were assessed using a longitudinal repeated-measures analysis, imputing EE patients as missing beyond week 16 (see online section 3 in the supplementary appendix). In addition, if there were still missing data (for EE patients between week 4 and week 16 and for non-EE patients between week 4 and week 24), patients were imputed as non-responders at the time point with missing data. However, if data were missing between two time points at which the patient had a response (eg, ACR20), then in such cases the response (eg, ACR20) was imputed at the time point with missing data.

Analyses up to week 44/52 used actual data at each time point for EE and non-EE patients. A non-responder imputation was done for all missing values regardless of escape status. As mentioned above, the denominators for all responder analyses up to week 44/52 were equal to all randomised and treated patients (ITT population). Most efficacy end points are reported only up to week 44, at which time EE patients had received 28 weeks and non-EE patients had received 20 weeks of open-label treatment. However, for analyses of enthesitis, dactylitis and radiographic data, week 44 data from EE patients were combined with week 52 data from non-EE patients, as these data were not collected at week 44 for non-EE patients. Continuous variables were analysed for this period using the longitudinal repeated-measures analysis model using the actual data including all patients in the ITT population. For SHS scores, adjusted mean change from baseline up to week 44/52 was calculated using the longitudinal repeated measures analysis model with the actual values for EE and non-EE patients (ITT population).

## RESULTS

### Patients

In total, 424 randomised patients received at least one dose of abatacept (n=213) or placebo (n=211). Patient characteristics at baseline are shown in table 1. The overall mean (SD) age was 50.4 (11.0) years, 55% were female and 60% reported current methotrexate use, with a mean (SD) dose of 17.1 (8.2) mg/week at baseline. Most patients (~60%) had previously received TNFi agents; of these, most (abatacept 60%, placebo 62%) had failed at least one TNFi due to inadequate efficacy. Overall, 69% of patients had psoriasis covering ≥3% of BSA. Numbers of non-biologic DMARDs used prior to study entry are described in online table 1 in the supplementary appendix. The baseline disease characteristics included mean (SD) disease duration of 8.5 (8.2) years; distal interphalangeal involvement in approximately half of the population (50.7%); presence of joint erosion on radiographs in 84% of patients, with a mean (SD) PsA-modified total SHS score of 18.8 (43.3); elevated serum CRP above upper limit of normal (3 mg/L) in 66% of patients, with a mean (SD) CRP of 14.1 (25.9) mg/L; and polyarticular disease in 98% of patients, with mean (SD) tender and swollen joint counts of 20.2 (13.3) and 11.6 (7.5), respectively.

Patient disposition is shown in figure 1. A total of 76 (35.7%) and 89 (42.2%) patients in the abatacept and placebo groups, respectively, were assigned to EE and switched to open-label abatacept at week 16. From the original abatacept and placebo arms, 197 (92.5%) and 185 (87.7%) patients, respectively, entered the open-label period.

### Musculoskeletal manifestations

#### Arthritis

Abatacept treatment resulted in a significantly higher proportion of patients achieving an ACR20 response at week 24 versus placebo (39.4% vs 22.3%; p<0.001; table 2; figure 2; online table 2 in the supplementary appendix). The mean change from baseline in each of the ACR core components was numerically greater for patients in the abatacept group than those in the placebo group at 24 weeks (see online table 3 in the supplementary appendix).

As the effect of abatacept on the first key secondary end point in the statistical hierarchy (HAQ-DI response rate) did not reach significance (see below), only nominal p values were generated for subsequent outcomes. Nominally higher ACR20 response rates with abatacept versus placebo were seen in both TNFi-naïve and TNFi-exposed subgroups at week 24 (table 2), with the largest treatment difference seen in TNFi-naïve patients. ACR20 responses at 24 weeks by number of prior TNFi received are shown in online table 4 in the supplementary appendix. Analysis (ITT population) up to week 44 showed that ACR20 responses were maintained for patients who continued abatacept and improved for those who switched from placebo to abatacept (placebo/abatacept) in the total population and in both TNFi-naïve and TNFi-exposed subgroups (table 2; figure 3; online table 5 in the supplementary appendix). Because the trial design allowed for early escape to open-label abatacept, improvement in the placebo-treated patients initiating active treatment would be expected starting at week 16. Similar trends were observed for ACR50 and ACR70 responses (table 2). In addition, patients with CRP elevated above the upper limit of normal at baseline showed the highest ACR20 responses at 24 weeks with abatacept treatment versus placebo (estimated differences (95% CI)): total population, 43.8% versus 23.7% (20.17 (9.32 to 31.02)); TNFi naïve, 50.0% versus 23.9% (26.09 (7.93 to 44.25)); TNFi exposed, 40.2% versus 23.5% (16.69 (3.21 to 30.17)).

The efficacy of abatacept in reducing arthritic manifestations was supported by the results of the post hoc analysis of greater improvement in DAS28 (CRP) from baseline to week 24 with abatacept versus placebo: adjusted mean change, –1.35 versus –0.94; adjusted difference (95% CI), –0.42 (–0.69 to –0.14). Continued improvement beyond week 24 in the ITT population was seen in adjusted mean changes from baseline in DAS28 (CRP) in both abatacept and placebo/abatacept groups, with changes from baseline to week 44 of –1.81 and –1.84, respectively (see online supplementary figure 1 and table 6 in the supplementary appendix).

#### Enthesitis and dactylitis

At week 24, complete resolution of enthesitis and dactylitis present at baseline was numerically more frequent with abatacept versus placebo. The proportions (95% CI) of patients with enthesitis resolution were 32.9% (25.1 to 40.6) versus 21.2% (14.2 to 28.2) and with dactylitis resolution were 44.3% (31.8 to 56.7) versus 34.0% (20.9 to 47.1), respectively. At week 44/52, an increased proportion of patients achieved complete resolution of baseline enthesitis (48.6% vs 43.9%) and dactylitis (68.9% vs 60.0%) in both the abatacept and placebo/abatacept groups, respectively.

#### Physical function

The proportion of patients with an HAQ-DI response (reduction from baseline score ≥0.35) at week 24 was numerically higher with abatacept versus placebo: 31.0% versus 23.7%;

**Table 1** Patient characteristics at baseline

| | Abatacept (n=213*) | Placebo (n=211*) |
|---|---|---|
| **Demographic characteristics** | | |
| Age, years | 51.0 (10.7) | 49.8 (11.3) |
| Sex, female, n (%) | 121 (56.8) | 112 (53.1) |
| Race, white, n (%) | 195 (91.5) | 198 (93.8) |
| Body mass index, kg/m$^2$ | 30.7 (6.3) | 31.3 (6.8) |
| Region, n (%) | | |
| South America | 95 (44.6) | 80 (37.9) |
| Europe | 53 (24.9) | 59 (28.0) |
| North America | 44 (20.7) | 40 (19.0) |
| Rest of World | 21 (9.9) | 32 (15.2) |
| **Disease characteristics** | | |
| PsA duration, years | 8.3 (8.1) | 8.8 (8.3) |
| TJC | 21.0 (13.4) | 19.3 (13.1) |
| SJC | 12.1 (7.8) | 11.1 (7.2) |
| DIP involvement,† n (%) | 114 (53.5) | 101 (47.9) |
| HAQ-DI | 1.3 (0.7) | 1.3 (0.7) |
| Patient Global Assessment of disease activity (VAS 0–100 mm) | 61.1 (23.5) | 62.6 (22.6) |
| Physician Global Assessment of disease activity (VAS 0–100 mm) | 53.9 (18.8) | 55.0 (19.6) |
| Patient Global Assessment of pain (VAS 0–100 mm) | 64.2 (23.5) | 64.4 (21.8) |
| CRP, mg/L | 14.0 (20.9) | 14.3 (30.3) |
| Elevated CRP (>ULN‡), n (%) | 146 (68.9) | 131 (62.7) |
| DAS28 (CRP) | 5.0 (1.1) | 4.9 (1.1) |
| PsA-modified total SHS | 20.0 (46.8) | 17.7 (39.6) |
| Psoriasis covering ≥3% BSA, n (%)§ | 146 (68.5) | 148 (70.1) |
| PASI score¶** | 7.4 (8.0) | 7.2 (7.8) |
| Enthesitis, n (%) | 140 (65.7) | 132 (62.6) |
| Dactylitis, n (%) | 61 (28.6) | 50 (23.7) |
| Anti-CCP positive (>10 U/mL), n (%) | 10 (5.1) | 2 (1.0) |
| **Medication use** | | |
| Prior TNFi, n (%) | 129 (60.6) | 130 (61.6) |
| 1 | 94 (44.1) | 92 (43.6) |
| 2 | 31 (14.6) | 36 (17.1) |
| ≥3 | 4 (1.9) | 2 (0.9) |
| Concomitant methotrexate, n (%) | 129 (60.6) | 127 (60.2) |
| Concomitant csDMARDs other than methotrexate, n (%) | 27 (12.7) | 25 (11.8) |
| Concomitant oral corticosteroids, n (%)** | 56 (26.3) | 51 (24.2) |

Data are presented as mean (SD) unless indicated otherwise.
*For the following assessments, patient numbers in the abatacept and placebo arms, respectively, were as follows: body mass index (212 and 210), HAQ-DI score (212 and 211), Patient Global Assessment of disease activity (211 and 210), Physician Global Assessment of disease activity (210 and 209), Patient Global Assessment of pain (213 and 210), elevated CRP (212 and 209), DAS28 (CRP) score (210 and 208), PsA-modified total SHS score (205 and 202), PASI score (145 and 148) and anti-CCP positive (196 and 198).
†One or more swollen or tender DIP joint.
‡ULN=3 mg/L.
§Of patients with psoriasis covering ≥3% of BSA in the abatacept and placebo arms, 55 and 51 were in the TNFi-naïve subgroup, and 91 and 97 were in the TNFi-exposed subgroup, respectively.
¶Measured only for patients with psoriasis covering ≥3% of BSA.
**Mean (SD) oral daily steroid dose at baseline (prednisone equivalent) abatacept, 6.8 (2.68); placebo, 6.3 (2.56).
BSA, body surface area; CCP, cyclic citrullinated peptide; CRP, C-reactive protein; csDMARD, conventional synthetic disease-modifying antirheumatic drug; DAS28 (CRP), Disease Activity Score 28 (C-reactive protein); DIP, distal interphalangeal; HAQ-DI, Health Assessment Questionnaire–Disability Index (range 0–3); PASI, Psoriasis Area and Severity Index (range 0–72); PsA, psoriatic arthritis; PsA-modified total SHS, psoriatic arthritis-modified total Sharp/van der Heijde score (range 0–528); SJC, swollen joint count (range 0–66); TJC, tender joint count (range 0–68); TNFi, tumour necrosis factor inhibitor; ULN, upper limit of normal; VAS, visual analogue scale.

estimated difference (95% CI), 7.2 (–1.1 to 15.6); p=0.097. However, as this did not reach statistical significance, only nominal p values were generated for lower-ranking end points in the hierarchical testing. HAQ-DI responses at 24 weeks in the abatacept versus placebo arms were 34.5% versus 19.8%, respectively, in the TNFi-naïve subgroup (estimated difference 14.8; 95% CI 1.7 to 28.0) and 28.7% versus 26.2%, respectively, in the TNFi-exposed subgroup (estimated difference 2.5; 95% CI –8.3 to 13.3). HAQ-DI responses were maintained to week 44 in the abatacept group and improved in the placebo/abatacept group (39.9% and 38.9%, respectively) in the ITT population.

Further analyses showed nominal improvements in adjusted mean change in HAQ-DI score from baseline to week 24 with abatacept versus placebo for all patients: –0.33 versus –0.20, respectively; estimated difference (95% CI), –0.13 (–0.25 to –0.01), and in both TNFi-naïve (–0.29 vs –0.17) and TNFi-exposed (–0.35 vs –0.18) subgroups. Continued improvements

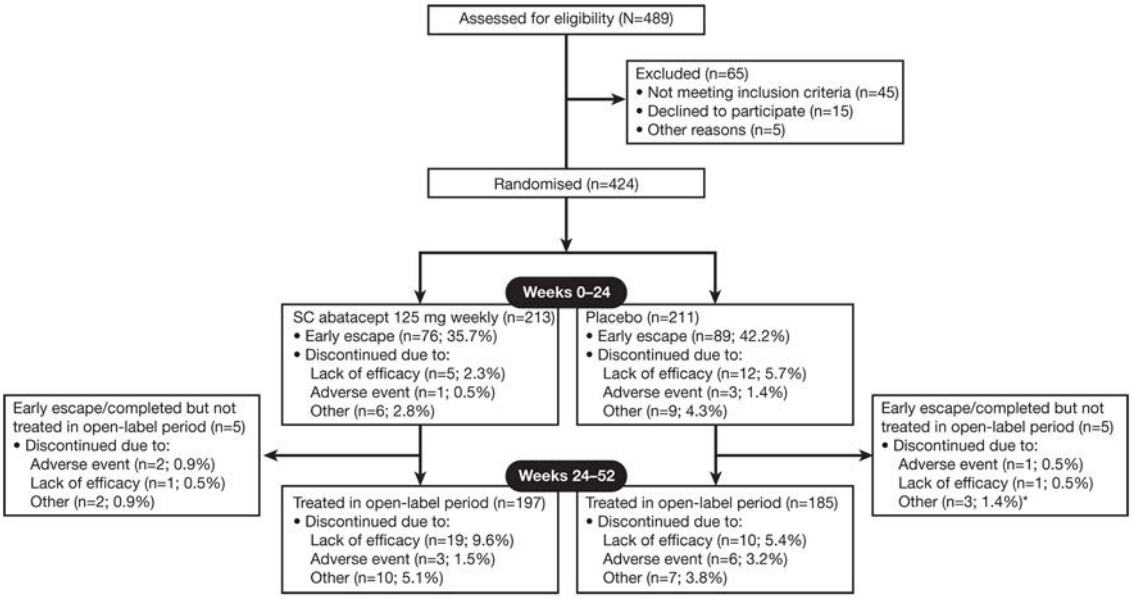


**Figure 1** Patient disposition. SC, subcutaneous. *Includes missing (n=2).

were seen in the total population and TNFi-naïve and TNFi-exposed subgroups at week 44 in the ITT analyses (see online supplementary figure 2 and table 7 in the supplementary appendix).

### Structural damage

The proportion of patients without radiographic progression at week 24 was 42.7% in the abatacept group versus 32.7% in the placebo group (estimated difference (95% CI), 10.0 (1.0 to 19.1); nominal p=0.034). The mean (SE) change from baseline in PsA-modified total SHS score was 0.30 (0.12) versus 0.35 (0.13) at week 24 for abatacept versus placebo and 0.18 (0.12) versus 0.30 (0.12) at week 44/52 for abatacept versus placebo/abatacept.

### Psoriatic skin responses

The psoriatic skin response was more modest compared with the musculoskeletal response. At week 24, there was a small numerical increase in the proportion of PASI 50 responders with abatacept compared with placebo: 26.7% versus 19.6% (estimated difference (95% CI), 7.3 (–2.2 to 16.7); nominal p=0.137). The proportion of patients with ≥75% improvement in PASI score from baseline (PASI 75 responders) with abatacept versus placebo at week 24 was 16.4% versus 10.1%, respectively.

The magnitude of improvement in both PASI 50 and PASI 75 response rates with abatacept versus placebo at week 24 was numerically greater in the TNFi-naïve compared with the TNFi-exposed subgroup: PASI 50 (TNF naïve, 32.7% vs 19.6%;

**Table 2** ACR20/50/70 responders in the total population and TNFi-naïve and TNFi-exposed subgroups (ITT population)

| | Week 24 | | | Week 44 | |
|---|---|---|---|---|---|
| | Abatacept | Placebo | Estimated difference (95% CI) | Abatacept/open-label abatacept | Placebo/open-label abatacept |
| **Total population** | n=213 | n=211 | | n=213 | n=211 |
| ACR20 | 39.4 | 22.3 | 17.2 (8.7 to 25.6)* | 48.4 | 49.3 |
| ACR50 | 19.2 | 12.3 | 6.9 (0.1 to 13.7) | 28.2 | 32.2 |
| ACR70 | 10.3 | 6.6 | 3.7 (–1.5 to 8.9) | 15.5 | 17.5 |
| **TNFi naïve** | n=84 | n=81 | | n=84 | n=81 |
| ACR20 | 44.0 | 22.2 | 21.9 (8.3 to 35.6)† | 54.8 | 56.8 |
| ACR50 | 25.0 | 14.8 | 10.2 (–1.5 to 22.0) | 35.7 | 38.3 |
| ACR70 | 11.9 | 8.6 | 3.3 (–5.8 to 12.4) | 14.3 | 23.5 |
| **TNFi exposed** | n=129 | n=130 | | n=129 | n=130 |
| ACR20 | 36.4 | 22.3 | 14.0 (3.3 to 24.8)‡ | 44.2 | 44.6 |
| ACR50 | 15.5 | 10.8 | 4.7 (–3.4 to 12.8) | 23.3 | 28.5 |
| ACR70 | 9.3 | 5.4 | 3.9 (–2.4 to 10.2) | 16.3 | 13.8 |

Data are presented as percentages of patients.
Early escape patients were imputed as non-responders in the week 24 analysis. Estimated differences between original treatment arms were not calculated in the week 44 analysis.
*p<0.001 versus placebo.
†Nominal p=0.003 versus placebo.
‡Nominal p=0.012 versus placebo.
ACR20, ≥20% improvement in American College of Rheumatology criteria; ACR50, ≥50% improvement in American College of Rheumatology criteria; ACR70, ≥70% improvement in American College of Rheumatology criteria; ITT, intent-to-treat; TNFi, tumour necrosis factor inhibitor.

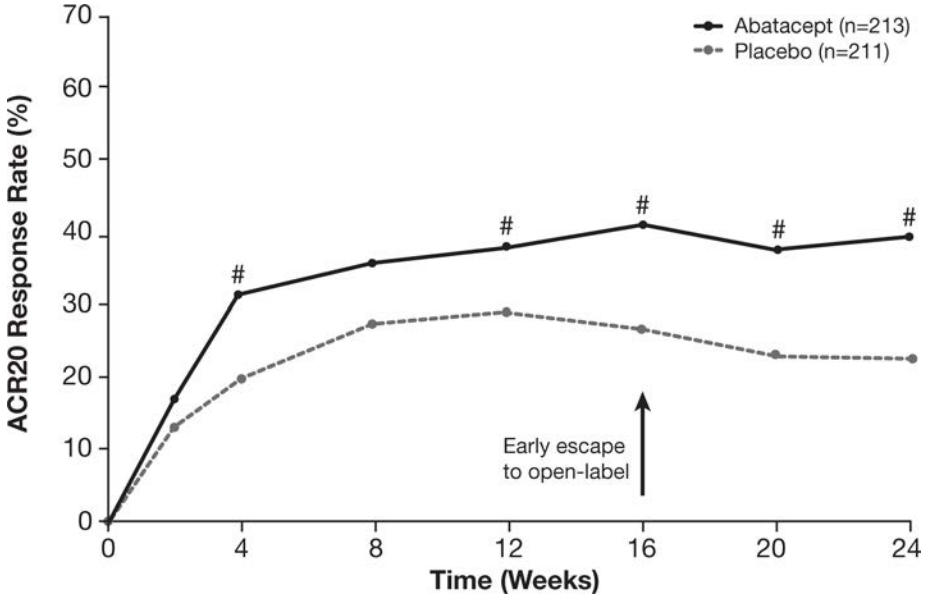


**Figure 2** ACR20 response over the 24-week double-blind period (non-responder imputation for early escape). Early escape patients switching to open-label abatacept at week 16 were imputed as non-responders at weeks 20 and 24. If there were still missing data, patients were imputed as non-responders, unless data were missing between two time points at which the patient had a response, in which case response was imputed. #Where 95% CI of estimate of differences in ACR20 responses for abatacept versus placebo do not contain zero. ACR20, ≥20% improvement in the American College of Rheumatology criteria.

TNFi exposed, 23.1% vs 19.6%) and PASI 75 (TNFi naïve, 18.2% vs 9.8%; TNFi exposed, 16.5% vs 10.3%).

In the ITT population at week 44, PASI 50 response rates were maintained for patients who continued on abatacept (total population, 30.1%; TNFi naïve, 36.4%; TNFi exposed, 26.4%) and improved for those who switched from placebo to abatacept (total population, 34.5%; TNFi naïve, 39.2%; TNFi exposed, 32.0%). PASI 75 responses were also maintained for patients who continued on abatacept (total population, 19.9%; TNFi naïve, 27.3%; TNFi exposed, 15.4%) and improved for the placebo/abatacept group (total population, 16.9%; TNFi naïve, 17.6%; TNFi exposed, 16.5%).

### Disease activity—composite measures

The proportion of patients with MDA at week 24 was numerically higher with abatacept versus placebo in the total population (11.7% vs 8.1%; nominal p=0.205). At week 52, the proportion of patients with MDA increased to 17.4% for patients who continued on abatacept and 18.5% for the placebo/abatacept group. Similar trends were observed in the modified Composite Psoriatic Disease Activity Index and Psoriatic Arthritis Disease Activity Score (see online section 4 in the supplementary appendix). There was a nominally significant improvement with abatacept versus placebo in adjusted mean change from baseline to week 24 in DAPSA score (–18.75 vs –13.00; adjusted difference –5.75; 95% CI –10.01 to –1.49; nominal p=0.008). At week 44, further improvements in adjusted mean change from baseline in DAPSA score were observed in the abatacept and placebo/abatacept groups (–24.58 and –25.18, respectively).

### Quality of life

At 24 weeks, mean improvements from baseline were numerically greater with abatacept versus placebo for SF-36 physical component summary and Dermatology Life Quality Index scores but were similar between the two groups for SF-36 mental component summary scores. Similar results were also seen at week 52 (see online table 8 in the supplementary appendix).

### Safety

Safety findings during the 24-week, double-blind period and for cumulative abatacept treatment over the 52-week study period are summarised in table 3. During the 24-week, double-blind period, the abatacept and placebo groups had similar safety profiles, with comparable incidences, respectively, of serious AEs (2.8% vs 4.3%), AEs (54.5% vs 53.1%) and infections (26.8% vs 29.9%). One serious infection (*Pneumocystis jirovecii*) was considered related to study drug by the investigator and led to treatment discontinuation. This event occurred during the double-blind period in a patient receiving abatacept who had a history of smoking and chronic obstructive pulmonary disease and had recently used high-dose corticosteroids. The episode resolved after 7 days of appropriate treatment.

## DISCUSSION

In this phase III study, selective modulation of T-cell costimulation with abatacept resulted in significantly higher ACR20 response rates in patients with PsA compared with placebo, with responses maintained to at least 1 year. Our findings support previous data suggesting a role for T cells in PsA: activated T cells are abundant in the synovial fluid of patients with PsA[31 32] and frequencies of interleukin (IL)-17-secreting CD8+ T cells are increased in erosive disease.[32] Furthermore, treatment with abatacept has been shown to reduce circulating IL-17-secreting CD4+ and CD8+ T cells in RA.[33] The data reported here suggest that selective inhibition of the CD28-dependent costimulatory pathway critical for T-cell activation[7] may offer a novel treatment option in PsA.

Our findings demonstrate that abatacept had an overall beneficial effect on musculoskeletal symptoms and was well tolerated in a relatively refractory population of patients with PsA (approximately 60% had received prior TNFi), confirming earlier results from a phase II, dose-ranging study in a less refractory population (approximately 30% of patients in the abatacept 10 mg/kg and placebo groups had received prior TNFi).[15] The primary end point was met, with a statistically significantly

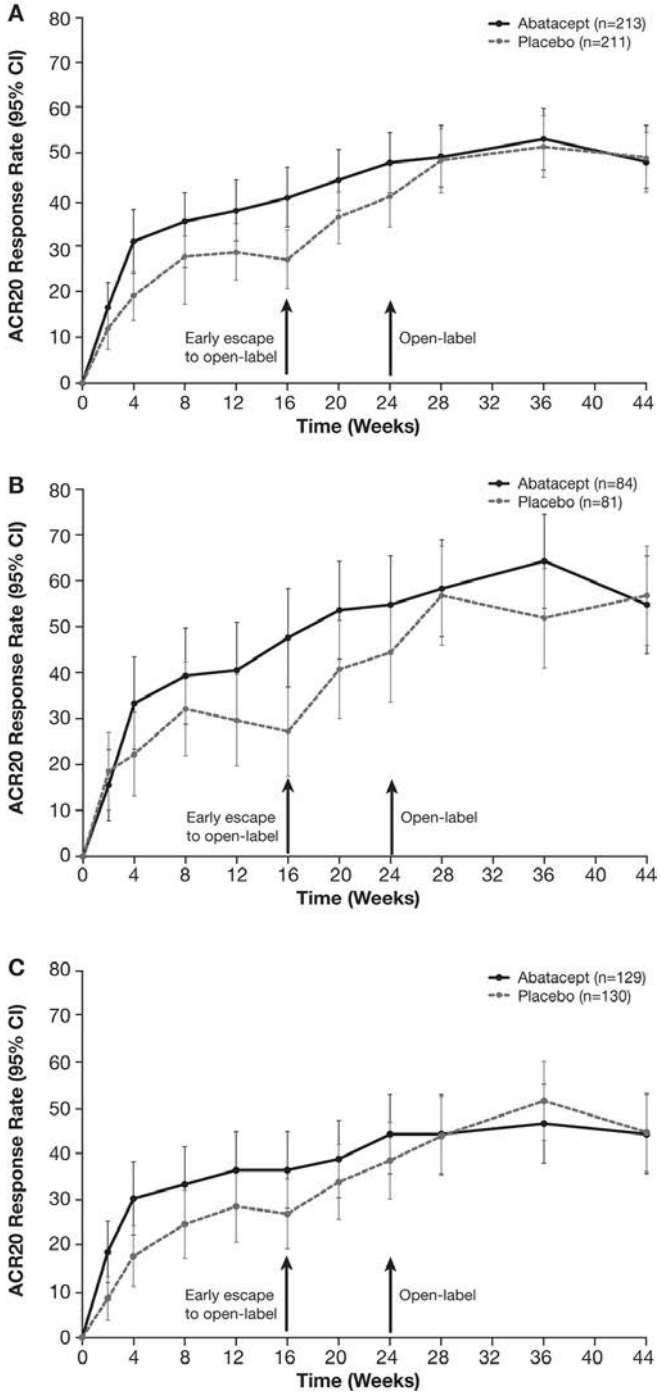


**Figure 3** Proportion of patients achieving ACR20 response (ITT analysis, actual data for early escape patients) over the combined double-blind and open-label periods in the total population (A) and the TNFi-naïve (B) and TNFi-exposed (C) subgroups. Error bars represent 95% CIs. For EE patients, measurements at weeks 20, 24, 28, 36 and 44 are actual measurements at weeks 4, 8, 12, 20 and 28 of open-label abatacept treatment. The increase in the proportion of patients with ACR20 response from week 16 to week 24 in the placebo group reflects the mixed population of EE patients who received abatacept between weeks 16 and 24 and non-EE patients who received placebo at week 24. If there were missing data, patients were imputed as non-responders, unless data were missing between two time points at which the patient had a response, in which case response was imputed. ACR20, ≥20% improvement in the American College of Rheumatology criteria; EE, early escape; ITT, intent to treat; TNFi, tumour necrosis factor inhibitor.

**Table 3** Summary of safety

| | Double-blind period* | | Week 52† |
|---|---|---|---|
| | Abatacept (n=213) | Placebo (n=211) | Cumulative abatacept population (n=398) |
| Deaths | 0 | 0 | 0 |
| SAEs | 6 (2.8) | 9 (4.3) | 34 (8.5) |
| Treatment related | 1 (0.5)‡ | 1 (0.5) | 5 (1.3)§ |
| Leading to discontinuation | 3 (1.4) | 3 (1.4) | 8 (2.0)¶ |
| AEs | 116 (54.5) | 112 (53.1) | 273 (68.6) |
| Treatment related | 33 (15.5) | 24 (11.4) | 81 (20.4) |
| Leading to discontinuation | 3 (1.4) | 4 (1.9) | 13 (3.3) |
| AEs reported in ≥5% of patients | | | |
| Nasopharyngitis | 9 (4.2) | 11 (5.2) | 25 (6.3) |
| Upper RTI | 6 (2.8) | 14 (6.6) | 28 (7.0) |
| Bronchitis | 7 (3.3) | 5 (2.4) | 26 (6.5) |
| AEs of special interest | | | |
| Infections | 57 (26.8) | 63 (29.9) | 162 (40.7) |
| Malignancies | 0 | 2 (0.9) | 4 (1.0) |
| Autoimmune events | 0 | 0 | 1 (0.3) |
| Local ISRs | 1 (0.5) | 1 (0.5) | 5 (1.3) |

Data are presented as n (%) of patients.
Investigators were instructed not to report psoriasis or psoriatic arthritis as AEs unless they were new forms of psoriasis or SAEs.
*Includes data up to 56 days after the last dose in the double-blind period or the first dose in the open-label period, whichever occurred first.
†Includes data from the first day of the double-blind period for patients in the abatacept group and from the first day of the open-label period for patients treated initially with placebo up to 56 days after the last abatacept dose up to week 52.
‡*Pneumocystis jirovecii* infection (see text).
§Pyelonephritis (n=1), dyspnoea (n=1), erythrodermic psoriasis (n=1), transitional cell carcinoma (n=1), plus the event of *P. jirovecii* infection in the double-blind period. The event of erythrodermic psoriasis occurred following treatment with topical corticosteroids and intramuscular dexamethasone in a female patient with severe plaque psoriasis at baseline (PASI score=27.6); the patient had discontinued earlier from the study due to lack of efficacy.
¶Gastroenteritis (n=1), *P. jirovecii* infection (n=1), prostate cancer (n=1), transitional cell carcinoma (n=1), uterine leiomyoma (n=1), colitis (n=1), biliary dilatation plus an AE of upper abdominal pain (n=1) and interstitial lung disease (n=1).
AE, adverse event; ISR, injection-site reaction; PASI, Psoriasis Area and Severity Index (range 0–72); RTI, respiratory tract infection; SAE, serious adverse event.

higher ACR20 response at 24 weeks with abatacept treatment versus placebo. Although numerical improvements in individual ACR core components were observed with abatacept versus placebo at 24 weeks, the CIs were overlapping. Due to the lack of significant effect on HAQ-DI response rates in the total population, it was not possible to attribute significance to lower-ranking outcomes in the statistical hierarchy. The efficacy in joints was supported by mean improvements in DAS28 (CRP) with abatacept versus placebo. In addition, disease improvement was evident when placebo-treated patients switched to abatacept. Outcomes tended to be better in the TNFi-naïve versus TNFi-exposed subgroups.

Across end points up to week 52, responses were maintained or improved for patients who continued on abatacept, demonstrating the durability of effects and accrual of benefits over time on some measures. For patients who switched from placebo to open-label abatacept, it is possible that observed improvements could at least partially be explained by patient awareness of receiving active treatment, or a continuation of trends during receipt of placebo, rather than a true treatment effect. However, the similar ACR20 response rates at 44 weeks for patients who

started on abatacept and those who switched from placebo to abatacept indicate a treatment effect and benefit after switching.

Skin responses to abatacept were modest. A small treatment effect on skin manifestations has previously been observed with the T-cell inhibitor, alefacept, in a phase III study in psoriasis.[34] It is also possible that a higher dose of abatacept may be required for optimal efficacy in skin versus musculoskeletal symptoms, similar to previous findings with the TNFi etanercept.[35]

Caution is advised when comparing the current efficacy data with findings from studies of TNFi and other agents in PsA. The ACR20 response rate at week 24 in this study was lower than that in previous studies of agents that target some of the known effector molecules in PsA.[36–38] However, this study included a higher proportion of TNFi failures compared with most studies,[36 37] which may indicate a more treatment-refractory population, as noted previously.[39] Higher efficacy in the TNF-naive compared with the TNFi-exposed subgroup across multiple end points in the current study confirmed the treatment resistance in the latter subpopulation. In contrast to findings with other agents with different mechanisms of action,[36–38] abatacept treatment demonstrated better efficacy on musculoskeletal versus skin end points. The reasons for this are unclear but may include differential dose requirements for optimal efficacy of abatacept in skin versus the joints, for example, due to less efficient drug penetration of skin versus synovial tissue, and distinct pathologies with divergent roles of T cells and T-cell subsets in skin versus synovial inflammation in PsA. Regarding the latter, it is interesting to note that, in PsA, agents targeting the IL-23/IL-17 axis can achieve complete clearing of psoriatic skin lesions without a similar level of efficacy in the joints.[40] We speculate that T-cell subsets driving pathology in the skin and joints may differ in their expression of CD28 and, hence, susceptibility to abatacept.

In this study, subcutaneous abatacept was well tolerated with no new safety signals, consistent with the phase II study of intravenous abatacept in PsA[15] and previous studies of subcutaneous and intravenous abatacept in RA.[41] Throughout the study, one serious opportunistic infection was reported. This case of *P. jirovecii* infection occurred in the abatacept arm in a patient who had a history of smoking and chronic obstructive pulmonary disease and had recently received high doses of corticosteroids. It has been recognised that patients with chronic obstructive pulmonary disease have an increased prevalence of *Pneumocystis* colonisation, which may predispose them to acute infection.[42]

Limitations of this study include the prespecified imputation method used to measure radiographic progression. Imputation as radiographic progressors of patients who escaped early to open-label abatacept, based on poor clinical response at week 16, led to a relatively high imputed rate of structural progression at week 24 in both groups. This imputation method that was designed initially is inappropriate as it assumed that the structural radiographic data behaved similarly to clinical data and obscured underlying rates of radiographic change. Overall, there was minimal progression based on the mean change from baseline in PsA-modified total SHS score over 24 and 44/52 weeks in both groups, making it difficult to detect meaningful treatment differences. In this context, it should be noted that, in the phase II study in PsA, abatacept demonstrated greater inhibition of structural damage versus placebo as well as improvements in joint inflammation on MRI over the same timeframe (24 weeks).[15]

In summary, abatacept treatment achieved the primary end point in ACR20 response rates in patients with PsA, of whom ~60% had prior exposure to TNFi agents. There were trends towards benefits in other musculoskeletal measures, with maximal effects seen in the TNFi-naïve patients. However, only modest benefit was demonstrated for psoriatic skin lesions. Abatacept was well tolerated with no new safety signals.

**Contributors** PM, ABG and DG were involved in the conception and design of the study and interpretation of data. DvdH and MN were involved in the conception and design of the study, acquisition of data and interpretation of data. OF was involved in interpretation of data. SB was involved in the acquisition of data and the analysis and interpretation of data. AJ was involved in the conception and design of the study, acquisition of data and the analysis and interpretation of data. All authors had full access to the study data, critically reviewed the manuscript and approved the final version prior to submission and take responsibility for the integrity and accuracy of the reported data.

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EXTENDED REPORT

# Effect of methotrexate discontinuation on efficacy of seasonal influenza vaccination in patients with rheumatoid arthritis: a randomised clinical trial

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## ABSTRACT

**Objective** To investigate whether temporary discontinuation of methotrexate (MTX) improves the efficacy of seasonal influenza vaccination in patients with rheumatoid arthritis (RA).

**Methods** In this prospective randomised parallel-group trial, patients with RA taking stable dose of MTX were randomly assigned at a ratio of 1:1:1:1 to continue MTX (group 1), suspend MTX for 4 weeks before vaccination (group 2), suspend MTX for 2 weeks before and 2 weeks after vaccination (group 3) or suspend MTX for 4 weeks after vaccination (group 4). All participants were vaccinated with trivalent influenza vaccine containing H1N1, H3N2 and B-Yamagata. The primary outcome was frequency of satisfactory vaccine response (≥4-fold titre increase 4 weeks postvaccination). Secondary endpoints included fold change in antibody titres from baseline.

**Results** The per-protocol population consisted of 199 patients (n=54, 44, 49 and 52 in groups 1, 2, 3 and 4, respectively). Group 3 achieved higher satisfactory vaccine response against all three antigens than group 1 (51.0% vs 31.5%, p=0.044). The anti-H3N2 antibody fold increase (95% CI) was significantly higher in groups 3 and 4 (12.2 (8.4 to 17.5), p <0.001 and 10.0 (6.8 to 14.8), p=0.043, respectively) than group 1 (5.9 (4.3 to 8.1)). The anti-B-Yamagata antibody responses of groups 3 and 4 were higher (4.7 (3.3 to 6.7), p=0.048; 6.1 (4.2 to 8.8), p <0.001, respectively) than group 1 (2.9 (2.2 to 3.8)). RA flare occurred in 24.1%, 21.2%, 34.1% and 38.8% in groups 1, 2, 3 and 4, respectively (p=NS).

**Conclusions** Temporary MTX discontinuation improves the immunogenicity of seasonal influenza vaccination in patients with RA.

**Trial registration** Trial registration number is: www.clinicaltrials.gov, NCT02748785.

## INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease in which the main target of inflammation is the joints. Patients with RA require chronic treatment with disease-modifying anti-rheumatic drugs (DMARDs), which constitute the mainstay of treatment. Patients with RA are more susceptible to infections because of their underlying immune dysfunction and the treatment-induced immune suppression.[1 2] Consequently, they are recommended to receive vaccines against preventable diseases, including influenza.[3 4]

Methotrexate (MTX) is highly effective and is recommended in almost all patients with RA.[5 6] However, MTX significantly decreases the satisfactory response of patients with RA to pneumococcal and seasonal influenza vaccination.[7–10] Moreover, it has a particularly negative effect on the response to presumably novel antigens such as new pandemic H1N1 influenza antigen.[10] Consequently, patients with RA are recommended to be vaccinated before they are treated with MTX.[11] However, most patients with RA are already receiving MTX at the time when vaccination is required. Therefore, a novel strategy to improve the vaccine response of such patients with RA is needed.

The present clinical trial aimed to investigate whether discontinuing MTX for 4 weeks before, during or after seasonal influenza vaccination improves the immunogenicity of the vaccine in patients with RA who are being treated with a stable dose of MTX.

## METHODS

### Study

This was a prospective single-centre randomised single-blind parallel-group intervention study that aimed to investigate the effects of temporary MTX discontinuation on vaccine response to seasonal influenza vaccination in patients with RA. The study started in September 2015 and was completed in July 2016.

After obtaining informed consent, the patients were screened for eligibility according to the inclusion and exclusion criteria described below. The study was approved by the institutional review board of the Seoul National University Hospital (IRB 1508-050-694) and was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines.[12] The study was registered at www.clinicaltrials.gov (protocol number NCT02748785).

### Patients

Patients with RA who were aged 18 years or older and had been on the same dose of MTX for 6 weeks or longer were eligible for inclusion. RA was defined on the basis of revised 1987 American College of Rheumatology criteria.[13] The exclusion criteria were as follows: pregnant or lactating women, patients with a previous anaphylactic response to vaccine components or to egg, evidence of an acute infection with temperature >38°C at the time of vaccination, history of Guillain-Barré syndrome or demyelinating syndromes, and previous vaccination

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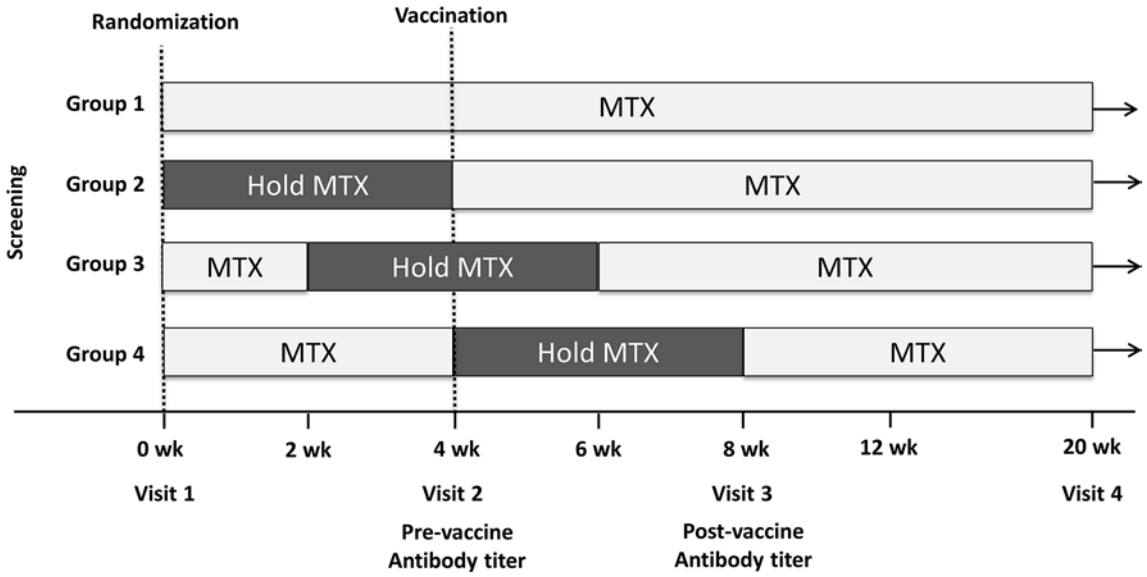


**Figure 1** Study design. MTX, methotrexate.

with any live vaccine 4 weeks before or any inactivated vaccine 2 weeks before start of the study. Patients with high disease activity that necessitated a recent change in their treatment regimen and patients with any other additional rheumatic disease except for secondary Sjogren's disease were also excluded.

### Randomisation/blinding

The eligible patients were randomly assigned to continue MTX (group 1), suspend MTX for 4 weeks before vaccination (group 2), suspend MTX for 2 weeks before and 2 weeks after vaccination (group 3), or suspend MTX for 4 weeks after vaccination (group 4) (figure 1). Patients were assigned to these treatment groups by a Central Interactive Web Response System at a 1:1:1:1 ratio according to the randomisation table. Information on the intervention was concealed from the investigators who enrolled and assessed the study patients. To measure the adherence to the study protocol, study participants were required to record their MTX administration in a diary.

### Intervention

The seasonal trivalent influenza vaccine (GC Flu, Green Cross, South Korea) contained 15 µg of A/California/72009 Reassortant virus NYMC X-181 (H1N1), 15 µg of A/Switzerland/9715293/2013 Reassortant virus NIB-88 (H3N2) and 15 µg of B/Phuket/3073/2013 (B-Yamagata). The vaccine was contained in a 0.5 mL prefilled syringe and was delivered as a single intramuscular injection in the deltoid muscle.

### Baseline, treatment and follow-up visits

There were four visits. Visit 1 (week 0) took place 4 weeks before vaccination. During visit 2 (week 4), the prevaccination sera were taken and all patients were vaccinated. Visit 3 (week 8) took place 4 weeks after vaccination, at which point the postvaccine antibody titres were measured. There was also a fourth visit 16 weeks after vaccination to assess disease activity (week 20) (figure 1).

### Concomitant medications

Adding or changing DMARDs were not allowed until the postvaccination antibody titre was obtained (ie, 4 weeks after the vaccination, visit 3). Medications for other comorbid conditions were continued. During MTX discontinuation, acetaminophen (650 mg up to three times per day)/nonsteroidal anti-inflammatory drugs (NSAIDs) (in standard dosing) and prednisolone (or its equivalent) up to 10 mg per day were allowed for RA flares.

### Efficacy and safety assessments

The primary outcome was the frequency of satisfactory vaccine response to influenza antigens 4 weeks after vaccination (ie, visit 3). A satisfactory vaccine response was defined as a ≥4-fold increase in haemaglutination inhibition (HI) antibody titre at visit 3 relative to the prevaccination HI antibody titre at visit 2. Secondary endpoints were (1) fold change in postvaccination HI antibody titres against each vaccine antigen at visit 3 relative to baseline, and (2) frequency of patients who lacked seroprotection at baseline (defined as HI titres of <1:40) who became seroprotected against each vaccine antigen at visit 3 (defined as HI titres of ≥1:40).[14]

The 28-joint disease activity score (DAS28) was measured and adverse events that were associated with vaccination were captured from the patients at each visit. An RA flare was defined as an increase in DAS28 of >1.2 (or >0.6 if the baseline DAS28 was ≥3.2).[15]

### Titre measurements

The HI antibody titres against each of the three influenza strains in the vaccine were measured in duplicate by an independent laboratory (GC Labs, Gyeonggi-do, South Korea) according to standard procedures. The average of the duplicate measurements for each antigen was calculated.

### Statistical analysis

#### Analysis and safety population

The primary analysis population (per-protocol population) included all study subjects who underwent the vaccination, discontinued or continued MTX according to the allocated regimen, and whose prevaccination and postvaccination titres were available.

#### Sample size calculation

The vaccine response to influenza, defined as a fourfold or more increase in HI antibody titres in two or more of three influenza

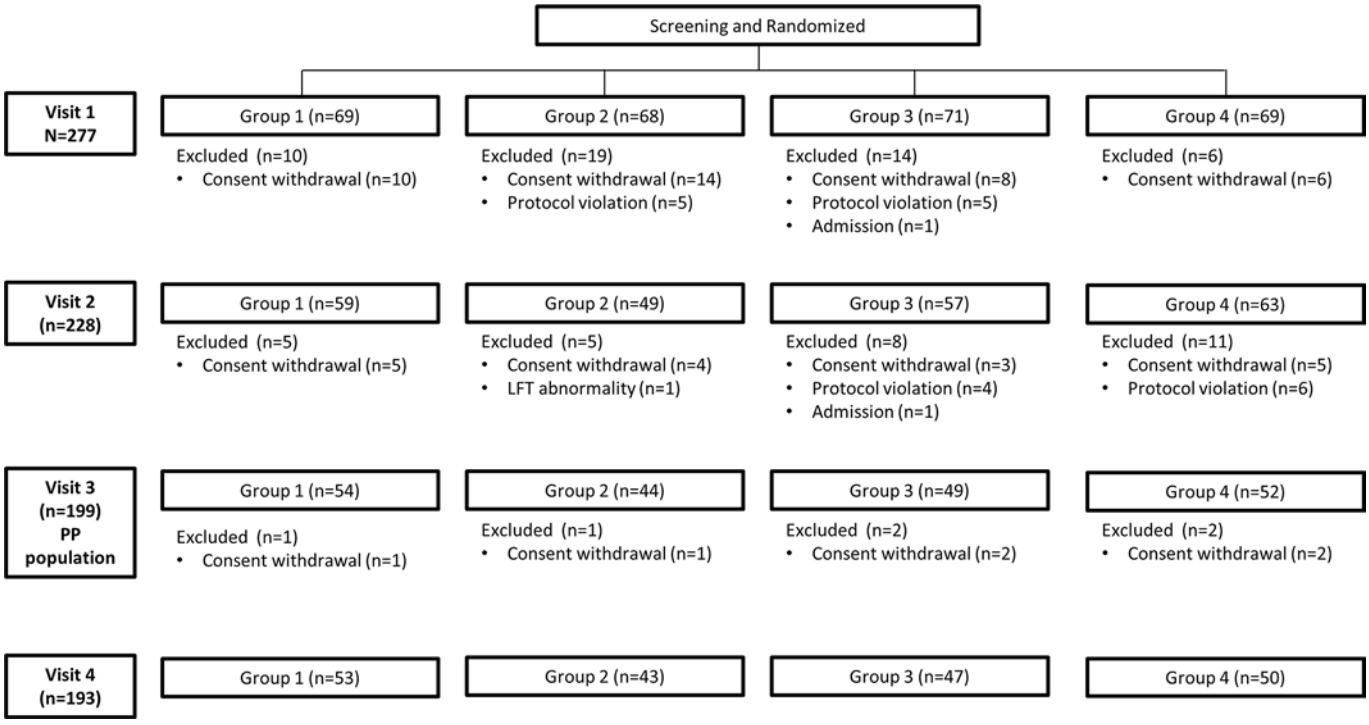


**Figure 2** Patient flow.

antigens, in patients with RA with and without concurrent MTX treatment has been reported to be 61.8% and 76.7%, respectively.[7] Assuming that 4 weeks of MTX discontinuation would improve the vaccination response to that seen in patients without MTX treatment, and assuming an alpha level of 0.05 (two-tailed), a power of 0.90 and dropout rate of 10%, 146 patients per group would be required for the study. Thus, the total target number was 584 patients.

Continuous variables were analysed by using a *t* test or Mann-Whitney U test, as appropriate. The binary secondary efficacy variables (frequency of satisfactory vaccine response and frequency of disease flare) were analysed by using $\chi^2$ tests or Fisher's exact test, as appropriate. $p<0.05$ was considered to indicate statistical significance. Analyses were not adjusted for multiple testing because these analyses were performed with exploratory rather than confirmatory intention. All analyses were performed by using SPSS (IBM SPSS Statistics V. 18).

## RESULTS

### Baseline characteristics of the patients

Patients were asked to participate during their longitudinal follow-up in the Rheumatology Clinic between September 2015 and November 2015. A total of 277 patients were randomly assigned to the four treatment groups. The target participant number (n=584) could not be reached due to the short enrolment period (3 months): it was short because the patients had to be vaccinated before the start of the influenza season (ie, by the end of December 2015). The per-protocol population consisted of the 199 patients who had at least completed visit 3 (figure 2). The patients were predominately female. The four groups did not differ at baseline in terms of demographic or disease characteristics, including DAS28 C reactive protein. The groups were also comparable in terms of their treatment regimen at baseline, including their use of oral corticosteroids and MTX (table 1).

### Effect of MTX discontinuation on influenza vaccine efficacy

The four groups were similar in terms of their baseline HI antibody titres against H1N1, H3N2 and B antigens. Four weeks after the vaccination (visit 3), the patients in all four groups mounted significant humoral immune responses against the three vaccine antigens (see online supplementary table S1).

#### Satisfactory vaccine response

The four groups were similar in terms of the frequency with which they mounted a satisfactory vaccine response (defined as an increase relative to baseline of at least fourfold) to at least one influenza antigen (figure 3A). However, group 3, in which MTX treatment was suspended 2 weeks before and 2 weeks after the vaccination, tended to respond more satisfactorily to at least two influenza antigens than group 1 (no MTX suspension) (figure 3B). Group 3 also responded significantly more satisfactorily to all three antigens than group 1 (figure 3C). Groups 2 (MTX suspension for 4 weeks before vaccination) did not differ significantly from group 1 in terms of these responses, whereas group 4 (MTX suspension for 4 weeks after the vaccination) tended to respond better than group 1 (figure 3).

#### Vaccine response to individual strain

In terms of the responses to individual vaccine antigens, groups 3 and 4 responded satisfactorily to B-Yamagata more frequently than group 1 (group 3 vs group 1: difference, 20.3%; 95% CI, 1.0% to 39.6%; p=0.040; group 4 vs group 1: difference, 22.6%; 95% CI, 3.6% to 41.7%; p=0.0197). Differences between the groups in responses to H1N1 or H3N2 were not observed (see online supplementary table S1 and supplementary figure S1A). Analysis of the subgroup of patients whose baseline HI antibody titre was <1:40 showed that group 3 responded (ie, HI antibody titre ≥1:40) to all three antigens significantly more frequently than group 1 (see online supplementary table S2, supplementary figure S1B).

**Table 1** Baseline characteristics of the study patients (the per-protocol population)

| | Group 1 (n=54) | Group 2 (n=44) | Group 3 (n=49) | Group 4 (n=52) | p Value |
|---|---|---|---|---|---|
| Female | 45 (83.3) | 39 (88.6) | 42 (85.7) | 43 (82.7) | 0.854 |
| Age, years | 59.1±13.1 | 58.5±113.3 | 58.1±10.9 | 58.1±11.7 | 0.854 |
| Body mass index, kg/m$^2$ | 22.5±3.0 | 22.6±3.7 | 23.1±3.7 | 23.4±3.2 | 0.418 |
| Duration of RA, years | 5.2±4.5 | 6.1±4.9 | 4.9±4.4 | 6.2±5.5 | 0.429 |
| RF positivity | 41/52 (78.8) | 37/43 (86.0) | 39/49 (79.6) | 41/51 (80.4) | 0.824 |
| Anti-CCP positivity | 37/46 (80.4) | 31/35 (88.6) | 39/46 (84.8) | 32/36 (88.9) | 0.675 |
| DAS28-CRP | 2.5±1.1 | 2.8±1.2 | 2.6±1.0 | 2.6±1.0 | 0.604 |
| Treatment | | | | | |
| GC | 25 (46.3) | 29 (65.9) | 32 (65.3) | 29 (55.8) | 0.152 |
| GC dose, mg/day | 2.2±2.8 | 3.0±2.5 | 2.9±2.4 | 2.4±2.3 | 0.320 |
| MTX dose, mg/week | 12.7±3.7 | 13.3±3.4 | 13.6±2.9 | 13.2±3.3 | 0.603 |
| Sulfasalazine | 5 (9.3) | 3 (6.8) | 5 (10.2) | 6 (11.5) | 0.878 |
| HCQ | 10 (18.5) | 5 (11.4) | 8 (16.3) | 5 (9.3) | 0.540 |
| Leflunomide | 14 (25.9) | 8 (18.2) | 8 (16.3) | 16 (30.8) | 0.288 |
| Biological DMARDs | | | | | |
| TNF inhibitor | 5 (9.3) | 4 (9.1) | 6 (12.2) | 4 (7.7) | 0.888 |
| Abatacept | 0 (0) | 2 (4.5) | 0 (0) | 1 (1.9) | 0.135 |
| Tocilizumab | 0 (0 | 2 (4.5) | 3 (6.1) | 2 (3.8) | 0.329 |
| Rituximab | 0 (0) | 0 (0) | 0 (0) | 1 (1.9) | 0.729 |
| Tofacitinib | 0 (0) | 0 (0) | 1 (2.0) | 0 (0) | 0.467 |
| Seroprotection | | | | | |
| H1N1 | 18 (33.3) | 20 (45.5) | 20 (40.8) | 19 (36.5) | 0.638 |
| H3N2 | 39 (72.2) | 23 (52.3) | 26 (53.1) | 28 (53.8) | 0.115 |
| B-Yamagata | 21 (38.9) | 14 (31.8) | 19 (38.8) | 11 (21.2) | 0.117 |

The data are expressed as absolute number (frequency) or mean±SD.
p Values were determined by using t test, Mann-Whitney U test, $\chi^2$ test or Fisher's exact test, as appropriate.
Anti-CCP, anti-cyclic citrullinated peptide; CRP, C reactive protein; DAS28, Disease Activity Score in 28 joints; DMARDs, disease-modifying antirheumatic drugs; ESR, erythrocyte sedimentation rate; GC, glucocorticoids; HCQ, hydroxychloroquine; LEF, leflunomide; MTX, methotrexate; RA, rheumatoid arthritis; RF, rheumatoid factor.

### Fold change in antibody titres

Compared with group 1, groups 3 and 4 had significantly higher fold increases in their antibody titres against H3N2 and B-Yamagata antigen, but not against H1N1 antigen (figure 4A). The anti-H3N2 antibody responses of groups 3 and 4, but not group 2, improved better relative to baseline (12.2-fold, 95% CI=8.4 to 17.5, p<0.001; 10.0-fold, 95% CI=6.8 to 14.8, p=0.043; and 6.1-fold, 95% CI=4.4 to 8.5, p=0.859, respectively) than group 1 (5.9-fold, 95% CI=4.3 to 8.1). Similarly, the anti-B-Yamagata antibody responses of groups 3 and 4, but not group 2, improved better (4.7-fold, 95% CI=3.3 to 6.7, p=0.048; 6.1-fold, 95% CI=4.2 to 8.8, p<0.001; and 2.8-fold, 95% CI=2.1 to 3.7, p=0.795, respectively) than group 1 (2.9-fold, 95% CI=2.2 to 3.8). Groups 3 and 4 also exhibited numerically greater fold changes in anti-H1N1 antibody titre (8.7-fold, 95% CI=5.3 to 14.5; 8.1-fold, 95% CI=5.3 to 14.4, respectively) than groups 1 and 2 (5.1-fold, 95% CI=3.4 to 7.8; 5.0-fold, 95% CI=3.2 to 7.8), although these differences did not achieve statistical significance.

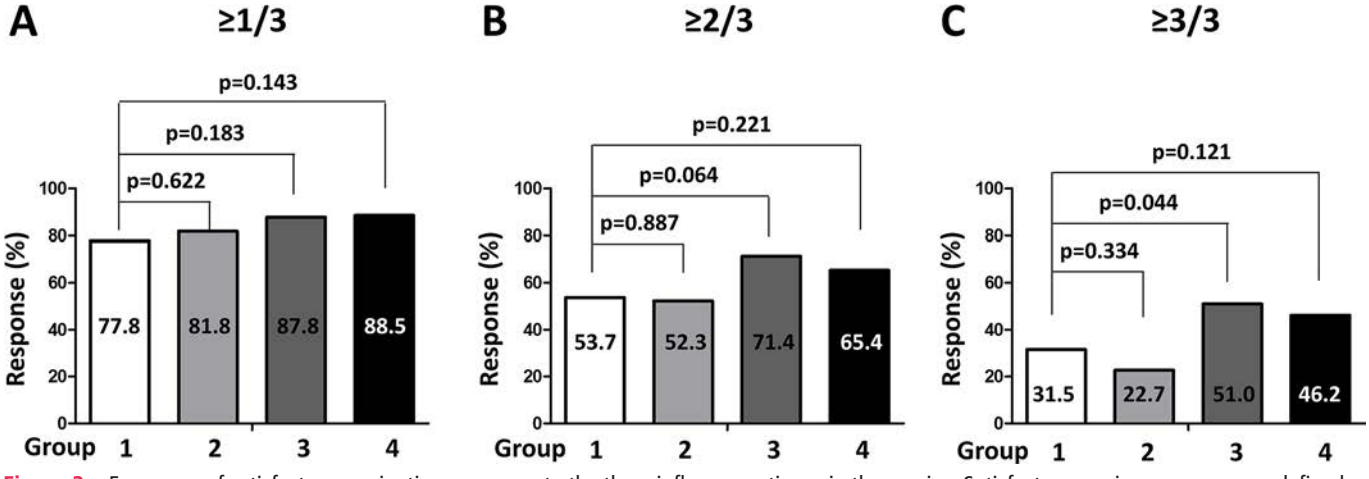


**Figure 3** Frequency of satisfactory vaccination responses to the three influenza antigens in the vaccine. Satisfactory vaccine response was defined as a ≥4-fold improvement in titres relative to baseline. The numbers in the bars indicate the percentage of satisfactory responders. p Values were generated by Mann-Whitney U tests.

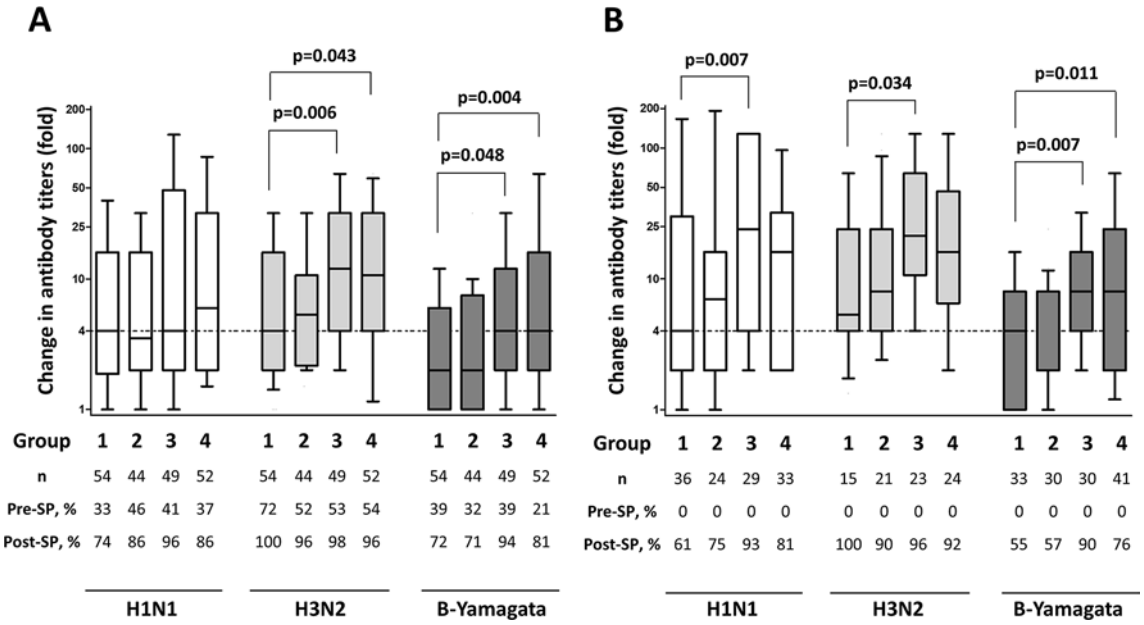


**Figure 4** Fold change in antibody titres relative to baseline (box-and-whisker plots) and seroprotection rates before and after vaccination (frequencies below the plot) in all patients (A) or only patients whose baseline antibody titre was <1:40 (B). Satisfactory vaccine response was defined as a ≥4-fold improvement in titres relative to baseline and is indicated by the dotted horizontal line in the box-and-whisker plots. The boxes represent the IQR. The median is represented by the horizontal line. The whiskers represent the 10th and 90th percentiles. The groups were compared in terms of fold change by using the Mann-Whitney U test. Seroprotection was defined as titres of ≥1:40. n, number; post-SP, postvaccination seroprotection rate; pre-SP, prevaccination seroprotection rate.

The differences between groups became more prominent when the analysis was restricted to patients who lacked seroprotection before vaccination (ie, HI antibody titres of <1:40): group 3 had significantly higher fold increases in antibody titres against all three antigens than group 1. Group 4 also exhibited significantly higher fold increases in antibody titres against B-Yamagata than group 1 (figure 4B). Differences between groups were not observed when only the patients with seroprotection at baseline were analysed (see online supplementary table S3). Group 2 did not differ from group 1, regardless of the vaccine efficacy variable.

### Seroprotection

Notably, the overall baseline seroprotection against H1N1 and H3N2 appeared to be higher than that against B-Yamagata (table 2). In terms of seroprotection after vaccination, >95% of the patients became seroprotected against H3N2 regardless of group (figure 4 and see online supplementary table S1). However, groups 3 and 4 became seroprotected against H1N1 and B-Yamagata more frequently than group 1. The difference was more prominent in the patients who lacked seroprotection at baseline (figure 4B, see online supplementary table S2).

### Safety

The vaccine was well tolerated. No severe adverse events that related to the vaccination were reported during follow-up. Of the 199 patients, 58 (29.1%) experienced a RA disease flare during the study. Flares tended to be more common in groups 2 (n=15; 34.1%) and 3 (n=19; 38.8%), while groups 1 and 4 had lower flare rates (n=13; 24.1% and n=11; 21.2%, respectively).

**Table 2** Adverse events

| | Group 1 (n=54) | Group 2 (n=44) | Group 3 (n=49) | Group 4 (n=52) |
|---|---|---|---|---|
| Any AE | 30 (55.6) | 27 (61.4) | 26 (53.1) | 17 (32.7) |
| SAE | | | | |
| Lung cancer | 0 (0) | 1 (2.3) | 0 (0) | 0 (0) |
| Fracture | 0 | 1 (2.3) | 1 (2.0) | 0 (0) |
| AE occurring in >5% of patients | | | | |
| Upper respiratory infection | 26 (48.1) | 18 (40.9) | 20 (40.8) | 16 (30.8) |
| Dizziness | 1 (1.9) | 3 (6.8) | 0 (0) | 1 (1.9) |
| Injection site reaction | 3 (5.6) | 1 (2.3) | 2 (4.1) | 0 (0) |
| Headache | 3 (5.6) | 1 (2.3) | 1 (2.0) | 0 (0) |
| RA flare at any visit | 13 (24.1) | 15 (34.1) | 19 (38.8) | 11 (21.2) |
| RA flare at visit 4 | 4 (7.5) | 6 (14.0) | 9 (19.1) | 6 (12.0) |
| RA flare at last visit | 0 (0) | 0 (0) | 0 (0) | 1 (1.9) |

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

However, these differences did not achieve statistical significance (p=0.224). Most patients recovered from the flare except one who exhibited a higher disease activity than at baseline at the end of the study (table 2).

## DISCUSSION

In this study, we showed that temporarily discontinuing MTX, especially when the vaccination occurred in the middle of the discontinuation period, significantly increased the efficacy of a seasonal influenza virus vaccine in patients with RA who were on a stable dose of MTX. To the best of our knowledge, this study is the first to demonstrate a novel strategy that increases the vaccine immunogenicity in such patients with RA.

The immunosuppressive effects of MTX might explain why this drug associates with impaired vaccine responses and, more beneficially, why it prevents the development of antidrug antibodies.[16–18] Notably, the therapeutic efficacy of MTX in RA takes weeks to months to achieve maximum efficacy and is sustained for several weeks after discontinuation. This suggests that MTX has a considerably long biological half-life.[19] While this suggests that short-term discontinuation of MTX would not boost the response of patients with RA to vaccines, we found that temporary MTX discontinuation (4 weeks) did improve the vaccine response of patients who had been on a stable dose of MTX. Furthermore, we showed that the time point of MTX discontinuation was critical: compared with patients without MTX discontinuation, the response to vaccine was greatest when MTX was suspended for 2 weeks before and 2 weeks after vaccination (group 3). By contrast, and somewhat counterintuitively, discontinuing MTX for 4 weeks before the vaccination (group 2) did not improve it. Moreover, discontinuing MTX for 4 weeks after the vaccination (group 4) was quite effective, although less effective than group 3. These data suggest that the effect of MTX on immune cells is actually immediate, whereas the disease-modifying effects of MTX (ie, the inhibition of inflammation in the joints) may take significantly longer to evolve.[20–22] The fact that discontinuing MTX improves immune responses to vaccines also supports the clinical practice that MTX be discontinued during acute (life-threatening) infections.

Notably, the overall baseline immunogenicity against H1N1 and H3N2 appeared to be higher than that against B-Yamagata (table 2). This suggests that patients had not been previously uniformly exposed to the three influenza antigens. Alternatively, B-Yamagata antigen is less immunogenic. In terms of seroprotection after vaccination, >95% of the patients were seroprotected against H3N2 regardless of group (table 2). In terms of H1N1 and B-Yamagata, groups 3 and 4 became seroprotected against more frequently than group 1. The difference was more prominent in the patients who lacked seroprotection at baseline.

In all groups, the vaccine response to the three influenza antigens varied markedly: there were strong responses to H3N2, lower responses to H1N1 and the weakest response was to B-Yamagata. The response to the less immunogenic B-Yamagata exhibited the best improvement (figure 4), indicating that vaccine response to less immunogenic antigens is suppressed by MTX. In addition, MTX discontinuation particularly improved the response to vaccine when the patients lacked protective antibody titres before vaccination (figure 4B). This suggests that the MTX discontinuation may have an even greater impact on the immune response to new, less immunogenic, antigens or vaccines that immune system has not encountered before or has not responded to appropriately previously. This possibility has important clinical implications. MTX discontinuation will improve responses to vaccines that are based on a new influenza pandemic strain and it will also help infected patients to generate immune responses to such strains more rapidly.[23] MTX discontinuation might aid vaccination against antigens with low immunogenicity, such as herpes zoster.[24] Further studies that assess the efficacy and safety of temporary MTX discontinuation in various clinical settings are warranted.

The majority of patients tolerated the influenza vaccination well without major complications. However, MTX discontinuation did associate with more RA flares compared with MTX continuation. However, nearly all of the patients recovered fully from their flare after MTX was reintroduced (table 2). Further studies that determine the optimal duration of MTX discontinuation that improves vaccine efficacy while avoiding RA flares are warranted.

The study has several limitations. First, since it was a single-centre study, it was not possible to enrol the optimal number of patients needed to identify the best MTX discontinuation regimen. The number of enrolled patients was fewer than the initial target sample size of 584, resulting in a power of 0.46 to detect a difference in vaccine response to at least two antigens and 0.52 for response to all three antigens. Second, we only enrolled patients with stable RA and low disease activity on a relatively low MTX dose. Moreover, all patients had the Korean ethnicity. Further studies testing the generalisability of our results to patients with moderate–high disease activity or with other ethnicities are needed. Last but not least, it remains unclear as yet whether a rise in antibody titre actually translates into decreased influenza incidence, although the HI antibody titres have been shown as a correlate of vaccine-induced protection.[25]

In conclusion, temporary discontinuation of MTX was associated with improved humoral vaccine response to a seasonal influenza vaccine in patients with RA who were receiving a stable dose of MTX. Further studies are needed to determine whether MTX discontinuation decreases the influenza incidence or alter the course of the disease.

**Contributors** EBL had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. EBL and JKP: study concept and design. JKP, MAL, EYL, YC, YWS, KLW and EBL: acquisition, analysis or interpretation of data. EBL, KLW and JKP: drafting of the manuscript. JKP, KHL, EYL, YWS, YC, KW and EBL: critical revision of the manuscript for important intellectual content. EBL, JKP, YC and KLW: statistical analysis. EBL: obtained funding, study supervision.

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**Competing interests** EBL has acted as a consultant to Pfizer. The other authors declare no conflicts of interest.

**Patient consent** Obtained.

**Ethics approval** The study was approved by the institutional review board of the Seoul National University Hospital [IRB 1508-050-694] and was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines. All patients provided written informed consent.

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EXTENDED REPORT

# Mortality in ANCA-associated vasculitis: a meta-analysis of observational studies

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## ABSTRACT

**Objective** To determine the magnitude of all-cause mortality risk in patients with antineutrophil cytoplasmic antibodies-associated vasculitis (AAV) compared with the general population through a meta-analysis of observational studies.

**Methods** We searched Medline and Embase databases from their inception to April 2015. Observational studies that met the following criteria were assessed by two researchers: (1) clearly defined AAV identified by either the American College of Rheumatology 1990 classification criteria or the 2012 Chapel Hill Consensus Conference disease definitions, and (2) reported standardised mortality ratios (SMR) and 95% CI. We calculated weighted-pooled summary estimates of SMRs (meta-SMRs) for all-cause mortality using random-effects model, tested for publication bias and heterogeneity.

**Results** Ten studies met the inclusion criteria, comprising 3338 patients with AAV enrolled from 1966 to 2009, and a total of 1091 observed deaths. Overall, we found a 2.7-fold increased risk of death in patients with AAV when compared with the general population (meta-SMR: 2.71 (95% CI 2.26 to 3.24)). Analysis on studies that included only granulomatosis with polyangiitis cases also indicated a similar mortality risk (meta-SMR: 2.63 (95% CI 2.02 to 3.43)). There was no significant publication bias or small-study effect. Subgroup analyses showed that mortality risks were higher in older cohorts, with a trend towards improvement over time (ie, those with their midpoint of enrolment periods that were between 1980–1993 and 1994–1999, vs 2000–2005).

**Conclusion** Published data indicate there is a 2.7-fold increase in mortality among patients with AAV compared with the general population.

## INTRODUCTION

Primary systemic vasculitides are a heterogeneous group of rare diseases characterised by the presence of necrotising inflammation of the blood vessel wall. Among the various hypotheses on the immunological mechanisms seeking to explain the nature of these diseases, the antineutrophil cytoplasmic antibodies (ANCA) appear to play a prominent role in the pathological pathways of a group of predominantly small vessel vasculitis, otherwise known as ANCA-associated vasculitis (AAV).[1 2] This distinctly pauci-immune form of vasculitis includes granulomatosis with polyangiitis (GPA, formerly Wegener's granulomatosis), microscopic polyangiitis (MPA) and eosinophilic granulomatosis with polyangiitis (EGPA, formerly Churg-Strauss syndrome).

The spectrum of AAV ranges from isolated organ involvement to life-threatening fulminant disease. The prognosis in untreated systemic GPA was initially poor, with mortality rates of 80% within 1 year and with a mean survival time of 5 months.[3] With the introduction of glucocorticoids and cyclophosphamide in the management of AAV in the 1960s, significant advances have been made in survival.[4] The 1-year, 5-year and 10-year survival rates in patients with GPA are now reported to range between 81%–95%, 73%–83% and 55%–75%, respectively.[5–13] Similar improvements were also noted in MPA and EGPA studies. With treatment, MPA survival rate at 1 year is 80%, 5 years 45%–85% and 10 years ~74%.[14–17] Recent EGPA studies have estimated 5-year survival rates at 89%–97%.[18 19]

Despite improving survival, patients with AAV still remain at a higher risk of death relative to the general population.[10] Standardised mortality ratio (SMR) provides an estimate of the true death risk, as it compares the number of observed patient deaths with the number of expected deaths of age-matched and sex-matched individuals from the general population. Several studies have reported an elevated SMR for patients with AAV, ranging from 1.6 to 4.8,[9 13 16 20–23] although others have found that contemporary mortality risks were not significantly different from the general population.[19 22 24 25] The conflicting results from these reports may be due to biases from small sample sizes and cohort types (eg, community-based vs clinic-based).

The purpose of our study was to estimate all-cause mortality risk of patients with AAV through a systematic review and meta-analysis from observational studies.

## METHODS

### Search strategies

A search was performed by an experienced research librarian (MDW) to identify primary studies and review literature using Medline and Embase databases on the Ovid platform. Records were captured for the full date range for each database through April 2015 (Medline from 1948, Embase from 1980) in any language. Database-specific indexing was used (Medline Medical Subject Headings (MeSH) and Embase subject headings), along with text words in titles and abstracts. Two search concepts were combined with the Boolean operator 'AND': (1) ANCA-associated vasculitis (AAV) or vasculitis, and (2) mortality or survival. Conference abstracts were captured with this approach, as they

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were not specifically excluded as a publication type. The exact search strategy is available as an online supplementary material (or available on request from the corresponding author).

Abstracts for all articles of interest were reviewed for relevance, that is those that reported mortality or survival data in AAV. Full papers of selected abstracts were retrieved and assessed for eligibility based on the inclusion criteria listed below. We also searched the reference lists of identified papers and conference abstracts for additional relevant publications.

All English-language peer-reviewed articles that met the following inclusion criteria were considered eligible: (1) clearly defined AAV identified by either the American College of Rheumatology (ACR) 1990 classification criteria[26 27] or the 2012 Chapel Hill Consensus Conference (CHCC) on disease definitions,[28] and (2) reported SMRs and 95% CI, or available data to calculate SMRs. In cases of duplicate data used in more than one study, the sample with the most up-to-date data was selected for review.

### Data extraction

Two authors (JAT and ND) independently reviewed and assessed the selected articles for eligibility. From eligible studies, JAT and ND extracted data on year of publication, enrolment period, study design, country, population setting, definition of AAV, sample size and demographics, proportion of ANCA positivity, proportion of renal involvement at diagnosis, and survival or mortality data. Gender-specific SMR was also noted, where available. In two studies, we calculated the 95% CI for SMR from available information.[9 20] In studies where the overall cohort was divided into time cohorts (by year of enrolment), each time cohort was computed as an individual cohort during meta-analysis.[22 24] One study provided 1-year and 5-year SMRs, and the latter was selected for the meta-analyses,[24] as the median or mean follow-up times for all studies were greater than 1 year. Any differences between the two authors (JAT and ND) were resolved by consensus together with a third author (JAA-Z).

### Quality scores of included studies

We assessed study quality based on a 12-point scale that was adapted from previously published scales for observational studies.[29 30] We used a similar scoring system in our previously published meta-analyses on the risk of mortality in rheumatoid arthritis[31 32] and systemic lupus erythematosus.[33] Points were allocated on an ordinal scale for each of the six items recorded: source of the study population (population based=2 points, clinic/hospital-based=1 point and undefined=0); cohort type (inception cohort=2, non-inception cohort=1 and undefined=0); definition of AAV (ACR or CHCC classification criteria=2, other validated classification criteria=1, and other predefined but non-validated classification criteria=0); ascertainment of death outcome (validated criteria=2, non-validated but clearly defined criteria (eg, death certificates)=1 and not mentioned=0); AAV exposure (≥10 years=2, ≥5 years and <10 years=1, and <5 years=0); and loss to follow-up (≤20%=2, >20% and ≤40%=1, and >40% or not mentioned=0). Studies with scores ≥7 points were considered higher quality and those with ≤6 points were lower quality studies. Two authors (JAT and ND) performed quality scoring independently, with differences resolved by consensus together with a third author (JAA-Z).

### Statistical analysis

We calculated the meta-SMR for all-cause mortality in AAV, which is a weighted-pooled summary estimate of SMRs (weighted by the sample size of each study) using HEpiMA statistical software, V.2.1.2.0.[34] A GPA meta-SMR was determined from study cohorts that included only GPA cases, excluding MPA and EGPA. Separate meta-SMRs were also calculated for men and women. Initial calculations were performed using SMRs from the individual studies on a log scale to approximate a normal sampling distribution. The resulting pooled values were then transformed back to the SMR scale. Results from the pooled statistics were based on the random-effects model. Statistical heterogeneity was assessed using the $I^2$ statistic, which indicates the proportion of variation in effect size due to heterogeneity.[35] Source of heterogeneity was determined by subgroup analysis. To do so, all included studies were stratified accordingly: population setting (population-based vs hospital/clinic-based samples), cohort type (inception vs non-inception), midpoint of enrolment periods (1980–1993, 1994–1999 and 2000–2005) and centre (single-centre vs multicentre). Furthermore, a univariate meta-regression analysis was then used to study and interpret the difference in meta-SMRs between the subgroups.[36] The time cut-offs for our enrolment period analysis were chosen as such because of the increased usage of ANCA testing in the mid-1990s, and because in the early 2000s there was a paradigm shift in treatment strategies, with an emphasis on improving the safety profile of induction therapy.[37]

We evaluated the robustness of the results using jackknife sensitivity analysis, by repeated meta-SMR analyses with removal of a single study in succession each time.[38]

### Assessment of publication bias/small-study effect

We constructed a funnel plot in which a measure of the study size is plotted as a function of the measure of interest.[39] We used the log of the SMRs from individual studies as well as the log of precision (1/variance). This was done to detect publication bias (ie, bias resulting from the greater likelihood of studies with positive results to be published compared with negative results) or the small-study effect (ie, a tendency for treatment effect estimates in small studies to differ from those in larger studies).[40] In the absence of publication bias and small-study effect, the distribution of the data points will be symmetric. Furthermore, we used Egger's regression as an objective, quantitative test statistic to test for the presence of asymmetry in the data.[41]

## RESULTS

We screened 570 abstracts published over the last 38 years (324 Medline and 238 Embase and 8 from reference lists). A total of 58 studies were retrieved for detailed evaluation and 10 studies met the inclusion criteria (figure 1 and table 1). Forty-eight studies were excluded: 43 did not provide SMRs or data to calculate them, 3 were review papers and 2 included only patients with renal vasculitis. The complete list of references reviewed is available on request from the corresponding author.

The 10 studies included 3338 patients with AAV (2619 with GPA, 501 with MPA, 185 with EGPA and 33 with renal limited vasculitis) enrolled from 1966 to 2009, and a total of 1091 observed deaths.[9 13 16 19–25] Three were population-based studies (n=1691), whereas seven were hospital/clinic-based studies (n=1647). Four of these studies included only patients with GPA (n=1987).

There were 14 unique cohorts available for the meta-analysis. Overall, the mortality risk in patients with AAV was significantly increased when compared with the general population (meta-SMR: 2.71 (95% CI 2.26 to 3.24)) (see figure 2). Analysis on patients with GPA alone also showed a similar increase in risk

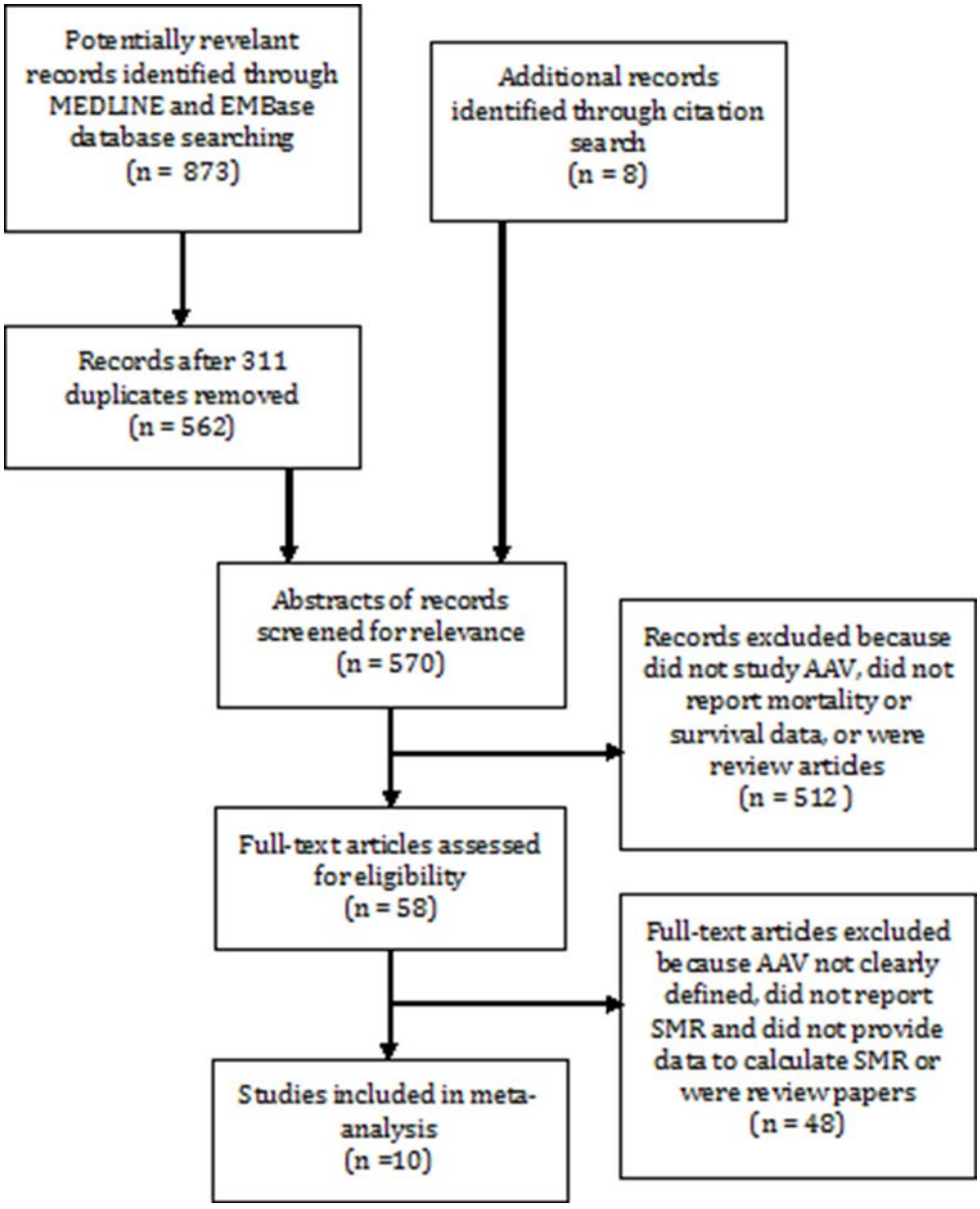


**Figure 1** Flow chart of study selection from literature search. AAV, ANCA-associated vasculitis; ANCA, antineutrophil cytoplasmic antibodies; SMR, standardised mortality ratio.

of mortality (GPA meta-SMR: 2.63 (95% CI 2.02 to 3.43)). Five studies reported sex-specific mortality estimates with no differences in mortality risks between sexes (meta-SMR: 3.36 (95% CI 2.10 to 5.38) and 3.11 (95% CI 2.21 to 4.36) for women and men, respectively).

There was significant heterogeneity among the studies ($I^2$=84.4%, 95% CI 72.6 to 96.3). Subgroup analyses showed that a number of factors might have influenced the mortality risk. Meta-SMRs were higher in population-based studies, in non-inception cohorts, in multicenter studies, and in cohorts enrolled prior to 2000 (table 2). All subgroups showed significantly increased mortality risk compared to the general population, although we observed a decreasing mortality trend in newer cohorts. Despite the differences in mortality within subgroups, only "center" was significantly associated with the observed heterogeneity using meta–regression analysis (p=0.05).

The results of the jackknife sensitivity analysis are shown in table 3. The meta-SMR remained significantly increased with every sequential study exclusion, with the point estimates ranging from 2.6 to 2.9 and the corresponding 95% CI remaining >1 in all analyses. This suggested that the meta-SMR result was robust and not skewed by a single dominant study.

The funnel plot is shown in figure 3. Each plot represents individual cohorts and the solid line is the log of the meta-SMR. The distribution of our data points was symmetrical; therefore, we concluded that there was no significant publication bias or small-study effect. The Egger's test for presence of asymmetry in the data was not significant (p=0.308).

## DISCUSSION

This is the first systematic review and meta-analysis of observational studies assessing the mortality risk in patients with AAV. We found a 2.7-fold increased risk of death in patients with AAV when compared with the general population, with no differences between sexes. Analysis on studies that included only GPA cases also indicated a similar mortality risk. Of interest, mortality

**Table 1** Summary of studies included in meta-analysis

| Authors/Year published | Country (single/ multicentre) | Study design | Enrolment period | Mean follow-up, years | Patients (n) | Female (%) | Setting | Cohort type | AAV classification criteria | Mean age at study entry, years | Death events, n (%) | Survival rate | Standardised mortality ratio, SMR (95% CI) | Quality score |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Matteson *et al*[9]/1996 | Canada, Mexico, USA (multicentre) | Retrospective cohort | 1978–1987 | 7.1 | 77 GPA | 29 (37.7) | Tertiary hospital/ clinic | Inception | ACR | N/A | 28 (36) | 5-year survival: 75% | 4.69 (3.41 to 5.96)<br>Female: 6.81 (3.73 to 9.89)<br>Male: 4.00 (2.72 to 5.27) | 10 |
| Knight *et al*[23]/2002 | Sweden (multicentre) | Retrospective cohort | 1969–1994 | Up to 31, December 1995 | 1065 GPA | 502 (47.1) | Population-based | Non-inception | ICD 8 and 9 | N/A | 516 (48.5) | N/A | 4.0 (3.6 to 4.3)<br>All cancer: 2.2 (1.7 to 2.8) | 10 |
| Booth *et al*[20]/2003 | UK (multicentre) | Retrospective cohort | 1995–2000 | 3.1 (median) | 246 AAV<br>– 82 GPA<br>– 120 MPA<br>– 33 RLV<br>– 11 EGPA | 106 (43) | Tertiary hospital/ clinic | Non-inception | CHCC | 66 (median) | 59 (24) | 1-year survival: 84%<br>5-year survival: 76% | 2.84 (2.53 to 3.18) | 5 |
| Lane *et al*[16]/2005 | UK (single-centre) | Retrospective cohort | 1988–2000 | 3.3 | 99 AAV<br>– 57 GPA<br>– 24 MPA<br>– 18 EGPA | 38 (38.4) | Secondary district general hospital/ clinic | Non-inception | ACR, CHCC and Lanham, plus case note reviews | 62.6 | 31 (31.3) | 1-year survival<br>GPA: 85.5%<br>MPA: 82.7%<br>EGPA: 83.2%<br>5-year survival<br>GPA: 75.9%<br>MPA: 45.1%<br>EGPA: 68.1% | 4.8 (2.9 to 6.6)<br>Female: 3.05 (1.2 to 4.9)<br>Male: 5.9 (3.1 to 8.8) | 8 |
| Mohammad *et al*[25]/2009 | Sweden (multicentre) | Retrospective cohort | 1997–2006 | 4.9 (median) | 140 AAV<br>– 63 GPA<br>– 65 MPA<br>– 6 EGPA<br>– 6 PAN (excluded from analysis) | 73 (52.1) | Population-based | Inception | EMEA algorithm, plus case note reviews | 67.6 (median) | GPA: 14 (22.2)<br>MPA: 29 (44.6)<br>EGPA: 1 (16.7) | 1-year survival<br>GPA: 95%<br>MPA: 80%<br>5-year survival<br>GPA: 83%<br>MPA: 55% | GPA:<br>1.77 (0.84 to 2.70)*<br>MPA: 3.95 (2.51 to 5.38)*<br>Female: 3.27 (1.99 to 5.04)<br>Male: 2.48 (1.60 to 3.65)<br>Renal SMR: 3.22 (2.21 to 4.23) | 7 |
| Eriksson *et al*[24]/2009 | Sweden (single-centre) | Retrospective cohort | 1978–2005<br>Old cohort: 1978–1996<br>Recent cohort: 1997–2005 | –<br>Old cohort: 11.1<br>Recent cohort: 4.4 | 95 AAV<br>Old cohort: 32 AAV (24 GPA, 8 MPA)<br>Recent cohort: 63 AAV (33 GPA, 30 MPA) | 43 (45.3)<br>Old cohort: 15 (46.9)<br>Recent cohort: 28 (44.4) | Tertiary hospital/ clinic | Inception | CHCC | –<br>Old cohort: 57.7<br>Recent cohort: 61.4 | 22 (23.2)<br>Old cohort: 15 (46.9)<br>Recent cohort: 7 (11.1) | –<br>Old cohort: 1-year survival, 91%<br>5-year survival, 81%<br>Recent cohort:<br>1-year survival, 95%<br>5-year survival, 87% | –<br>Old cohort:<br>1-year SMR, 5.2 (1.07 to 15.14)<br>5-year SMR, 2.5 (0.93 to 5.52)†<br>Recent cohort:<br>1-year SMR, 2.1 (0.43 to 6.09)<br>5-year SMR, 1.6 (0.6 to 3.2)† | 7 |
| Takala *et al*[13]/2010 | Finland (multicentre) | Retrospective cohort | 1981–2000<br>Old cohort: 1981–1990<br>Recent cohort: 1991–2000 | Up to 30 July 2005 | 492 GPA<br>Old cohort: 126 GPA<br>Recent cohort: 366 GPA | 249 (50.6)<br>Old cohort: 67 (53.2)<br>Recent cohort: 182 (49.7) | Population-based | Non-inception | ICD 8, 9 and 10, plus case note reviews with ACR criteria | –<br>Old cohort: 49.3<br>Recent cohort: 54.5 | 203 (41.3)<br>Old cohort: 67 (53.2)<br>Recent cohort: 136 (37.2) | 1-year survival: 83%<br>5-year survival: 74%<br>Old cohort:<br>1-year survival, 4.9%<br>5-year survival: 6.2%<br>Recent cohort:<br>1-year survival, 82%<br>5-year survival, 74% | 3.43 (2.98 to 3.94)<br>Female: 4.38 (3.59 to 5.61)<br>Male: 2.80 (2.28 to 3.41) | 8 |

Continued

**Table 1** Continued

| Authors/Year published | Country (single/ multicentre) | Study design | Enrolment period | Mean follow-up, years | Patients (n) | Female (%) | Setting | Cohort type | AAV classification criteria | Mean age at study entry, years | Death events, n (%) | Survival rate | Standardised mortality ratio, SMR (95% CI) | Quality score |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Flossmann *et al*[21]/2011 | 15 European countries (multicentre) | Prospective cohort | 1995–2002 | 5.2 (median) | 535 AAV<br>– 281 GPA<br>– 254 MPA | 247 (46.2) | Tertiary hospital | Inception | CHCC | 61 (median) | 133 (24.9) | 1-year survival: 88%<br>5-year survival: 78% | 2.6 (2.2 to 3.1) | 8 |
| Holle *et al*[22]/2011 | Germany (single-centre) | Retrospective cohort | 1994–2002<br>Cohort 1: 1966–1993<br>Cohort 2: 1994–1998<br>Cohort 3: 1999–2002 | –<br>(median)<br>Cohort 1: 6.6<br>Cohort 2: 7.3<br>Cohort 3: 3.9 | 445 GPA<br>Cohort 1: 155 GPA<br>Cohort 2: 123 GPA<br>Cohort 3: 167 GPA | 222 (49.9)<br>Cohort 1: 79 (51)<br>Cohort 2: 61 (49.6)<br>Cohort 3: 82 (49.1) | Tertiary hospital | Inception | ACR | –<br>(median)<br>Cohort 1: 48<br>Cohort 2: 52<br>Cohort 3: 55 | 43 (9.6)<br>Cohort 1: 22 (14.2)<br>Cohort 2: 13 (10.6)<br>Cohort 3: 8 (4.8) | N/A | 1.58 (1.14 to 2.13)<br>Cohort 1: 2.1 (1.34 to 3.25)‡<br>Cohort 2: 1.41 (0.75 to 2.42)‡<br>Cohort 3: 1.03 (0.44 to 2.03)‡<br>Female: 1.23 (0.66 to 2.11)<br>Male: 1.8 (1.22 to 2.58)<br>Young patients: 5.77 (2.6 to 10.95)<br>Young men: 8.87 (4.05 to 16.8)<br>Cancer mortality: 0.65 (0.24 to 1.43) | 9 |
| Moosig *et al*[19]/2013 | Germany (single-centre) | Retrospective cohort | 1990–2009 | 5.2 | 150 EGPA | 74 (49.3) | Tertiary hospital | Non-inception | ACR | 49.1 | 12/142 (8.5) | 5-year survival: 97%<br>10-year survival: 89% | 1.29 (0.66 to 2.12)<br>EGPA-associated heart failure SMR: 3.06 (1.10 to 6.00) | 10 |

*Computed into meta-SMR as two cohorts.
†5-year SMRs computed into meta-SMR as two cohorts.
‡Computed into meta-SMR as three cohorts studies, in non-inception cohorts, in multicentre studies and in cohorts enrolled prior to 2000 (table 2). All subgroups showed significantly increased mortality risk compared with the general population, although we observed a decreasing mortality trend in newer cohorts. Despite the differences in mortality within subgroups, only 'center' was significantly associated with the observed heterogeneity using meta-regression analysis (p=0.05).
AAV, ANCA-associated vasculitis; ACR, American College of Rheumatology; ANCA, antineutrophil cytoplasmic antibodies; CHCC, Chapel Hill Consensus Conference; EGPA, eosinophilic granulomatosis with polyangiitis; EMEA, European Medicines Evaluation Agency; GPA, granulomatosis with polyangiitis; ICD, International Classification of Diseases (8, 9 and 10 denotes 8th, 9th and 10th revisions, respectively); MPA, microscopic polyangiitis; N/A, not available; PAN, polyarteritis nodosa; RLV, renal limited vasculitis; SMR, standardised mortality ratio.

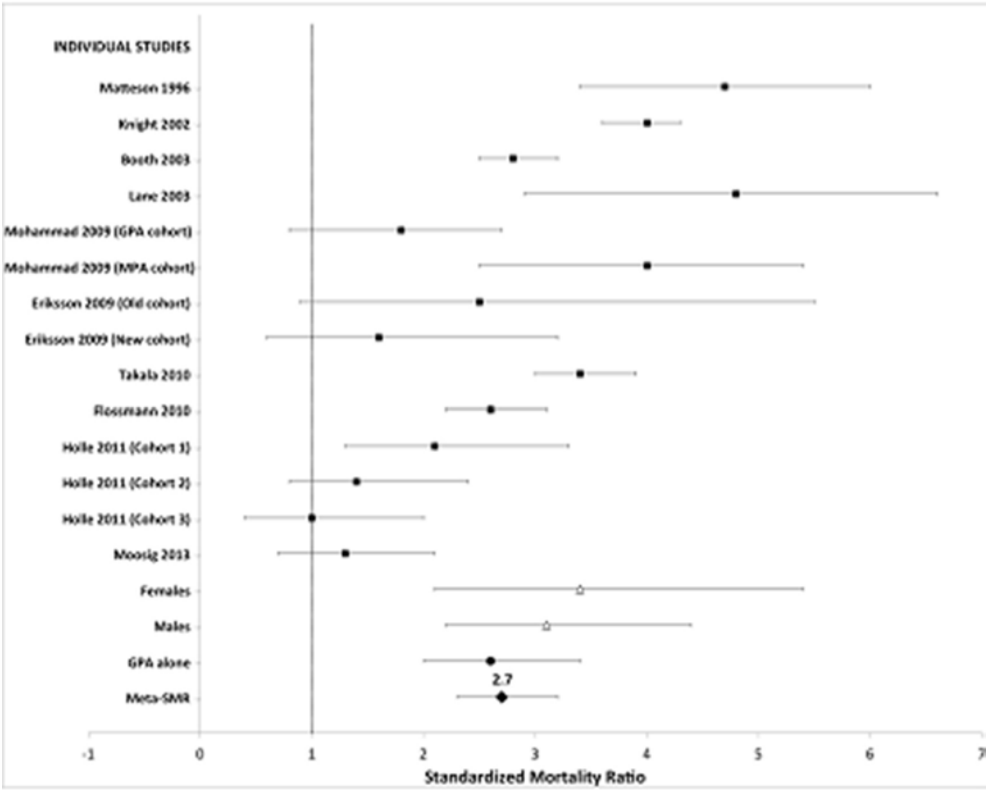


**Figure 2** Meta-analysis of 10 studies on all-cause mortality in patients with ANCA-associated vasculitis. ANCA, antineutrophil cytoplasmic antibodies; GPA, granulomatosis with polyangiitis; meta-SMR, weighted-pooled summary estimates of standardised mortality ratios.

risks were higher in earlier cohorts, that is, those with their midpoint of enrolment periods that were between 1980–1993 and 1994–1999, relative to those between 2000–2005, with a trend towards improvement over time.

Our meta-analyses did not show any significant difference in mortality between women and men. Individual studies have reported contrasting mortality risks between genders, with some favouring women[16 22] and others favouring men.[9 13 25] It was interesting to note that in the study by Holle *et al*, young patients with AAV (median age: 31.7 years) were almost six times more likely to die than the age-matched general population, with the entire risk contributed by young men (SMR: 8.87 (95% CI

**Table 2** Overall mortality and sensitivity analyses for the 10 studies (14 unique cohorts) in patients with AAV

| Study subset | No. cohorts | No. patients | No. death events | Random-effects meta-SMR (95% CI) | p |
|---|---|---|---|---|---|
| All studies | 14 | 3338 | 1091 | 2.71 (2.26 to 3.24) | |
| Disease definition | | | | | NS |
| GPA only (homogeneous) | 7 | 1987 | 804 | 2.63 (2.02 to 3.43) | |
| AAV mixed (heterogeneous) | 6 | 1125 | 257 | 2.59 (1.99 to 3.37) | |
| Sex | | | | | NS |
| Female | 5 | 611 | 147 | 3.36 (2.10 to 5.38) | |
| Male | 5 | 636 | 172 | 3.11 (2.21 to 4.36) | |
| Study population | | | | | NS |
| Population-based | 4 | 1691 | 763 | 3.37 (2.73 to 4.17) | |
| Hospital/clinic-based | 10 | 1647 | 328 | 2.39 (1.86 to 3.09) | |
| Cohort type | | | | | NS |
| Inception | 9 | 1286 | 270 | 2.30 (1.69 to 3.13) | |
| Non-inception | 5 | 2052 | 821 | 3.22 (2.57 to 4.05) | |
| Midpoint of enrolment period | | | | | NS |
| 1980–1993 | 5 | 1821 | 784 | 3.43 (2.79 to 4.21) | |
| 1994–1999 | 4 | 1003 | 236 | 2.82 (2.14 to 3.72) | |
| 2000–2005 | 5 | 514 | 71 | 1.92 (1.12 to 3.29) | |
| Centre | | | | | 0.05 |
| Multicentre | 7 | 2549 | 983 | 3.27 (2.73 to 3.91) | |
| Single-centre | 7 | 789 | 108 | 1.89 (1.17 to 3.07) | |

AAV, ANCA-associated vasculitis; ANCA, antineutrophil cytoplasmic antibodies; GPA, granulomatosis with polyangiitis; SMR, standardised mortality ratio; NS, not significant.

**Table 3** Sensitivity analysis using the jackknife approach

| Authors/Year published | All-cause mortality SMR (95% CI) | Study excluded, meta-SMR (95% CI) |
|---|---|---|
| All studies | 2.7 (2.3 to 3.2) | Not applicable |
| Matteson *et al*[9]/1996 | 4.7 (3.4 to 6.0) | 2.6 (2.1 to 3.1) |
| Knight *et al*[23]/2002 | 4.0 (3.6 to 4.3) | 2.6 (2.1 to 3.1) |
| Booth *et al*[20]/2003 | 2.8 (2.5 to 3.2) | 2.7 (2.2 to 3.3) |
| Lane *et al*[16]/2005 | 4.8 (2.9 to 6.6) | 2.6 (2.1 to 3.1) |
| Mohammad *et al*[25]/2009 (GPA cohort) | 1.8 (0.8 to 2.7) | 2.8 (2.3 to 3.4) |
| Mohammad *et al*[25]/2009 (MPA cohort) | 4.0 (2.5 to 5.4) | 2.6 (2.2 to 3.2) |
| Eriksson *et al*[24]/2009 (old cohort) | 2.5 (0.9 to 5.5) | 2.7 (2.3 to 3.3) |
| Eriksson *et al*[24]/2009 (new cohort) | 1.6 (0.6 to 3.2) | 2.8 (2.3 to 3.3) |
| Takala *et al*[13]/2010 | 3.4 (3.0 to 3.9) | 2.6 (2.1 to 3.2) |
| Flossmann *et al*[21]/2011 | 2.6 (2.2 to 3.1) | 2.7 (2.2 to 3.3) |
| Holle *et al*[22]/2011 (cohort 1) | 2.1 (1.3 to 3.3) | 2.8 (2.3 to 3.3) |
| Holle *et al*[22]/2011 (cohort 2) | 1.4 (0.8 to 2.4) | 2.8 (2.4 to 3.4) |
| Holle *et al*[22]/2011 (cohort 3) | 1.0 (0.4 to 2.0) | 2.8 (2.4 to 3.4) |
| Moosig *et al*[19]/2013 | 1.3 (0.7 to 2.1) | 2.9 (2.4 to 3.4) |

GPA, granulomatosis with polyangiitis; MPA, microscopic polyangiitis; SMR, standardised mortality ratio.

4.05 to 16.8)) as there were no deaths among the 80 women within the same cohort.[22] The authors postulated that the higher mortality risk in young men was due to a higher frequency of renal involvement at diagnosis.

The secular decline in mortality risks was an interesting observation. Although the overall comparison between the cohorts was non-significant, there was a trend towards significance when we compared the earliest with the most recent cohorts (1980–1993 vs 2000–2005, p=0.06). A similar finding was reported in a recent mortality study in patients with GPA.[42] In that study, 465 patients with GPA were followed over a 20-year period, and the authors found significantly improved HRs for mortality between an early cohort (1992–2002) and a late cohort (2003–2013) (4.34 (95% CI 2.72 to 6.92) vs 2.41 (95% CI 1.74 to 3.34), respectively, p=0.04). We hypothesise that this observation may have resulted from therapeutic improvements, earlier diagnosis with increased availability of ANCA testing and increased physician awareness, as well as improved overall patient care in terms of cardiovascular disease (CVD) risk modification, drug toxicity prevention strategies and cancer surveillance. Significant changes in the past decade on the way we treat patients with AAV include the use of pulsed cyclophosphamide and rituximab as less toxic therapeutic options.[43–45] There were insufficient data to directly assess impact of treatment strategies on mortality in this meta-analysis. Future studies will be needed to confirm the improvement in mortality.

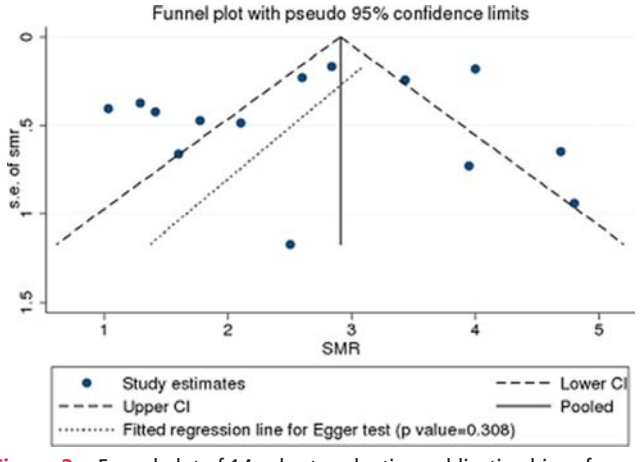


**Figure 3** Funnel plot of 14 cohort evaluating publication bias of mortality studies in ANCA-associated vasculitis. ANCA, antineutrophil cytoplasmic antibodies.

We found a significant difference in reported mortality risks from multicentre studies compared with single-centre studies. In fact, single-centre studies had the lowest meta-SMR of 1.89 (95% CI 1.17 to 3.07). The observed mortality difference between single-centre and multicentre studies was likely due to clinical differences in the respective patient populations, particularly in terms of the proportion and severity of renal involvement. Unfortunately, we were unable to test this hypothesis given that not all of the primary studies adequately described this type of data.

Unexpectedly, there was a trend towards higher mortality in the non-inception cohorts when compared with inception cohorts, although this did not reach statistical significance. One might expect higher mortality to be associated with inception cohorts as they capture the entire natural history up until the end of follow-up. However, inception cohorts may not follow patients for sufficiently long periods of time to capture late mortality risks, that is, deaths due to long-term disease or treatment-related complications such as cancer, cardiovascular disease or chronic renal failure. Non-inception cohorts by design would include prevalent as well as incident cases, and late mortality may be captured as the observation time begins at any point of the natural history. Unfortunately, we were unable to compare mean disease duration for the inception versus non-inception cohorts given that some reported mean times (n=5), some median times (n=6) and others none provided (n=2).

It was also interesting to note the trend for increased risk of death in studies that were population-based compared with those that were hospital/clinic-based. The risk estimates from population-based studies were more consistent, whereas there was wider variability in the estimates from hospital/clinic-based studies. The variability in the latter subgroup was not unexpected, given the likelihood of biases inherent in selected or referral cohorts. We suggest that further research in population-based cohorts is necessary to add to the current pool of knowledge.

Our study has several limitations. A common issue with meta-analyses is the comparability of the cohorts and the appropriateness of the comparison. We included cohorts that were clinically different in terms of enrolment period, AAV subgroups, classification criteria, follow-up, disease severity and study design. We adopted the random-effects model to incorporate the between-study heterogeneity into the analysis and provided an objective measure of the heterogeneity in the form of $I^2$. Significant heterogeneity was detected, as expected in meta-analyses of observational studies.[40] From the univariable meta-regression analysis, 'center' and 'enrolment period' were possible explanations for the heterogeneity (p=0.05 and p=0.06 (cohorts 1980–1993 vs 2000–2005), respectively). Furthermore, we performed a limited multivariable meta-regression analysis using these two variables. However, both variables were not significant predictors in the multivariable model. For this reason, our findings suggest that study centre is associated with between-study heterogeneity, but its effects may be confounded by enrolment period.

The remaining between-study heterogeneity may be partially explained by the variability of renal involvement in the study cohorts. However, the lack of uniformity in the definition of

'renal involvement' in the studies did not allow for grouping into a categorical 'renal characteristic', which would be necessary for meta-regression analysis. In addition, we were also unable to include 'quality score' in our meta-regression analysis as we only had one study scored as a lower quality study (≤6).

Current available data allowed us to report a meta-SMR on GPA, but not MPA or EGPA. A report on SMRs for each disease subcategory would be more clinically relevant than an overall SMR for AAV as they are clinically distinct diseases. However, the SMR for AAV may serve as a reference point for future studies seeking to compare mortality risk differences over time.

In our meta-analysis, the SMR evaluated the mortality risk adjusted only for age and gender but did not account for other confounders. However, there is no method for adjusting the results of meta-analyses using SMRs. Meta-analyses on studies assessing risk factors or predictors of mortality in AAV are required to address these issues.

In summary, our meta-analysis indicated that there was a 2.7-fold increase in mortality among patients with AAV compared with the general population. The pooled SMR for only patients with GPA was elevated at 2.6 times the general population. The risk of death was elevated for both male and female patients with AAV, with no significant difference between the genders. Furthermore, there was a trend towards improvement in mortality risks over time, which warrants further investigation. There is a need for longitudinal studies in contemporary cohorts to evaluate mortality benefits of modern therapies.

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**Contributors** JAT and JAA-Z conceived and designed the study. JAT and ND screened titles and abstracts for inclusion and extracted the data. All authors were responsible for data analysis and interpretation. JAT drafted the manuscript. All authors contributed to critical review of the manuscript and have read and approved the final manuscript.

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EXTENDED REPORT

# Enteric-coated mycophenolate sodium versus azathioprine in patients with active systemic lupus erythematosus: a randomised clinical trial

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## ABSTRACT

**Objective** To compare the efficacy and safety of enteric-coated mycophenolate sodium (EC-MPS) versus azathioprine (AZA) in patients with active systemic lupus erythematosus (SLE) disease.

**Methods** A multicentre, 24-month, superiority, open-label, randomised controlled trial (NCT01112215) was conducted with 240 patients (120 per arm) receiving either EC-MPS (target dose: 1440 mg/day) or AZA (target dose: 2 mg/kg/day) in addition to prednisone and/or antimalarials. The primary endpoint was the proportion of patients achieving clinical remission, assessed by SLE Disease Activity Index 2000 (SLEDAI-2K) and British Isles Lupus Assessment Group (BILAG), at 3 and 24 months. Secondary endpoints included time to clinical remission, BILAG A and B flare rates, time to flare, corticosteroid reduction and adverse events (AEs).

**Results** Proportion of patients achieving clinical remission (clinical SLEDAI=0) was higher in the EC-MPS group at 3 (32.5% vs 19.2%; treatment difference, 13.3 (CI 2.3 to 24), p=0.034) and 24 months (71.2% vs 48.3%; treatment difference, 22.9 (CI 10.4 to 34.4), p<0.001). EC-MPS was superior with respect to time to clinical remission (HR 1.43; 95% CI 1.07 to 1.91; p=0.017). BILAG A/B and B flares occurred more frequently in the AZA group (71.7% vs 50%, p=0.001 and 21.67% vs 8.3%, p=0.004, respectively). EC-MPS was superior with respect to time to first BILAG A/B (HR 1.81; 95% CI 1.3 to 2.56; p=0.0004) and BILAG A flare (HR 2.84; 95% CI 1.37 to 5.89; p=0.003). AEs were similar in both groups except for leucopenia that occurred more frequently with AZA.

**Conclusions** EC-MPS was superior to AZA in treating SLE and preventing further relapses.

**Trial registration number** NCT01112215; Results.

## INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic multisystem autoimmune disease characterised by heterogeneous clinical manifestations and a relapsing–remitting course. Although there have been significant improvements in its prognosis and management, the treatment of moderate-to-severe SLE remains unsatisfactory with a significant proportion of patients still having morbidity, poorer quality of life and increased mortality.[1]

Controlled clinical trials in SLE have focused primarily on lupus nephritis (LN) and generally have not analysed non-renal manifestations.[2–4] Standard initial therapy for extrarenal disease consists of oral corticosteroids and antimalarials, although immunosuppressive drugs are needed to control disease activity, minimise SLE organ damage and reduce corticosteroids. To date, data on the efficacy, safety and steroid-sparing effects of non-biological therapies are limited and provided mainly by small open-label studies and few randomised controlled trials (RCTs).[5–11] Historically, azathioprine (AZA) has been one of the most frequently used immunosuppressants with the advantage of its safety during pregnancy. There is modest evidence supporting its use, and side effects sufficient to discontinue the drug have been described in about one-third of cases.[10] However, evidence suggests these might be mitigated by measuring 6-thioguanine (6-TGN) levels.[7 8 10 12] Results from the RCTs conducted in non-renal SLE have shown that low-dose ciclosporin is as effective as AZA in severe SLE as a steroid-sparing agent,[10] and that leflunomide[9] and methotrexate[5] are more effective than placebo in mild-to-moderate active disease.

Mycophenolate mofetil (MMF) and enteric-coated mycophenolate sodium (EC-MPS) have become widely used for SLE, although most data come from LN studies using MMF. Initial RCTs have shown MMF to be at least as effective as cyclophosphamide (CYC) for induction therapy and equal or superior to AZA in maintaining renal response.[4 13–15] EC-MPS has been shown to have similar efficacy to MMF but with fewer gastrointestinal side effects[16] and has been increasingly used for adult and paediatric patients.[17 18] To date, there have been no robust trials assessing the non-renal effects of these agents. However, limited data from open-label studies and underpowered RCTs[17–24] in refractory SLE have shown MMF to be as effective as CYC for ameliorating non-renal symptoms in patients with LN,[23] comparatively better in dermatological and haematological manifestations[19] and to reduce disease activity and act as a steroid-sparing agent.[21]

To confirm the relative efficacy and safety of EC-MPS to AZA for active non-renal lupus disease, we conducted this 24-month clinical trial.

## METHODS

The study was conducted at 12 teaching hospitals in Spain between May 2010 and January 2016 in accordance with the Declaration of Helsinki and Good Clinical Practice principles. All participants

provided written informed consent. The study protocol was reviewed and approved by every participant centre.

### Patient eligibility and enrolment

Eligible patients were aged ≥18 years, had an SLE according to the revised ACR classification criteria[25] and moderate-to-severe active disease defined as: a SLE Disease Activity Index 2000 (SLEDAI-2K)[26] total score ≥6 or at least 1 British Isles Lupus Assessment Group (BILAG) A or 2 BILAG B domain scores at screening.[27] Key exclusion criteria were immunosuppressant therapy 12 weeks before randomisation; active nephritis or non-lupus-related significant laboratory abnormalities. See

**Table 1** Baseline demographic and disease characteristics of patients

| Characteristic | Azathioprine (n=120) | EC-MPS (n=120) |
|---|---|---|
| Women, n (%) | 111 (92.5) | 108 (90) |
| Age, mean (SD), years | 40.9 (12.9) | 42.1(13.9) |
| Race or ethnic group, n (%) | | |
| White | 120 (100) | 119 (99.2) |
| Asian | 0 (0) | 1 (0.8) |
| Hispanic/Latin American origin | 10 (8.3) | 12 (10) |
| Duration of SLE disease, mean (SD), years | 5.1 (5.6) | 6.2 (7.1) |
| Disease duration ≤2 years at inclusion, n (%) | 52 (43.3) | 49 (40.8) |
| Previous lupus nephritis, n (%)* | 11 (9.2) | 13 (10.9) |
| SLE disease activity | | |
| Total SLEDAI-2K score, mean (SD) | 9.5 (2.9) | 9.9 (4.2) |
| SLEDAI-2K score ≥10, n (%) | 48 (40) | 58 (48.3) |
| PGA score (0–3 VAS), mean (SD) | 1.9 (0.3) | 1.9 (0.4) |
| Total BILAG-2004 index score, mean† (SD) | 19.4 (5.3) | 21.7 (8.2) |
| At least one BILAG A score, n (%) | 51 (42.5) | 63 (52.5) |
| BILAG A/B organ domain score at baseline, n (%) | | |
| Constitutional | 30 (25) | 41 (34.2) |
| Musculoskeletal | 93 (77.5) | 91 (75.8) |
| Mucocutaneous | 88 (73.3) | 78 (65) |
| Cardiorespiratory | 32 (26.7) | 38 (31.7) |
| Renal | 0 (0) | 0 (0) |
| Neuropsychiatric | 3 (2.5) | 4 (3.3) |
| Haematological | 18 (15) | 21 (17.5) |
| Gastrointestinal | 2 (1.7) | 3 (2.5) |
| Ophthalmic | 0 (0) | 2 (1.7) |
| Overall SLICC-DI, median (IQR) | 0.32 (0–4) | 0.43 (0–4) |
| Autoantibody status, n (%) | | |
| ANA titre ≥1/80, no (%) | 120 (100) | 120 (100) |
| Anti-dsDNA antibodies ≥15 IU/mL, n (%) | 65 (54) | 66 (55) |
| Anti-dsDNA antibodies, mean (SD), IU/mL | 194.3(405) | 247.8 (574) |
| Serum C3, mean (SD), mg/dL | 87.3 (34) | 86.9 (28.3) |
| C3 below lower limit of normal (<85 mg/dL), n (%) | 48 (40) | 49 (41) |
| Serum C4, mean (SD), mg/dL | 13.7 (8.8) | 13.1 (8) |
| C4 below lower limit of normal (<10 mg/dL), n (%) | 36 (30) | 44 (37) |
| Previous immunosuppressive therapy, n (%) | | |
| Cyclophosphamide | 6 (5) | 6 (5) |
| Methotrexate/leflunomide | 27 (21.5) | 29 (24.2) |
| Calcineurin inhibitors | 4 (3.3) | 4 (3.3) |
| Mycophenolate mofetil‡ | 5 (4.2) | 5 (4.2) |
| Azathioprine‡ | 4 (3.3) | 3 (2.5) |
| Antimalarial agents, n (%) | 105 (85.3) | 93 (77.5) |
| Corticosteroids use, n (%) | 114 (95) | 116 (96.7) |
| Daily prednisone dose, mean (SD), mg/day | 23.9 (18.1) | 28.6 (21.2) |
| >7.5 mg/day at baseline, n (%) | 103 (85.8) | 98 (81.6) |

*Lupus nephritis was diagnosed in a median time of 9 years (range 6 to 13) before inclusion.
†The total BILAG-2004 index score was based on the updated numerical score proposed for the BILAG-2004 index after the study was initiated (A=12; B=8; C=1; D=0; E=0).[34] BILAG A/B in the renal domain was not present at baseline because active nephritis was an exclusion criterion.
‡Previous mycophenolate mofetil and azathioprine therapy were given mainly for the treatment of lupus nephritis and withdrawn in a median time of 3.5 years (range 3 to 6) before inclusion.
ANA, antinuclear antibodies; BILAG, British Isles Lupus Assessment Group; dsDNA, double-stranded DNA; EC-MPS, enteric-coated mycophenolate sodium; PGA, physician's global assessment of disease activity; SLE, systemic lupus erythematosus; SLEDAI-2K, Systemic Lupus Erythematosus Disease Activity Index 2000; SLICC-DI, Systemic Lupus International Collaborating Clinics Damage Index.
Reference ranges are as follows: anti-dsDNA antibodies, <15 IU ml; serum C3 (mg/dL), 85 to 110; serum C4 (mg/dL), 10 to 40.

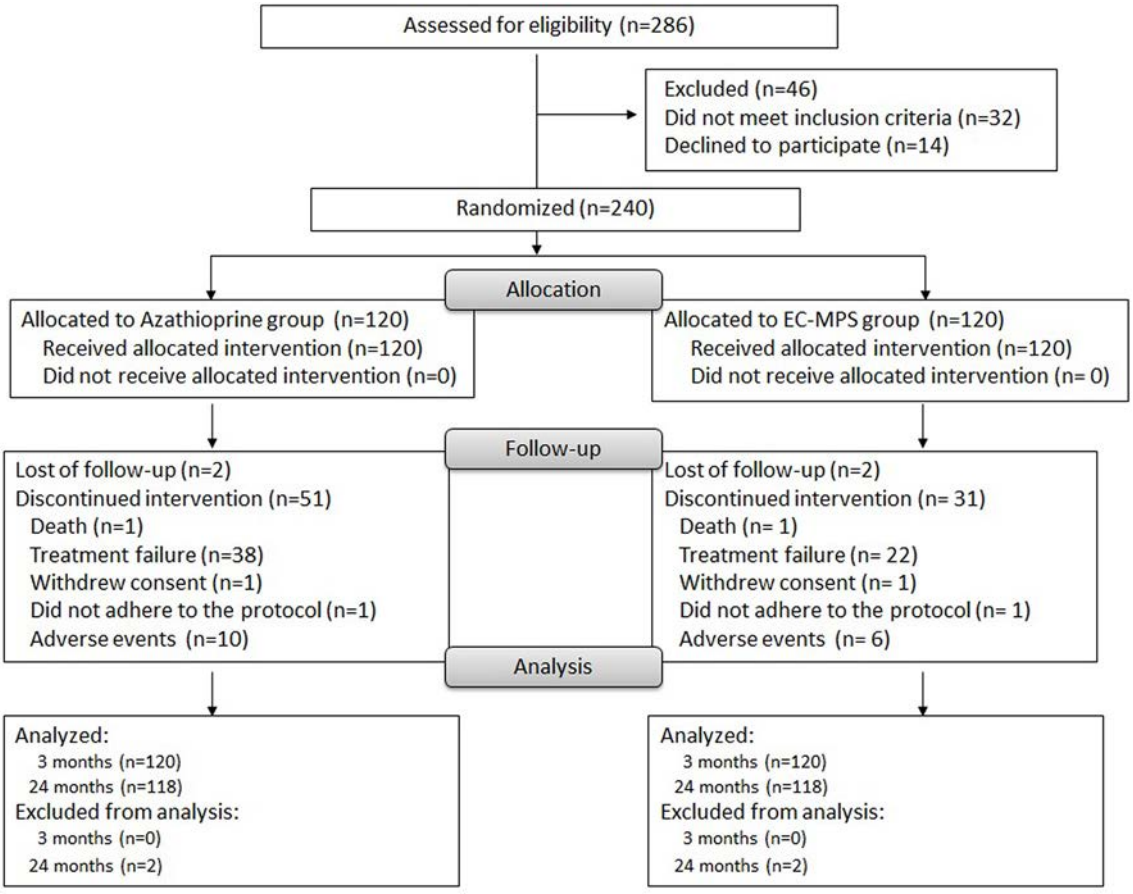


**Figure 1** Study flow diagram.

online supplementary material for detailed inclusion and exclusion criteria.

### Randomisation

The randomised list, stratified by centre and SLEDAI-2K score (6–9 vs ≥10), was created using computer-generated random-number sequences in blocks of 10 (C4 Study Design Pack Software, GlaxoSmithKline) by the Vall d'Hebrón Hospital investigational pharmacist, who was blind to patient enrolment. Sequentially numbered, concealed envelopes containing group assignment were provided to the investigators.

Eligible patients were randomised (1:1) to receive EC-MPS (target dose: 1440 mg/day) or AZA (target dose: 2 mg/kg, per thiopurine methyltransferase levels (TPMT)) in addition to background oral prednisone and antimalarial agents. Patients unable to tolerate the target dose or whose weight was below 50 kg remained in the study if they tolerated a minimum daily dose of either 720 mg of EC-MPS or 50 mg of AZA during the first 6 months. Progressive immunosuppressant dose reduction was allowed after week 24 on a 3- to 6-monthly basis per clinical judgement. Changes in antimalarial and prednisone doses were not restricted (see online supplementary text).

### Outcomes and follow-up

The primary efficacy endpoints were the proportion of patients achieving at 3 and 24 months, at least 8 consecutive weeks of clinical remission (CR), defined as a clinical SLEDAI-2K=0, where serology was permitted (maximum SLEDAI=4) following the later Zen *et al*[28 29] equivalent definition, in the absence of any BILAG A, B or C score.

Secondary endpoints included: the overall proportion of patients in CR and partial clinical response (PR) (≥50% reduction in the total SLEDAI-2K score with a BILAG C score or better, without new BILAG A/B scores); treatment failure (premature discontinuation necessitated by protocol-prohibited rescue therapy due to worsening or persistent disease activity (see online supplementary text)); time to CR; rates of SLE flares, defined as a new BILAG A (severe flare) or B (moderate flare) score in any organ system following a BILAG C, D or E score[30]; time to flare; changes in total mean SLEDAI-2K and BILAG-2004 scores, prednisone dose and serological activity (anti-double-stranded DNA (dsDNA) antibodies and C3). Comparative SLE Responder Index (SRI)[4 31] and Lupus Low Disease Activity State (LLDAS)[32] measurements were added post-hoc. The outcomes were adjudicated by an independent assessor.

Patients were evaluated monthly for the first 6 months and every 3 months thereafter. At each visit, the SLEDAI-2K[26] and BILAG-2004[30 33] scores, physician's global assessment (PGA), changes in concomitant medications and adverse events (AEs) were recorded. To assess the BILAG index global response, the scores were converted to numeric values (A=12, B=3, C=1, D=0, E=0).[34] SLICC Damage Index (SDI)[35] was scored at baseline and month 24. Patients were followed up for 24 months, regardless of outcome.

Safety assessments included the incidence and severity of AEs classified using the Medical Dictionary for Regulatory Activities (MedDRA version 12) (online at http://www.meddra.org/). SLE flares were not considered AEs.

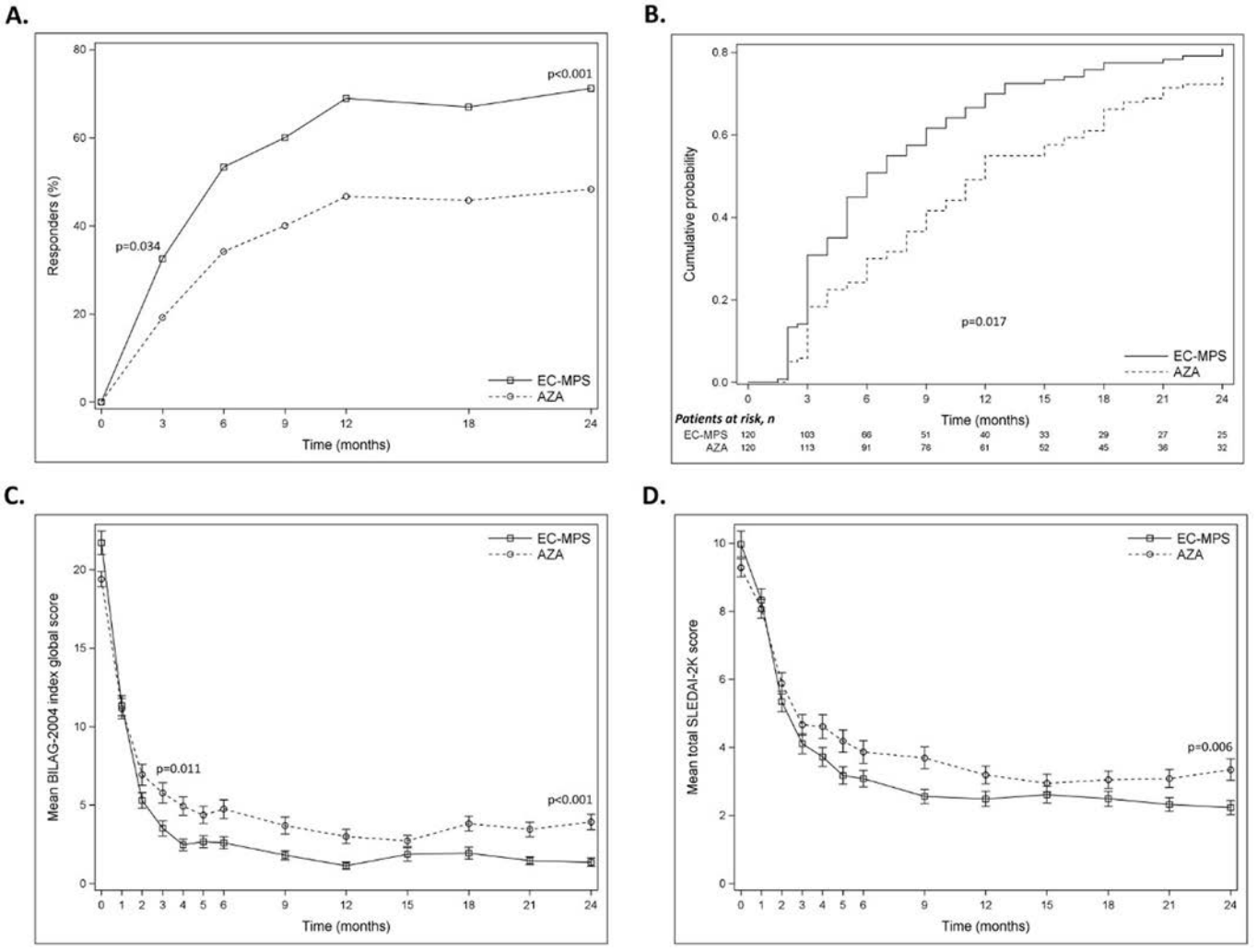


**Figure 2** Results for primary efficacy endpoints. Rates of clinical remission during the 24-month study period (A). Cumulative probability for time to clinical remission (B). Mean BILAG index (C) and SLEDAI-2K (D) global scores during the study. Bars in (C) and (D) represent the SEM. AZA, azathioprine; BILAG, British Isles Lupus Assessment Group; EC-MPS, enteric-coated mycophenolate sodium; SLEDAI-2K, Systemic Lupus Erythematosus Disease Activity Index 2000.

### Statistical analysis

The primary efficacy analysis was an intention-to-treat analysis that included all randomised patients who received at least one dose of the study agents, had at least one measurement prior to administration and had at least one efficacy assessment. The safety population comprised all patients who received at least one dose of study medication. Continuous variables are presented as mean and SD, and categorical variables as count and percentage. The main comparisons of proportions, at 3 and 24 months, have been estimated using generalised estimating equations to account for the repeated measurement design. To adjust for multiple comparisons (3 and 24 months), a Bonferroni correction of the significance level was applied. Continuous data comparisons were performed with t-test. In the time-to-CR analysis, the proportional hazards (PH) assumption of the Cox model regression did not hold. However, the curves clearly did not cross throughout the study. Thus, to provide an easier interpretation, the results are presented as HRs, which may be understood as an 'average effect'. The time to flare analyses did not show any issue with the PH assumption. Subgroup analyses of the primary endpoint by selected baseline characteristics are presented. Additional comparisons were carried out with log-rank tests. Statistical analyses were performed using SAS software V.9.3. Differences were determined to be statistically significant when two-sided p value was less than 0.05.

The sample size estimation was based on an overall remission rate in AZA-treated patients at 24 months of 45%.[6 7 10] Under this assumption, 120 patients would need to be assigned to each group to have 80% power to detect significant differences with a bilateral alpha level of 0.05, assuming a 20% difference between treatments and allowing a 20% dropout rate.

## RESULTS

### Patients

A total of 240 patients were enrolled between May 2010 and December 2013. Of the patients in this intention-to-treat population, 120 were randomised to each treatment group. Baseline demographics are shown in table 1. Seven patients had neurological manifestations: peripheral polyneuropathy[1] and transverse myelitis[2] in the AZA group; and organic brain syndrome,[2] lupus-related Parkinsonism[1] and Guillain-Barré[1] in the EC-MPS group. A total of 154 patients (64.2%) completed the study: 87 (72.5%) in the EC-MPS group and 67 (55.8%) in the AZA group. Fifty-three patients in the AZA group and 33 in the

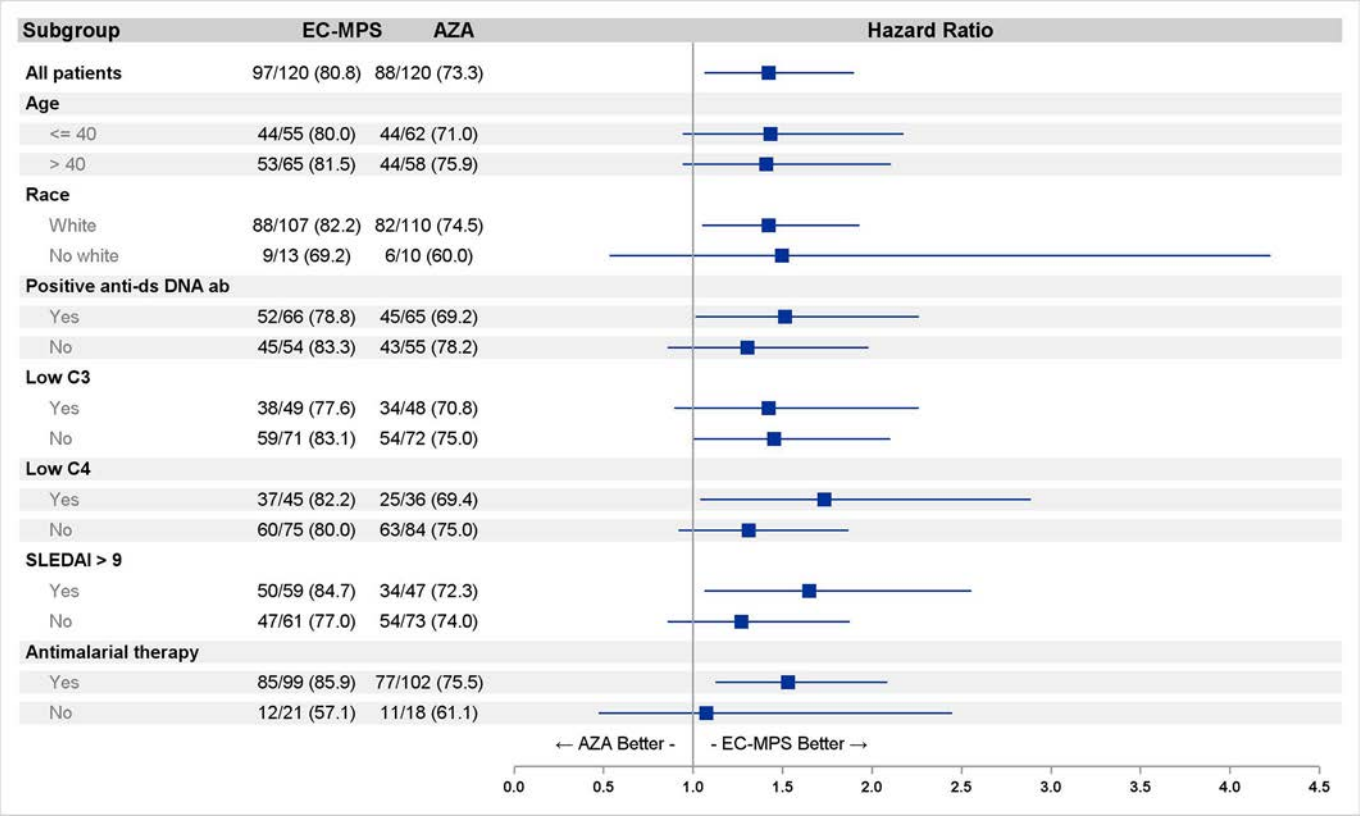


**Figure 3** Risk of clinical remission in patient subgroups. The HR was derived from a Cox model, with treatment as the only factor, according to subgroup. AZA, azathioprine; dsDNA, double-stranded DNA; EC-MPS, enteric-coated mycophenolate sodium.

EC-MPS group discontinued the study. The main reasons for early withdrawal were treatment failure and AEs (figure 1).

## Treatment

Mean (SD) doses of EC-MPS and AZA during the study were 1.18 (0.29) g and 123.2 (22) mg, respectively. The proportion of patients whose average daily dose was 80% or more of the target dose at 3 months was 79.2% for EC-MPS and 75.8% for AZA. Mean doses at withdrawal for patients with treatment failure were 1.35 (0.223) g and 133 (20.9) mg, respectively. The mean treatment duration was 575 (min 30 and max 730) days for EC-MPS and 496 (30–730) for AZA. Few patients in CR could discontinue the study agents, 4 (5.8%) in the AZA group and 10 (11.4%) in the EC-MPS group. Antimalarials were discontinued in eight patients (6.7%) in the EC-MPS and in two (1.7%) in the AZA group (see online supplementary table S1).

## Outcomes

### Primary endpoint

Clinical remission rates were higher in the EC-MPS group by month 3 (32.5% (39/120 patients)) compared with the AZA group (19.2% (23/120); percentage difference 13.3% (95% CI 2.3 to 24), p=0.034) and sustained throughout the study to month 24 (71.2% (84/118) vs 48.3% (57/118); percentage difference 22.9% (95% CI 10.4 to 34.4), p<0.001) (figure 2A, online supplementary table S2). Median time to CR was 6 months (95% CI 5 to 9) in the EC-MPS group and 12 months (95% CI 9 to 16) in the AZA group (p=0.002). The HR for time to CR with EC-MPS use was 1.42 (95% CI 1.07 to 1.90; p=0.017) (figure 2B). There were also more SRI4 and LLDAS responders at 3 months (p=0.053) and at 24 months (p<0.0001) in the EC-MPS group (see online supplementary table S2).

SLEDAI-2K and BILAG-2004 scores showed an improvement over time reflecting the reduction in disease activity in both groups. This reduction was superior in the EC-MPS group. BILAG score difference was already statistically significant at month 3 (p=0.011) (figure 2C) whereas SLEDAI-2K score reached statistical significance at month 24 (p=0.006) (figure 2D). Resolution of disease activity (from BILAG A/B to BILAG D) in most individual body systems was similar in both groups, except for the cardiorespiratory domain with more EC-MPS-treated patients reaching CR at 3 months (p=0.015) (see online supplementary figure S1). Subgroup analysis did not show evidence of different clinical response (figure 3). Cumulative rates of treatment failure at 24 months were higher in the AZA group (31.7% (38/120 patients)) compared with the EC-MPS group (18.3% (22/120)) (p=0.099)).

### Secondary endpoints

#### *SLE flares*

BILAG A/B flares were more common in the AZA group (71.7% (86/120 patients)) compared with the EC-MPS group (50% (60/120)) (p<0.001). In the AZA and EC-MPS groups, 34.2% and 35% patients had 1 disease flare; 21.7% and 13.3% had 2 flares; and 16.7% and 5% had >2 flares, respectively. Mucocutaneous and renal flares were more frequent in the AZA group (p=0.003 and p=0.031, respectively) (figure 4A). Flares were associated with medication reduction in 38 patients (31.7%) of the AZA group and 29 (24.2%) of the EC-MPS group. Rates of new BILAG A flares were low, but significantly higher in AZA (21.7% (26/120) vs 8.3% EC-MPS (10/120), p=0.004) (figure 4B). BILAG A biopsy-proven glomerulonephritis occurred in 5.8% (7/120; all type III–IV) of patients in the AZA group compared with 0.8% (1/120; type V) in the EC-MPS group (p=0.031). The HR for time to first BILAG A/B and

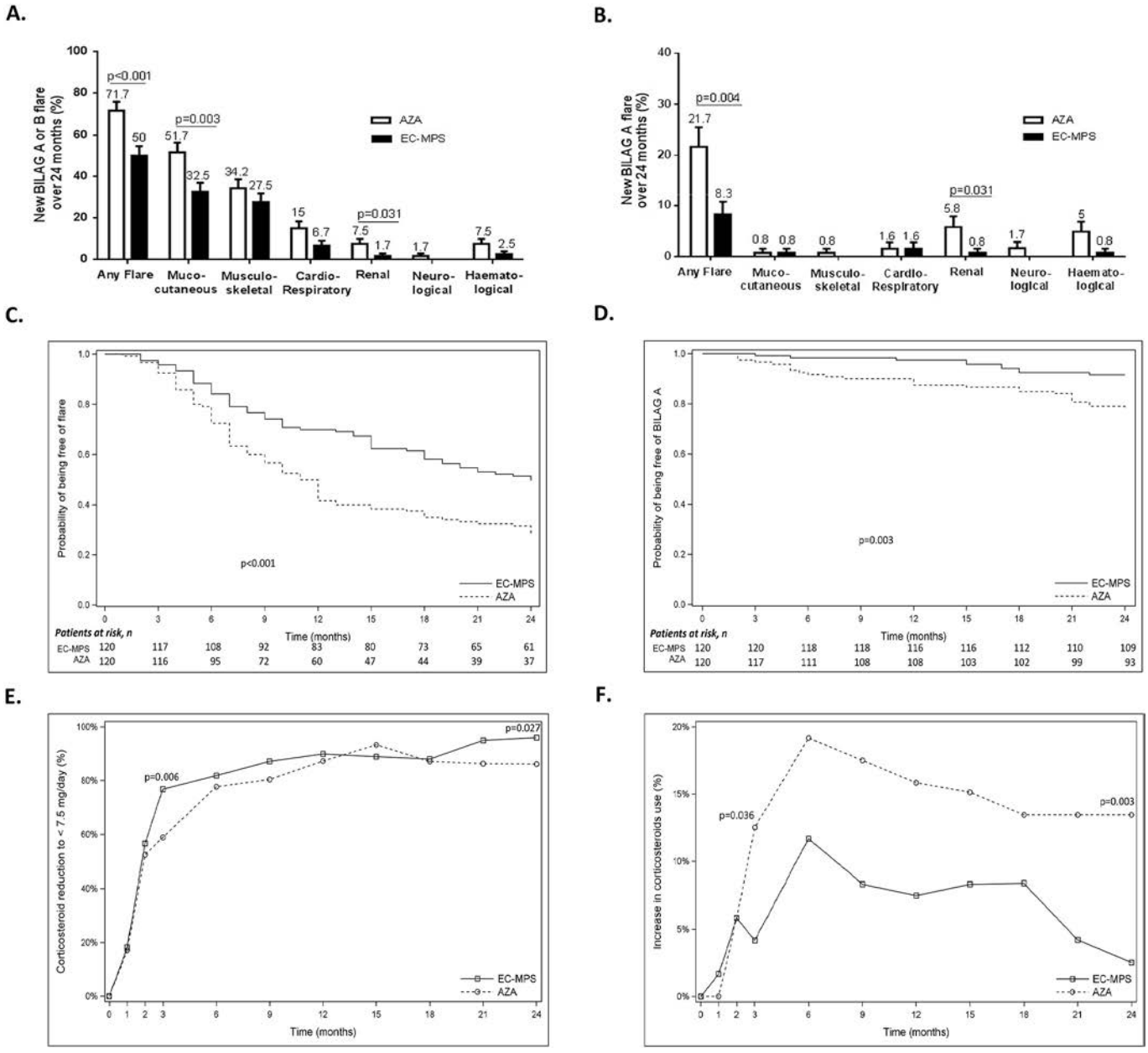


**Figure 4** Results for secondary efficacy endpoints. Proportion of patients with a new flare of SLE over the 24-month study period. Flare is determined by a BILAG index A or B (A). Flare is determined by a BILAG score of A only (B). Cumulative probability of being free of BILAG A/B flare (C) and BILAG A only flare (D). Percentage of patients with corticosteroid dose reduced to ≤7.5 mg/day from ≥7.5 mg/day at baseline (n= 103 in the AZA group and n=98 in the EC-MPS group) (E), and percentage with increased corticosteroid use over 24 months (F). Analyses are based on the intention-to-treat population. Values at the top of the bars in (A) and (B) are actual percentages, with SE represented. AZA, azathioprine; BILAG, British Isles Lupus Assessment Group; EC-MPS, enteric-coated mycophenolate sodium; SLE, systemic lupus erythematosus.

BILAG A was 1.84 (95% CI 1.32 to 2.57; p<0.001) and 2.81 (95% CI 1.36 to 5.84; p=0.003), respectively (figure 4C,D). No association with anti-dsDNA antibody positivity or complement levels was found.

### *Corticosteroid use*

Reduction of the prednisone dose (<7.5) by month 24 among those patients taking ≥7.5 mg/day at inclusion was higher in the EC-MPS group (94.9% (93/98) patients) compared with the AZA group (83.5% (86/103), p=0.027) (figure 4E). During the study, mean prednisone dose decreased from 28.6 (21.2) to 4.2 (2.3) mg/day in the EC-MPS group compared with 23.9 (18.1) to 6.8 (9.2) mg/day in the AZA group (p=0.037). Fewer rescue increments (≥7.5 mg/day) were required with EC-MPS (figure 4F) (see online supplementary table S1). Prednisone discontinuation occurred in 10.5% (12/114) and 17.2% (20/116) of the AZA and EC-MPS groups, respectively.

### *Changes in immunological parameters*

Mean anti-dsDNA antibody level reductions from baseline were greater in the EC-MPS group at month 3 (p<0.001). No differences in mean C3 level increments were observed (see online supplementary figure S2).

## Adverse events

The incidence of AEs was similar in both groups: 59.2% (71/120) patients given EC-MPS and 57.5% (69/120) patients

**Table 2** Incidence of AEs that emerged during treatment and serious AEs

| Event | EC-MPS (n=120) | AZA (n=120) | p Value |
|---|---|---|---|
| | *No of patients (%)* | | |
| All AEs* | 71 (59.2) | 69 (57.5) | 0.793 |
| All serious AEs | 11 (9.2) | 13 (10.8) | 0.667 |
| All AEs leading to withdrawal | 4 (3.3) | 10 (8.3) | 0.098 |
| Frequent non-serious AEs | | | |
| Upper gastrointestinal symptoms | 9 (7.5) | 16 (13.3) | 0.139 |
| Liver toxicity | 0 | 3 (2.5) | 0.081 |
| Infectious | 34 (28.3) | 26 (21.6) | 0.233 |
| Upper respiratory tract infection | 16 (13.3) | 12 (10) | 0.421 |
| Pneumonia | 3 (2.5) | 1 (0.8) | 0.313 |
| Urinary tract infection | 4 (3.3) | 4 (3.3) | 1.00 |
| Herpes varicella zoster | 5 (4.2) | 3 (2.5) | 0.472 |
| Skin and soft tissue infection | 2 (1.7) | 2 (1.7) | 1.00 |
| Influenza | 3 (2.5) | 2 (1.7) | 0.651 |
| Oral candidiasis | 1 (0.8) | 2 (1.7) | 0.313 |
| Leucopenia | 0 | 5 (4.2) | 0.024 |
| Serious AEs | | | |
| Death | 1 (0.8) | 1 (0.8) | 1.00 |
| Malignant conditions | 1 (0.8) | 3 (2.5) | 0.313 |
| Pneumonia | 4 (3.3) | 3 (0.8) | 0.701 |
| Pyelonephritis | 1 (0.8) | 2 (1.7) | 0.313 |
| Soft tissue infection | 0 | 2 (1.7) | 0.157 |
| Acute coronary syndrome | 0 | 1 (0.8) | 0.316 |
| CVA | 3 (2.5) | 1 (0.8) | 0.313 |
| Subarachnoid haemorrhage | 1 (0.8) | 1 (0.8) | 1.00 |

The terms used to describe the events are those preferred by the Medical Dictionary for Regulatory Activities, version 12.0. Only AEs that occurred during treatment are listed; these include any serious and non-serious AE that occurred between the date of the first dose and the date of last visit plus 30 days. Multiple occurrences of the same AE in one person were counted only once. SLE flares were not considered AEs. This category includes all patients who had at least one AE.
AE, adverse event; AZA, azathioprine; CVA, cerebrovascular accident; EC-MPS, enteric-coated mycophenolate sodium; SLE, systemic lupus erythematosus.

given AZA (p=0.793) (table 2). The rate of serious events was also similar in both groups. Infections were the most common AEs with an overall rate of 32.5% (39/120 patients) in EC-MPS and 27.5% (33/120) in the AZA group (p=0.398). The rate of serious infections was low in both groups: 4.2% (5/120) patients in the EC-MPS group and 5.8% (7/120) in the AZA group.

The proportion of patients with AEs leading to withdrawal was slightly higher with AZA (8.3% (10/120)) than with EC-MPS (2.5% (4/120), p=0.06). Leucopenia was more frequent in the AZA group. One death occurred in each group, both due to complicated pneumonia. Three cases of cancer (two breast cancers and one thymoma) occurred in the AZA group and one (cervix carcinoma) in the EC-MPS group.

## DISCUSSION

There are few data on the use of non-biological agents for the management of extrarenal lupus disease. This is the first multicentre randomised long-term trial to demonstrate the superiority of EC-MPS over AZA in achieving better clinical remission rates in moderate-to-severe active non-renal lupus disease. Most patients achieved their target dose and remained in the study for the full 24 months. The study was adequately powered to assess the primary outcome, which was achieved across the treatment groups and provided valuable data of two frequently prescribed therapies in SLE.

To date, there are limited data from controlled clinical trials about the use of MMF in non-renal disease.[19–24] A systematic review has identified 24 relevant studies including approximately 850 patients. Although the studies were mainly case series or open-label trials, the data suggest MMF to be effective for refractory haematological and dermatological manifestations.[19–22] The only RCT by Ginzler *et al*[23] showed similar efficacy between MMF and cyclophosphamide in reducing non-renal disease activity, measured by BILAG score. However, results should be interpreted with caution as the study was designed for LN, high-dose corticosteroids were used as induction treatment and the BILAG index was not the primary endpoint measure in the Aspreva Lupus Management Study (ALMS).[13] Our results support our hypothesis that EC-MPS would be more effective than AZA for attaining remission and maintaining clinical response during the 24-month study period. Higher rates of CR were observed as early as 12 weeks in the EC-MPS group and continued to increase over time. Secondary endpoint results including time to CR, reduction in lupus disease activity indices and the dose of corticosteroids also confirmed the superiority of EC-MPS over AZA. Although the study was not powered to demonstrate the treatment efficacy in individual organs, both study agents showed a similar profile of individual organ response except for an early remission in the cardiorespiratory domain with EC-MPS. Neurological symptoms also seemed to respond earlier in the EC-MPS group, but low sample size, the heterogeneity of neurological manifestations and the fact that some more severe patients may have been excluded prevented drawing conclusions.

We found that EC-MPS was also more effective at preventing relapses and its effect was consistent across moderate and severe flares. Moderate-to-severe SLE flares occurred in 50% of patients receiving EC-MPS compared with 71.7% of patients given AZA. Most flares were articular and mucocutaneous and in 25%–30% of cases occurred while reducing the dose of concomitant medication. Rates of severe flares were low but higher in the AZA group and mainly of haematological and renal nature. EC-MPS reduced by 45% and 65% the risk of developing any SLE flare and severe flare, respectively. The superiority of MMF over AZA in preventing LN flares has been previously reported during the maintenance phase of the ALMS.[14]

The occurrence of AEs and serious events was similar in both groups except for gastrointestinal side effects, including liver toxicity, and haematological events, which were more common in the AZA group, consistent with previous findings[7 8 10 14 15] and were readily controlled by dose adjustments. Frequency of serious infections was low and similar in both groups. Two patients died during the study. One death occurred in each group due to pneumonia complications.

The study has some limitations. First, this is an investigator-led clinical trial with mainly Caucasian patients rather than a large international multiethnic study. Second, it was an open-label and not double-blinded trial. However, given that outcomes were strictly evaluated by an independent assessor they are unlikely to have been influenced by knowledge of patient allocation. Third, serum measures of the active metabolites of AZA (ie, 6-TGN) or EC-MPS (mycophenolic acid) were not routinely performed, leaving open the possibility that patients who failed treatment were underdosed or non-adherent to medication. Fourth, the liberty to adjust corticosteroids during the study could have confounded SLE disease activity assessments. Finally, although the trial is substantially long, potential outcomes that might

appear later in time (eg, cardiovascular complications) cannot be determined.

Despite AZA being shown to be less effective, its safety profile during pregnancy is a significant advantage over EC-MPS. MMF/EC-MPS are absolutely contraindicated. MMF is likely to be a human teratogen based on the reported malformations observed among exposed offspring (microtia, orofacial clefts, external auditory canal atresia and cardiovascular malformations).[36] When long-term immunosuppression is required in young women planning pregnancy this issue needs to be considered.

We conclude that EC-MPS is superior to AZA in achieving long-term clinical remission and in preventing relapse in patients with active non-renal lupus disease.

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**Competing interests** None declared.

**Patient consent** Patient enrolled in the study signed the patient consent form approved by the Ethic Committee of Vall d'Hebron Hospital and by the Agencia Espaola del medicamento y productos sanitarios (AEMPS).

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OPEN ACCESS

EXTENDED REPORT

# Serious adverse events and the risk of stroke in patients with rheumatoid arthritis: results from the German RABBIT cohort

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## ABSTRACT

**Objective** In the general population, the incidence of stroke is increased following other serious events and hospitalisation. We investigated the impact of serious adverse events on the risk of stroke in patients with rheumatoid arthritis (RA), taking risk factors and treatment into account.

**Methods** Using data of the German biologics register RABBIT (Rheumatoid Arthritis: Observation of Biologic Therapy) with 12354 patients with RA, incidence rates (IRs) and risk factors for stroke were investigated using multi-state and Cox proportional hazard models. In addition, in a nested case–control study, all patients with stroke were matched 1:2 to patients with identical baseline risk profile and analysed using a shared frailty model.

**Results** During follow-up, 166 strokes were reported. The overall IR was 3.2/1000 patient-years (PY) (95% CI 2.7 to 3.7). It was higher after a serious adverse event (IR: 9.0 (7.3 to 11.0)), particularly within 30 days after the event (IR: 94.9 (72.6 to 121.9)). The adjusted Cox model showed increased risks of age per 5 years (HR: 1.4 (1.3 to 1.5)), hyperlipoproteinaemia (HR: 1.6 (1.0 to 2.5)) and smoking (HR: 1.9 (1.3 to 2.6)). The risk decreased with better physical function (HR: 0.9 (0.8 to 0.96)). In the case–control study, 163 patients were matched to 326 controls. Major risk factors for stroke were untreated cardiovascular disease (HR: 3.3 (1.5 to 7.2)) and serious infections (HR:4.4 (1.6 to 12.5)) or other serious adverse events (HR: 2.6 (1.4 to 4.8)).

**Conclusions** Incident adverse events, in particular serious infections, and insufficient treatment of cardiovascular diseases are independent drivers of the risk of stroke. Physicians should be aware that patients who experience a serious event are at increased risk of subsequent stroke.

## INTRODUCTION

Cerebrovascular diseases are a major health concern worldwide representing the second most common cause of death and the most frequent reason for disability.[1] Two main types are distinguished—ischaemic and haemorrhagic strokes—depending on their aetiology. In the general population, risk factors for stroke are divided into *non-modifiable* such as age, gender, family predisposition or genotype and *modifiable* such as management of underlying comorbidities (eg, hypertension) or lifestyle (eg, smoking).[2 3] Recently, elevated levels of the cytokines tumour necrosis factor (TNF)-alpha and interleukin 6, as well as of high-sensitivity C reactive protein (CRP) were discussed additionally as ischaemic stroke promoters.[3]

Compared with the general population, the risk of stroke is higher in patients with rheumatoid arthritis (RA). A recently published meta-analysis states significantly higher risks for ischaemic (OR: 1.64) and haemorrhagic (OR: 1.68) strokes in patients with RA.[4] Nonetheless, investigations of risk factors for stroke in RA are scarce. In a matched case–control study, ischaemic stroke was predicted by RA severity and prevalent comorbidities.[5] Other authors identified elevated erythrocyte sedimentation rate (ESR)[6 7] and CRP values[7] as risk factors for ischaemic stroke.

Novel approaches in the general population have taken precedent adverse events (AEs) into account and showed significant associations of incident stroke with infections,[8 9] hospitalisation[10] and cancer.[11] The authors hypothesised pathogenic mechanisms of serious infections, dehydration during hospitalisation and pathophysiological complications of cancer as triggering events for stroke.

These findings suggest that prior AEs should also be considered in RA as possible triggers for stroke in addition to known risk factors. Calabrese *et al*[12] found a time-dependent risk for stroke after herpes zoster, being highest within the first 90 days after diagnosis. So far, it is unclear whether similar mechanisms or pathways also apply to other AEs in patients with RA.

The aim of our study was to investigate risk factors for non-haemorrhagic stroke in patients with RA using data of a large observational cohort study. We were interested in the impact of RA-specific disease characteristics such as inflammation, treatment with conventional synthetic (cs) or biological (b) disease-modifying antirheumatic drugs (DMARDs) and the role of other AEs regarding the risk to develop stroke. To address confounding by different risk profiles in patients with and without stroke, we performed a nested case–control study which allowed controlling for known risk factors.

## PATIENTS AND METHODS

### Data source and assessments

Data of the German biologics register RABBIT (Rheumatoid Arthritis: Observation of Biologic Therapy), a prospective cohort study, were used. Patients with RA are enrolled when starting treatment with a bDMARD or csDMARD after at least

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one csDMARD failure. Clinical-derived and patient-derived data are reported at predefined time points of follow-up (baseline, at 3 and 6 months, thereafter every 6 months). Regularly collected data comprise disease activity measures, treatment details (eg, start/stop dates of DMARDs and dosages of glucocorticoids) and AEs. Rheumatologists are requested to give additional information about serious AEs (SAEs) and to provide hospital discharge letters.

Comorbidities and whether they were medically treated were reported by the rheumatologists at baseline. Among others, patients specified their physical function (Hannover Functional Status Questionnaire (FFbH)[13]) and their global health. Further details of RABBIT were reported elsewhere.[14–16] The study protocol of RABBIT was approved by the ethics committee of the Charité University Medicine Berlin. Patients have to give their written informed consent prior to enrolment.

### Outcome definition

All incident cerebrovascular events reported until 31 October 2015 were reviewed by the study physician of RABBIT (AS). Events were categorised as ischaemic, haemorrhagic and unclassified strokes as well as transient ischaemic attacks (TIAs) and subarachnoid haemorrhages. Only the first event of a non-haemorrhagic stroke (ischaemic or unclassified strokes or TIAs) in a patient was considered in this analysis.

In addition, all reported AEs apart from stroke classified as being serious according to the International Council for Harmonisation (ICH) definition[17] with event dates either reported by rheumatologists or from hospital discharge letters were investigated. We categorised the SAEs into: infections, cardiovascular (CV) events but not stroke, surgeries and all remaining SAEs.

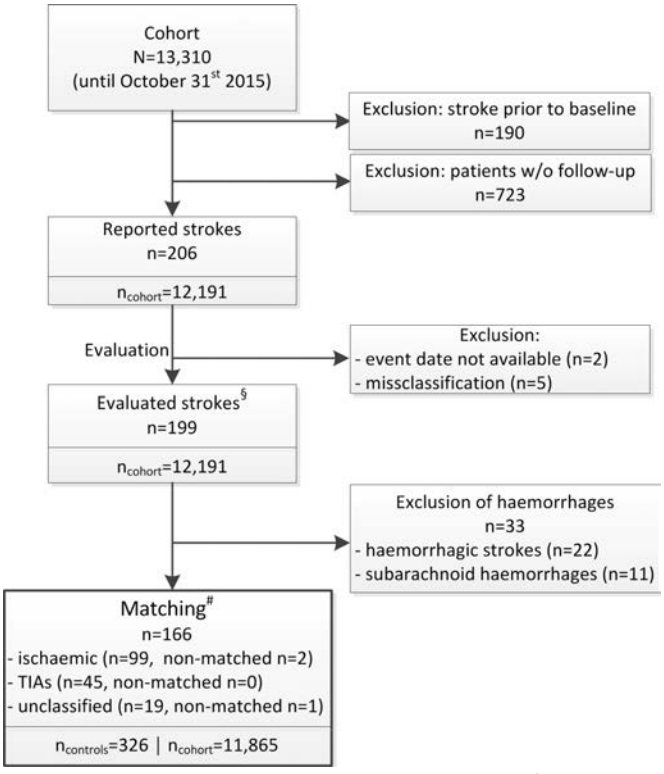


**Figure 1** Flow chart for patient selection and matching. §Diagnoses of evaluated events are listed in the online Supplementary table 1. #Cases were matched to potential controls in a 1:2 manner using the following criteria: gender, age at baseline (±5 years), enrolment episode (2001–2006 and 2007–2015), four baseline comorbidities (hypertension, coronary heart disease, heart failure and diabetes) and smoking habits (never and ever/unknown). Patients with no possible matching are listed in the online Supplementary table 2. TIA, transient ischaemic attack; w/o, without.

### Study design: cohort study and nested case–control study

Risk factors for stroke were first analysed with data from the entire cohort. Second, we performed a nested case–control study. Patients who developed a stroke were selected as cases. We applied an extensive matching algorithm with a 1:2 ratio (one case: two controls; they form one cluster). Exact agreement of cases and their controls was required regarding gender, hypertension, coronary heart disease, heart failure, diabetes, smoking habits (never vs ever/unknown) and enrolment episode (2001–2006 and 2007–2015). Age had to be similar in cases and controls (±5 years). Eligible controls had to be under observation at the date of stroke of the matching case (index date).

### Definition of treatment exposure

Treatment with DMARDs was categorised into (1) TNF-inhibitors (TNFi) (adalimumab, certolizumab, etanercept, golimumab and infliximab), (2) other bDMARDs (abatacept, anakinra, rituximab and tocilizumab) and (3) csDMARDs. In (1) and (2), a combination with csDMARDs was possible; group (3) was exclusively treated with one or more csDMARD(s). Patients were considered to be exposed to a certain bDMARD up to 3 months after treatment discontinuation (rituximab: 9 months after last infusion).

Current and cumulative treatment was investigated for the use of non-steroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids. The cumulative treatment with NSAIDs was calculated for each patient as the portion of observation time exposed to NSAIDs (range: 0–1). Similarly, cumulative treatment with glucocorticoids was calculated, but additionally weighted for different doses: each month with a dose of >5–10 mg/day was considered with a weight of 0.5 and each month with a dose of >10 mg/day with a weight of 1. The total sum over all weights was divided by the number of follow-up months (range: 0–1).

Patients with hypertension, coronary heart disease, heart failure or hyperlipoproteinaemia but without drug treatment for this condition(s) were labelled as having 'no CV treatment'. Patients with diabetes or osteoporosis and no treatment were marked accordingly.

### Statistical analysis

For baseline comparisons in the cohort study, t-test and $\chi^2$ test were applied. In the matched case–control study, univariate linear mixed effects models with a random component for each cluster were used to test for differences between cases and controls.

Risk factors for stroke were investigated using two different approaches: In approach 1, we applied univariate and multiple Cox proportional hazard (PH) models in (1) the whole cohort and (2) the nested case–control study. In the case–control study, we considered the matching structure by the application of a shared frailty Cox regression model,[18] which can be interpreted like Cox-PH models (for further explanations see online Supplementary text).

In approach 2, we adapted the idea of *multi-state models*[19 20] (online Supplementary figure 1). In brief, we were interested in the cumulative incidence of stroke in patients who (1) did not develop or (2) developed an SAE other than cerebrovascular prior to stroke. Exact Poisson confidence intervals

**Table 1** Baseline characteristics of the RABBIT cohort, cases (patients who developed stroke during follow-up) and their matched controls

| | Remainder of the cohort, n=11 865 | Controls, n=326 | Cases, n=163 |
|---|---|---|---|
| Matching criteria | | | |
| Gender, female | 9071 (76.5) | 244 (74.8) | 122 (74.8) |
| Age (years), mean (SD) | 55.8 (12.5)* | 62.6 (10.2) | 63.4 (10.7) |
| Hypertension | 4354 (36.7)* | 184 (56.4) | 92 (56.4) |
| Coronary heart disease | 672 (5.7) | 30 (9.2) | 15 (9.2) |
| Heart failure | 262 (2.2) | 6 (1.8) | 3 (1.8) |
| Diabetes mellitus | 1157 (9.8)* | 54 (16.6) | 27 (16.6) |
| Smoking, never | 5153 (43.4) | 132 (40.5) | 66 (40.5) |
| Smoking, ever and unknown | 6712 (56.6) | 194 (59.5) | 97 (59.5) |
| Enrolment period (prior 2007) | 4773 (40.2)* | 174 (53.4) | 87 (53.4) |
| Unmatched criteria | | | |
| Time to event/index date (months), mean (SD) | – | 46.6 (31.9) | 46.6 (32.0) |
| Observation time (months), mean (SD) | 48.9 (33.0)* | 73.7 (32.8) | 68.3 (32.3) |
| Disease duration (years), mean (SD) | 9.7 (9.0) | 11.3 (9.7) | 10.9 (9.2) |
| Rheumatoid factor positive | 8379 (71.2)* | 250 (77.2) | 128 (79.0) |
| CRP (mg/L), mean (SD) | 18.4 (26.0)* | 21.4 (39.6) | 24.2 (31.3) |
| ESR (mm/hour), mean (SD) | 30.7 (22.7)* | 33.2 (23.3) | 35.6 (25.6) |
| DAS28, mean (SD) | 5.1 (1.3)* | 5.4 (1.4) | 5.4 (1.3) |
| % of full physical function, mean (SD) | 64.0 (23.1)* | 60.3 (22.8) | 54.0 (23.8) |
| NRS patient global health 0–10, mean (SD) | 6.0 (2.1)* | 6.1 (2.1) | 6.5 (2.2) |
| BMI ≥30 kg/m$^2$ | 2818 (23.8) | 76 (23.3) | 41 (25.2) |
| Hyperlipoproteinaemia | 921 (7.8)* | 39 (12) | 27 (16.6) |
| Chronic renal disease | 437 (3.7)* | 24 (7.4) | 12 (7.4) |
| Osteoporosis | 2089 (17.6)* | 77 (23.6) | 49 (30.1) |
| ≥2 comorbidities | 4634 (39.1)* | 181 (55.5)† | 102 (62.6) |
| No CV treatment | 1038/4849 (21.4)* | 41/195 (21.0)† | 35/104 (33.7) |
| No diabetes treatment | 226/1157 (19.5) | 16/54 (29.6) | 4/27 (14.8) |
| No osteoporosis treatment | 325/2089 (15.6) | 13/77 (16.9) | 6/49 (12.2) |
| No of previous csDMARDs, mean (SD) | 2.2 (1.4) | 2.6 (1.4) | 2.6 (1.5) |
| No of previous bDMARDs, mean (SD) | 0.3 (0.7) | 0.3 (0.6) | 0.4 (0.9) |
| Enrolment therapy: csDMARD | 3874 (32.9) | 110 (34.2) | 47 (29.6) |
| Enrolment therapy: TNFi | 6009 (51.0) | 157 (48.8) | 81 (50.9) |
| Enrolment therapy: other bDMARD | 1907 (16.2) | 55 (17.1) | 31 (19.5) |
| Glucocorticoids, <5 mg/day | 4748 (40.0) | 136 (41.7) | 49 (30.1) |
| Glucocorticoids, 5–10 mg/day | 4718 (39.8) | 133 (40.8) | 82 (50.3) |
| Glucocorticoids, ≥10 mg/day | 2351 (19.8) | 54 (16.6) | 32 (19.6) |
| Any NSAID | 6150 (51.8) | 189 (58) | 89 (54.6) |

Values are numbers of patients (%) unless otherwise specified.
*p<0.05 in unpaired tests versus cases (t-test or $\chi^2$ test).
†p<0.05 in paired tests versus cases (linear mixed effects model with a random component, differences in comorbidity treatment were analysed with $\chi^2$ test).
bDMARD, biological disease-modifying anti-rheumatic drug; BMI, body mass index; CRP, C reactive protein; csDMARD, conventional synthetic disease-modifying antirheumatic drug; CV, cardiovascular; DAS28, disease activity based on 28 joint count; ESR, erythrocyte sedimentation rate; NRS, numeric rating scale; NSAID, non-steroidal antirheumatic drug; TNFi, inhibitors of tumour necrosis factor alpha.

were calculated for incidence rates (IRs). Furthermore, we estimated cause-specific hazards to investigate risk factors for stroke in patients without prior SAEs. In this model, patients were censored at the end of the observation (index date) or when other SAEs occurred, whatever came first.

Due to the skewed distribution of CRP values, we used a log-transformation (logCRP) in all models.

Missing data at baseline most frequently concerned smoking status (10.2%), CRP (6.5%), disease activity based on 28 joint count (DAS28) (4.6%) and ESR (3.6%). To analyse the course of disease activity and inflammation, we applied five multiple imputations of missing values. Missing smoking status was coded as a separate category (smoking unknown).

Estimates are shown with 95% CI. Matching was applied using the R-package Optmatch.[21] Data were analysed using SAS V.9.4 software.

## RESULTS

Until 31 October 2015, 206 incident cerebrovascular events were reported (figure 1). We excluded two patients without an available event date and five patients with a misclassification of the reported event. Of 199 events, the majority were ischaemic strokes (n=101, 50.8%), followed by TIAs (n=45, 22.6%), haemorrhagic (n=22, 11.1%) and unclassified strokes (n=20, 10.1%) as well as subarachnoid haemorrhages (n=11, 5.5%).

**Table 2** Disease characteristics in the cohort and in the nested case–control study in different time periods

| | Cohort study | Nested case–control study | |
|---|---|---|---|
| | Remainder of the cohort | Controls | Cases |
| **Disease activity and inflammation** | | | |
| Averages during the first year of follow-up after enrolment | | | |
| DAS28 (95% CI) | **4.25 (4.23 to 4.27)** | 4.37 (4.24 to 4.51) | 4.62 (4.45 to 4.80) |
| CRP (mg/L) (95% CI) | **13.42 (13.10 to 13.73)** | 14.27 (12.23 to 16.31) | 18.50 (14.32 to 22.68) |
| ESR (mm/hour) (95% CI) | **25.69 (25.36 to 26.02)** | 27.03 (24.94 to 29.12) | 30.79 (27.61 to 33.96) |
| Values within a 6 months risk window before the event/index date | | | |
| DAS28 (95% CI) | | **3.50 (3.31 to 3.69)** | 4.06 (3.79 to 4.34) |
| CRP (mg/L) (95% CI) | | 8.02 (6.12 to 9.93) | 16.19 (8.12 to 24.26) |
| ESR (mm/hour) (95% CI) | | 21.45 (18.93 to 23.97) | 27.98 (23.68 to 32.29) |
| **Treatment** | | | |
| Time from baseline until event/index date | | | |
| Cumulative doses of GC | | | |
| Exposure to 0–5 mg/day | | 77.7% (74.2 to 81.3) | 73.0% (68.2 to 77.7) |
| Exposure to ≥10 mg/day | | 4.1% (2.5 to 5.8) | 5.2% (2.8 to 7.5) |
| Cumulative use of Cox-2-Inh. | | 0.12% (0.09 to 0.15) | 0.15% (0.11 to 0.19) |

Bold indicates significant values compared to case patients.
Cox-2-Inh., inhibitors of cyclooxygenase-2; CRP, C-reactive protein; DAS28, disease activity based on 28 joint count; ESR, erythrocyte sedimentation rate; GC, glucocorticoids; NSAIDs, non-steroidal anti-inflammatory drugs.

Corresponding baseline characteristics are presented in the online Supplementary table 3. Of the 166 events considered in this analysis (ischaemic strokes, unclassified strokes and TIAs), 163 could be matched to controls (n=326). For one female and two male cases, matching was not possible (online Supplementary table 2).

### Patient characteristics at baseline

Case and control patients were 7 years older than the average cohort patient and differed significantly in comorbid hypertension, diabetes, enrolment period and disease activity at baseline (table 1).

A significant difference between cases and controls was found regarding the treatment of comorbidities. Of 104 case patients with at least one baseline CV comorbidity, 35 (34%) did not receive CV drug treatment, compared with 21% in controls and in the remaining cohort. Thereof, major gaps were seen regarding hyperlipoproteinaemia (no drug treatment in 78% of cases, 44% of controls and 47% in the cohort) and coronary heart disease (no drug treatment in 40% of cases, 30% of controls and 19% in the cohort). These significant differences in the management of comorbid conditions were not found for diabetes and osteoporosis.

### Cumulative incidence of stroke and influences of SAEs in the cohort

The overall rate of incident non-haemorrhagic strokes (n=166) in the RABBIT cohort was 3.2/1000 patient-years (PY) (95% CI 2.7 to 3.7) (online Supplementary figure 2). The IR in patients with no prior SAE was 2.2/1000 PY (95% CI 1.8 to 2.8] and with prior SAE 9.0/1000 PY (95% CI 7.3 to 11.0).

We found a linear increase in the cumulative incidence of stroke in patients who did not experience any SAE prior to stroke (figure 2, left). In contrast, there was an excess risk within the first 30 days after SAEs (figure 2, right). In this interval, the IR was 94.9/1000 PY (95% CI 72.6 to 121.9), dropping

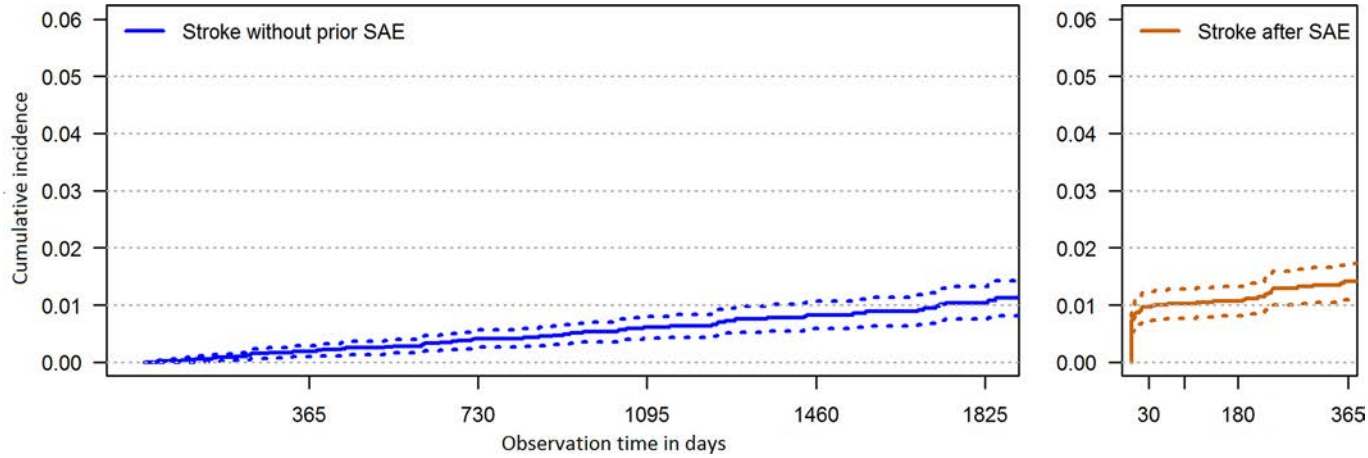


**Figure 2** Probability of stroke in patients with and without prior SAE. (Left) The probability of stroke after enrolment in patients without any serious adverse event (SAE) prior to stroke; time in days from baseline. (Right) The probability of stroke after SAE; time in days after SAE.

significantly to 3.4 (95% CI 2.4 to 4.8) in the period thereafter. Of all reported SAEs, 87.0% led to hospitalisation.

### Disease characteristics and treatment of cases, controls and the remaining cohort during follow-up

Patients with stroke presented with significantly higher DAS28 and inflammation markers during the first year of follow-up compared with the remaining cohort in unadjusted analyses. In the nested case–control study, values were insignificantly higher in cases than in controls (table 2). Within 6 months before the event/index date, the mean DAS28 was significantly higher in cases compared with controls.

No differences were observed in the cumulative doses of glucocorticoids, or the use of non-selective NSAIDs and Cox-2 inhibitors.

### Risk factors for stroke

In the cohort study, univariate analysis showed a significantly lower risk for stroke in patients with better physical function (FFbH) (table 3). Older age, high values of CRP, ESR and the DAS28 were significantly associated with a higher risk for stroke. Comorbidities such as hypertension, hyperlipoproteinaemia, diabetes, osteoporosis and particularly chronic renal

**Table 3** Investigation of risk factors for stroke

| | Cohort study | | Nested case–control study | |
|---|---|---|---|---|
| | Univariate analysis | Adjusted Cox-PH model | Univariate analysis | Adjusted shared frailty model |
| | HR (95% CI) | HR (95% CI) | HR (95% CI) | HR (95% CI) |
| Age per 5 years* | 1.42 (1.32 to 1.54) | 1.37 (1.25 to 1.50) | | |
| Gender, male* | 1.19 (0.84 to 1.70) | 1.03 (0.70 to 1.52) | | |
| logCRP | 1.30 (1.12 to 1.52) | 1.16 (0.99 to 1.35) | 1.41 (1.17 to 1.70) | 1.17 (0.98 to 1.40) |
| ESR | 1.07 (1.04 to 1.11) | | 1.06 (1.02 to 1.09) | |
| DAS28 | 1.28 (1.15 to 1.42) | | 1.33 (1.17 to 1.51) | |
| % of full physical function per 10 points | 0.83 (0.78 to 0.88) | 0.90 (0.84 to 0.96) | 0.92 (0.86 to 0.98) | 0.85 (0.78 to 0.93) |
| Hypertension* | 2.47 (1.81 to 3.37) | 1.33 (0.95 to 1.86) | | |
| Coronary heart disease* | 1.91 (1.12 to 3.25) | | | |
| Heart failure* | 1.12 (0.36 to 3.52) | | | |
| Hyperlipoproteinaemia | 2.57 (1.70 to 3.89) | 1.60 (1.04 to 2.45) | 1.60 (1.05 to 2.44) | |
| Diabetes mellitus* | 2.13 (1.41 to 3.21) | 1.26 (0.82 to 1.94) | | |
| Chronic renal disease | 2.91 (1.61 to 5.25) | 1.28 (0.69 to 2.36) | 1.92 (1.06 to 3.49) | |
| Osteoporosis | 1.84 (1.32 to 2.57) | 1.09 (0.77 to 1.56) | 1.47 (1.05 to 2.06) | |
| ≥2 comorbidities | 2.89 (2.10 to 3.97) | | 1.99 (1.44 to 2.76) | |
| No CV disease (Reference) | | | | |
| CV disease with therapy | 2.41 (1.70 to 3.42) | | 1.51 (0.98 to 2.32) | 1.81 (0.85 to 3.82) |
| CV disease and no therapy | 4.31 (2.83 to 6.54) | | 3.11 (1.89 to 5.10) | 3.31 (1.52 to 7.19) |
| csDMARD (Reference) | | | | |
| TNFi | 0.82 (0.60 to 1.12) | 0.85 (0.60 to 1.20) | 1.23 (0.87 to 1.73) | 0.82 (0.52 to 1.28) |
| Other bDMARDs | 0.89 (0.60 to 1.31) | 0.89 (0.58 to 1.37) | 0.83 (0.55 to 1.27) | 0.64 (0.37 to 1.13) |
| No of previous bDMARDs | 1.16 (0.96 to 1.39) | | 1.26 (1.05 to 1.51) | 1.34 (1.00 to 1.79) |
| No of previous csDMARDs | 1.00 (0.89 to 1.12) | | 0.88 (0.79 to 0.98) | |
| Glucocorticoids, current by 5 mg/day | 1.11 (1.00 to 1.24) | | 1.25 (0.99 to 1.58) | 0.90 (0.71 to 1.14) |
| Glucocorticoids, weighted† | 1.72 (0.85 to 3.44) | 1.17 (0.56 to 2.45) | 0.80 (0.22 to 3.00) | |
| Non-selective NSAIDs, weighted† | 1.04 ([0.74 to 1.47) | | 1.19 (0.85 to 1.68) | |
| Cox-2 inhibitors, weighted† | 1.34 (0.85 to 2.13) | 1.30 (0.82 to 2.06) | 1.22 (0.77 to 1.93) | |
| Smoking, never* (Reference) | | | | |
| Smoking, ever | 1.37 (0.99 to 1.89) | 1.87 (1.33 to 2.64) | | |
| Smoking, unknown | 1.14 (0.60 to 2.17) | 1.19 (0.63 to 2.28) | | |
| SAEs, 6 months prior stroke | | | | |
| Overall | | | 3.31 (2.18 to 5.02) | |
| Serious infections | | | 4.23 (2.03 to 8.81) | 4.39 (1.55 to 12.46) |
| CV events (other than stroke) | | | 3.02 (1.38 to 6.65) | 2.87 (0.94 to 8.74) |
| Surgeries | | | 1.00 (0.49 to 2.04) | 0.87 (0.33 to 2.27) |
| All other SAEs | | | 3.36 (2.10 to 5.37) | 2.61 (1.42 to 4.81) |

Baseline information was used for age, all comorbidities, CV treatment and smoking.
*Matching criteria were not considered in the model of the case–control study.
†The weighted approach is explained in the methods section.
bDMARD, biological disease-modifying antirheumatic drug; COX-2 inhibitors, inhibitors of cyclooxygenase-2 ; CRP, C reactive protein; csDMARD, conventional synthetic disease-modifying antirheumatic drug; CV, cardiovascular; DAS28, disease activity based on 28 joint count; ESR, erythrocyte sedimentation rate; NSAID, non-steroidal antirheumatic drug; SAE, serious adverse event; TNFi, inhibitors of tumour necrosis factor alpha.

disease were associated with a higher risk for stroke. Except for the current treatment with glucocorticoids, none of the treatments with csDMARDs or bDMARDs, non-selective NSAIDs or Cox-2 inhibitors were associated with the risk for stroke. The highest risk for stroke was found in patients with untreated CV diseases (HR 4.3 (95% CI 2.8 to 6.5)). In the adjusted cohort analysis, only the impact of higher age, physical function and hyperlipoproteinaemia were affirmed. Smoking (ever vs never) was additionally identified as risk factor.

In the nested case–control study, univariate analysis showed likewise that high levels of CRP, ESR and DAS28 as well as a poor physical function were significantly associated with a higher risk for stroke (table 3). Significant but smaller effects were found for the comorbidities hyperlipoproteinaemia, chronic renal disease and osteoporosis. Untreated CV comorbidities and the development of SAEs ≤6 months prior to stroke had the strongest association with the risk for stroke. This effect was confirmed in the adjusted shared frailty model with a HR of 3.3 (95% CI 1.5 to 7.2) for untreated CV disease. Regarding SAEs, we found the largest impact for prior serious infections with an HR of 4.4 (1.6 to 12.5). Further significant influences were found for physical function and the number of bDMARD treatments before entering RABBIT. In contrast, current treatment with TNFi, other bDMARDs and glucocorticoids had no association.

To investigate the impact of risk factors in patients without a prior SAE, a cause-specific hazard model was applied (approach 2, table 4). In this model, the associations between physical function as well as untreated CV comorbidities and stroke remained significant. However, the effect size of untreated CV diseases was attenuated to an HR of 2.3 (1.2 to 4.5). The influence of the number of bDMARD treatments before cohort entry was no longer significant.

## DISCUSSION

We examined the incidence and risk for stroke in a large cohort of patients with RA. The known risk factors age and smoking as well as hyperlipoproteinaemia and a poor physical function were associated with an increased risk. The IR for stroke was highest in patients who experienced another SAE within 30 days prior to stroke. In a nested case–control study with patients at comparable risk for stroke, the absence of CV treatment despite CV comorbidity was associated with a high risk for incident stroke. The highest impact was found for prior serious adverse events, particularly serious infections.

Our results support findings in the general population[8 9] and in patients with autoimmune diseases,[12] which suggest that stroke may be triggered by other adverse events. Compared with the overall IR of 3.1 strokes per 1000 PY, we observed a high IR of 8.7/1000 PY for patients with a previous adverse event other than cerebrovascular. The association was clearly time-dependent being highest within 30 days (IR: 93.3/1000 PY) after the serious event and dropping thereafter to 3.2. This is in line with results from Smeeth *et al* who reported an IR ratio (IRR) of 3.2 (95% CI 2.8 to 3.6) during the first 3 days after respiratory tract infections, gradually decreasing in the following weeks.[22] Others observed more strokes within 6 days after hospital admission.[10] In patients with autoimmune diseases, the risk was highest within 90 days after herpes zoster with an IRR of 1.4 (95% CI 1.1 to 1.7).[12]

Reasons for the contribution of SAEs to the occurrence of stroke may be diverse. Patients may rest in bed during their illness, with consequences of dehydration and hypercoagulability that can promote embolic events. Previous studies characterised patients with in-hospital onset ischaemic strokes, indicating, among others, fever, high blood pressure, dehydration,[10] female gender and atrial fibrillation as risk factors.[23]

Our data revealed a more than fourfold risk for stroke after serious infections, followed by other SAEs. For CV events, the estimator did not reach statistical significance. Interestingly, surgeries had no effect on the occurrence of stroke (adjusted HR 0.9 (95% CI 0.3 to 2.3)).

Insufficient treatment of CV diseases[24 25] and inadequate risk management in RA[26 27] were debated widely in recent years. We found that patients who experienced a stroke had been treated less often for their underlying CV diseases compared with control patients or the remaining cohort. This finding is in line with our study on myocardial infarction.[28] To preclude a general underreporting of treatment for comorbidity in patients with stroke, we examined the reporting of other comorbidities. Osteoporosis and diabetes were more stringently managed in patients with a future stroke, indicating that awareness for comorbidities differs. However, the guidelines consider the rheumatologist responsible for risk management of CV diseases in RA, in collaboration with cardiologists and other disciplines.[29]

The treatment with bDMARDs did not influence the occurrence of strokes which is consistent with previous findings.[5 30–33] Regarding the effect of glucocorticoids we did not find an association with stroke in the adjusted model and in the nested case–control study. This is in line with previous studies that did not find a negative effect of glucocorticoids on the risk for stroke.[5 34 35]

Inflammation is discussed as a risk factor for stroke in the general population and in patients with RA,[3 6 7] and even considered in the current guidelines for primary stroke prevention of the American Heart Association.[36] The association between markers of inflammation and disease activity with the incidence of stroke persisted in our study only in unadjusted analyses. This is in contrast to findings for myocardial infarction.[28] However, it implies the possibility of an SAE-driven elevation of inflammation markers. In the cause-specific model, which estimates the risk for stroke without the influence of SAEs, the estimator of

**Table 4** Cause-specific hazard ratios of stroke in patients without prior SAE

| Nested case–control study | |
|---|---|
| | **HR (95% CI)** |
| logCRP | 1.14 (0.90 to 1.45) |
| % of full physical function, per 10 points | 0.88 (0.79 to 0.97) |
| No CV disease (Reference) | |
| CV disease with therapy | 1.13 (0.65 to 1.98) |
| CV disease and no therapy | 2.27 (1.15 to 4.49) |
| csDMARD (Reference) | |
| TNFi | 0.73 (0.43 to 1.24) |
| Other bDMARDs | 0.65 (0.30 to 1.41) |
| No of previous bDMARDs | 1.18 (0.86 to 1.62) |
| Glucocorticoids, current by 5 mg/day | 0.74 (0.52 to 1.04) |
| Non-selective NSAIDs | 1.34 (0.78 to 2.32) |
| Cox-2 inhibitors | 1.38 (0.70 to 2.71) |

Patients are censored at the end of the observation (index date) or at the occurrence of other SAEs, whatever comes first.
bDMARD, biological disease-modifying anti-rheumatic drug; COX-2 inhibitors, inhibitors of cyclooxygenase-2.; CRP, C reactive protein; csDMARD, conventional synthetic disease-modifying antirheumatic drug; CV, cardiovascular; NSAID, non-steroidal antirheumatic drug; SAE, serious adverse events; TNFi, inhibitors of tumour necrosis factor alpha.

logCRP was non-significant (HR 1.1 (95% CI 0.9 to 1.5)) not supporting the idea of CRP as a risk factor for stroke.

Our study has several strengths and limitations. The large RABBIT cohort with well-monitored follow-up data[16] enabled us to analyse patients with similar baseline risk for incident stroke, using a nested case–control design. Stroke is a slowly evolving event,[37] and controls were required to have a minimum observation time corresponding to their matching case. Requesting the same observation time as matching criteria is a limitation of the study design too, which may imply a selection bias of patients with better controlled disease and less frequent SAEs. This peculiarity may bias the cumulative incidence of strokes after the development of SAEs. Therefore, we omitted this criterion in a sensitivity analysis which confirmed the findings of the main analysis (data not shown). A remaining limitation of shared frailty models rests with the lack of diagnostic tools for evaluation of model assumptions beyond the distribution of random effects.

## CONCLUSION

Aside from traditional risk factors, we found that insufficient CV treatment and the occurrence of other SAEs increased the risk for stroke in patients with RA. These findings, on the one hand, underline the need for rigorous management of CV diseases, on the other hand support results found in the general population which suggest expanding the traditional risk model for stroke by incident other adverse events. This could help to identify patients and clinical situations at increased risk for stroke.

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**Contributors** YM, AR, JL, AZ and AS: had full access to all data of this study and take responsibility for data integrity and accuracy of the analysis. YM, AR, JL, AZ and AS: study concept and design. BM, HPT and EW: acquisition of the data. YM, AR, and AS: analysis and interpretation of the data. YM: drafting the manuscript. YM, AR, BM, HPT, EW, JL, AZ and AS: critical revision of the manuscript for important intellectual content. AZ and AS: obtaining funding. All authors read and approved the manuscript.

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EXTENDED REPORT

# Incidence and prevalence of psoriatic arthritis in Denmark: a nationwide register linkage study

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## ABSTRACT

**Objectives** To examine the incidence and temporal trends of psoriatic arthritis (PsA) in the general population in Denmark.

**Methods** Using nationwide registry data, we estimated the number of patients with incident PsA within each 1-year period between 1997 and 2011 and calculated the rate of PsA cases within gender and age subgroups. Incidence rates were presented per 100 000 person-years.

**Results** There was a female predominance ranging from 50.3% (1998) to 59.2% (2010), and the mean age at time of diagnosis was 47–50 years. We identified a total of 12 719 patients with PsA (prevalence=0.22%), including 9034 patients where the PsA diagnosis was made by a rheumatologist (prevalence=0.16%). Incidence rates of PsA (per 100 000 person-years) increased from 7.3 in 1997 to a peak incidence of 27.3 in 2010. Incidence rates were highest for women and patients aged 50–59 years, respectively. The use of systemic non-biologic agents, that is, methotrexate, leflunomide, ciclosporin or sulfasalazine increased over the 15-year study course and were used in 66.3% of all patients. Biologic agents (etanercept, infliximab, adalimumab, certolizumab pegol, golimumab or ustekinumab) were used in 17.7% of patients with PsA.

**Conclusions** We found a clear trend of rising PsA incidence on a national level. While the cause remains unclear, our findings might be explained by increased attention by patients and physicians.

## INTRODUCTION

Psoriatic arthritis (PsA) is a chronic inflammatory musculoskeletal disease, which frequently develops in patients with cutaneous psoriasis.[1] PsA is characterised by inflammation of the peripheral and axial joints as well as at the sites of tendon and ligament insertion into bone (enthesitis) and inflammation of the whole digit (dactylitis) and extra-articular manifestations, including nail dystrophy.[2 3] Severity and prevalence of the different disease manifestations vary greatly, and the clinical picture may overlap with that of seronegative rheumatoid arthritis and ankylosing spondylitis.[3]

Although the prevalence of PsA is unclear, primarily due to lack of consensus on diagnostic criteria, it is estimated to occur in 0.04–0.1% of the general population; however, this figure may be underestimated.[4] Studies have suggested that approximately 30% of patients with cutaneous psoriasis suffer from PsA, and one study reported that 42% of Danish patients with psoriasis had PsA when examined by rheumatologists, albeit that this may be limited to patients seen in a hospital setting.[5] Although PsA may occur at any age, the onset typically begins in the patients mid-to-late 30s and affects men and women equally.[2 3]

Studies on the incidence of PsA in the general population remain scarce. While recent data suggest an incidence rate ranging from 3.6 to 7.2 per 100 000 person-years, older studies have reported a much wider incidence range (ie, from 0.1 to 23.1 per 100 000 person-years).[6–10] The most recent study and the only prospective study of PsA in patients with psoriasis demonstrated an annual incidence of 2.8%.[11] Consequently, the incidence and temporal trends of PsA in the general population remain poorly understood. In the present work, we therefore examined the incidence and prevalence of PsA in a Danish nationwide cohort.

## MATERIALS AND METHODS

### Data sources and study population

Study approval was obtained from the Danish Data Protection Agency (ref. 2007-58-0015, int. ref. GEH-2014–018, I-Suite 02736) and approval from an ethics committee is not required for registry studies in Denmark. The study was conducted in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology recommendations.[12]

Using the unique personal identification number assigned to all Danish citizens, we linked individual-level information from nationwide administrative registers. The Civil Registration System[13] contains information on sex, date of birth and updated information on vital status and emigration, thus minimising loss to follow-up. All inpatient and outpatient (ambulatory) hospital consultations are recorded in the Danish National Patient Register[14] (DNPR), including 1 primary and up to 19 secondary diagnoses coded by discharging physicians according to the International Classification of Diseases, eighth revision (ICD-8) (prior to 1994) and according to the tenth revision (ICD-10) thereafter. The primary diagnosis is the main reason for the hospital consultation or hospitalisation, and secondary diagnoses are additional conditions, including complications. Since 1994, detailed and accurate information on all pharmacy-dispensed medications has been registered in the Danish Registry of Medicinal Products Statistics according to the Anatomical Therapeutic

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**Table 1** Characteristics of patients diagnosed with first-time psoriatic arthritis (PsA) in Denmark, all specialties

| PsA—diagnoses from all specialties | 1997 | | 1999 | | 2001 | | 2003 | | 2005 | | 2007 | | 2009 | | 2011 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | % | n | % | n | % | n | % | n | % | n | % | n | % | n | % |
| **Population** | 5 799 626 | | 5 796 301 | | 5 790 440 | | 5 775 643 | | 5 763 587 | | 5 748 752 | | 5 730 728 | | 5 699 167 | |
| **Women** | 2 929 404 | 50.5 | 2 925 814 | 50.5 | 2 920 591 | 50.4 | 2 910 527 | 50.4 | 2 902 514 | 50.4 | 2 893 295 | 50.3 | 2 882 814 | 50.3 | 2 866 063 | 50.3 |
| **Men** | 2 870 222 | 49.5 | 2 870 487 | 49.5 | 2 869 849 | 49.6 | 2 865 116 | 49.6 | 2 861 073 | 49.6 | 2 855 457 | 49.7 | 2 847 914 | 49.7 | 2 833 104 | 49.7 |
| **PsA** | | | | | | | | | | | | | | | | |
| **Mean age, years** | 47.7 | | 48.0 | | 48.0 | | 47.8 | | 47.2 | | 48.6 | | 48.7 | | 49.7 | |
| **Any** | 376 | 0.01 | 466 | 0.01 | 447 | 0.01 | 566 | 0.01 | 714 | 0.01 | 894 | 0.02 | 1118 | 0.02 | 1128 | 0.02 |
| **Women** | 215 | 57.2 | 268 | 57.5 | 242 | 54.1 | 320 | 56.5 | 396 | 55.5 | 497 | 55.6 | 659 | 58.9 | 655 | 58.1 |
| **Men** | 161 | 42.8 | 198 | 42.5 | 205 | 45.9 | 246 | 43.5 | 318 | 44.5 | 397 | 44.4 | 459 | 41.1 | 473 | 41.9 |
| **Age groups** | | | | | | | | | | | | | | | | |
| **0–19** | 8 | 2.1 | 10 | 2.1 | 17 | 3.8 | 29 | 5.1 | 25 | 3.5 | 35 | 3.9 | 44 | 3.9 | 42 | 3.7 |
| **20–29** | 27 | 7.2 | 41 | 8.8 | 43 | 9.6 | 45 | 8.0 | 67 | 9.4 | 62 | 6.9 | 77 | 6.9 | 85 | 7.5 |
| **30–39** | 75 | 19.9 | 90 | 19.3 | 78 | 17.4 | 96 | 17.0 | 136 | 19.0 | 143 | 16.0 | 191 | 17.1 | 184 | 16.3 |
| **40–49** | 95 | 25.3 | 119 | 25.5 | 104 | 23.3 | 124 | 21.9 | 166 | 23.2 | 219 | 24.5 | 255 | 22.8 | 257 | 22.8 |
| **50–59** | 109 | 29.0 | 107 | 23.0 | 112 | 25.1 | 156 | 27.6 | 192 | 26.9 | 241 | 27.0 | 302 | 27.0 | 254 | 22.5 |
| **60–69** | 39 | 10.4 | 58 | 12.4 | 51 | 11.4 | 72 | 12.7 | 86 | 12.0 | 122 | 13.6 | 179 | 16.0 | 192 | 17.0 |
| **≥70** | 23 | 6.1 | 41 | 8.8 | 42 | 9.4 | 44 | 7.8 | 42 | 5.9 | 72 | 8.1 | 70 | 6.3 | 114 | 10.1 |
| **Systemic Tx*** | | | | | | | | | | | | | | | | |
| **Any** | 163 | 43.4 | 168 | 36.1 | 183 | 40.9 | 257 | 45.4 | 337 | 47.2 | 431 | 48.2 | 536 | 47.9 | 504 | 44.7 |
| **Non-biologic** | 163 | 43.4 | 168 | 36.1 | 183 | 40.9 | 257 | 45.4 | 337 | 47.2 | 416 | 46.5 | 496 | 44.4 | 479 | 42.5 |
| **Biologic** | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 6 | 0.8 | 32 | 3.6 | 98 | 8.8 | 79 | 7.0 |

*Systemic Tx: methotrexate, leflunomide, ciclosporin, sulfasalazine, etanercept, infliximab, adalimumab, certolizumab pegol, golimumab, ustekinumab.
Tx, therapy.

Chemical classification.[15] Hospital-administered pharmacotherapy is coded in the DNPR as treatment procedure (SKS) codes. We defined patients with incident PsA as those recorded with a corresponding first-time ICD-10 code (M07.0–3 and M09.0) and thus excluded all patients with a history of PsA before 1 January 1997. In estimations of prevalence, these patients were not excluded. The study period was divided into 1-year groups from 1997 to 2011. We identified the use of systemic therapy, that is, methotrexate, leflunomide, ciclosporin, sulfasalazine, etanercept, infliximab, adalimumab, certolizumab pegol, golimumab and ustekinumab. Although uncommon, methotrexate, sulfasalazine and ciclosporin can be prescribed by general practitioners in Denmark, whereas leflunomide and biologics are only prescribed by specialists. We did not consider corticosteroids, as these may be used primarily for short-term management and did not include non-steroidal anti-inflammatory drugs as these may also be purchased over the counter in Denmark.

### Statistical analysis

We estimated the number of incident PsA cases within each of the predefined 1-year periods and calculated the frequency of PsA cases within gender and age subgroups (0–19, 20–29, 30–39, 40–49, 50–59, 60–69, 70+ years). We computed the incidence rate within each 1-year period as the number of newly diagnosed PsA cases divided by the risk time of the underlying population. We estimated the population size in each of the 1-year periods as the number of Danes alive in the mid-year of each period, as recorded in the Civil Registration System, and the risk time as 1 year times the estimated number of Danes in each period. The prevalence of PsA was estimated among all Danes alive and resident in the source population on 31 December 2012. Since it is possible that younger individuals may have been coded as having juvenile idiopathic arthritis (JIA) instead of PsA, we performed additional analyses to examine the incidence of having either PsA or JIA in individuals aged 0–19 years. Due to data security requirements, data on one or two subjects are shown as 'less than 3', and the derived percentages are not shown. SAS statistical software version 9.4 (SAS Institute, Cary, North Carolina, USA) was used for statistical analysis.

## RESULTS

During the study period, the total Danish population comprised approximately 5.7 million individuals with equal gender distribution. Among patients diagnosed with PsA, there was a female

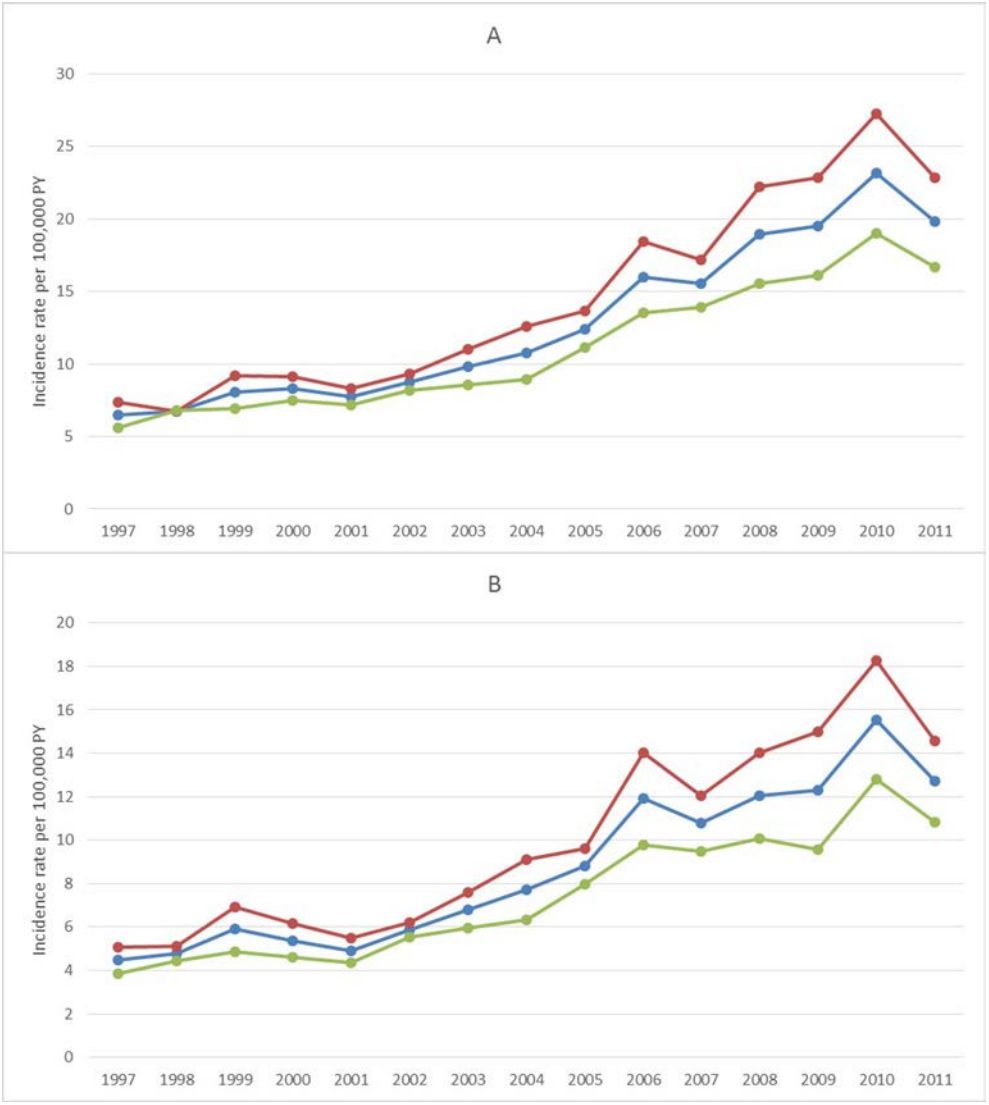


**Figure 1** Incidence rates of psoriatic arthritis (PsA). Total and gender-specific incidence rates per 100 000 person-years of PsA over the study period 1997–2011. (A) Diagnoses from all specialties. (B) Diagnoses made by rheumatologists. Blue,Overall; Red, Women; Green, Men.

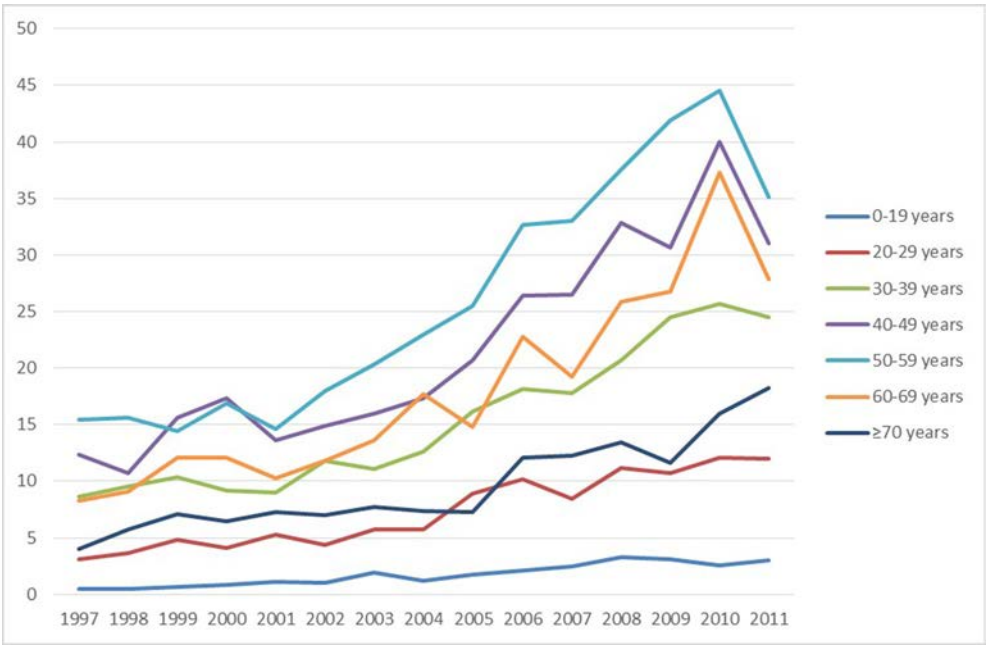


**Figure 2** Age-specific incidence rates per 100 000 person-years of psoriatic arthritis over the study period 1997–2011.

predominance ranging from 50.3% (1998) to 59.2% (2010), and the mean age at time of diagnosis was 47–50 years (table 1 and see online Supplementary table S1). The incidence of PsA (presented as incidence rates per 100 000 person-years) increased almost fourfold, from 7.3 in 1997 to a maximum of 27.3 in 2010 (see online Supplementary Table S2 and figure 1). The highest percentage of patients with incident PsA was among individuals aged between 50 and 59 years, whereas PsA was the least frequent among younger individuals aged 0–19 years (table 1 and figure 2). Similarly, age-specific incidence rates revealed the greatest absolute increase in PsA incidence among subjects aged 50–59 years and the lowest increase among individuals aged 0–19 years (see online Supplementary table S2 and supplementary figure S1).

The use of systemic non-biologic agents, that is, methotrexate, leflunomide, ciclosporin or sulfasalazine increased over the 15-year study course. The first recorded use of biologic agents (etanercept, infliximab, adalimumab, certolizumab pegol, golimumab or ustekinumab) for patients diagnosed with PsA within the same year occurred in 2004 from whereon their use steadily increased (table 1 and see online supplementary table S1).

As shown in table 2, we identified a total of 12 719 patients with PsA (prevalence=0.22%), including 9034 patients where the PsA diagnosis was made by a rheumatologist (prevalence=0.16%). When limited to adults (≥18 years), the PsA prevalence was 0.28% across specialties and 0.20% when the diagnosis was made by a rheumatologist. Among patients not diagnosed by a rheumatologist, the majority received their diagnosis from a dermatologist. Patients were predominantly women (58%) with a mean age of 47 years. The highest prevalence was among patients aged between 50 and 59 years, followed by those aged 60–69 years (table 2 and figure 3). Approximately two-thirds of patients had received treatment with systemic non-biologic agents, whereas biologics were used in one-fifth of patients (table 2). Similar characteristics were observed when analyses were limited to patients diagnosed by a rheumatologist (see online Supplementary figures S2-3). The trend in incidence of PsA and JIA combined for individuals aged 0–19 years are shown in online Supplementary figure S4.

**Table 2** Characteristics and prevalence of patients diagnosed with psoriatic arthritis (PsA)

| | All specialties | | Rheumatologists | |
|---|---|---|---|---|
| | n | % | n | % |
| **Population** | 5 677 138 | | 5 677 138 | |
| Women | 2 854 985 | 50.29 | 2 854 985 | 50.29 |
| Men | 2 822 153 | 49.71 | 2 822 153 | 49.71 |
| **PsA** | | | | |
| Mean age, years | 46.7 | | 47.4 | |
| Any | 12 719 | 0.22 | 9034 | 0.16 |
| Women | 7318 | 57.54 | 5257 | 58.19 |
| Men | 5401 | 42.46 | 3777 | 41.81 |
| **Age groups** | | | | |
| 0–19 | 183 | 1.44 | 26 | 0.29 |
| 20–29 | 501 | 3.94 | 318 | 3.52 |
| 30–39 | 1256 | 9.87 | 916 | 10.14 |
| 40–49 | 2505 | 19.69 | 1833 | 20.29 |
| 50–59 | 3168 | 24.91 | 2314 | 25.61 |
| 60–69 | 3124 | 24.56 | 2303 | 25.49 |
| ≥70 | 1982 | 15.58 | 1324 | 14.66 |
| **Systemic Tx*** | | | | |
| Any | 8519 | 66.98 | 6307 | 69.81 |
| Non-biologic | 8434 | 66.31 | 6246 | 69.14 |
| Biologic | 2250 | 17.69 | 1759 | 19.47 |

*Systemic Tx: methotrexate, leflunomide, ciclosporin, sulfasalazine, etanercept, infliximab, adalimumab, certolizumab pegol, golimumab and ustekinumab.

## DISCUSSION

In this nationwide study of the Danish population, we observed an increasing incidence of PsA between 1997 and 2011. This finding is in contrast to cutaneous psoriasis which appears to have a stable or even slightly decreasing incidence in Northern Europe.[16] Notably, the increasing incidence was most pronounced among older individuals, and a strong female predominance was observed. Overall, the prevalence of PsA in Denmark was 0.22%, whereas the prevalence was 0.16% when limited to diagnoses

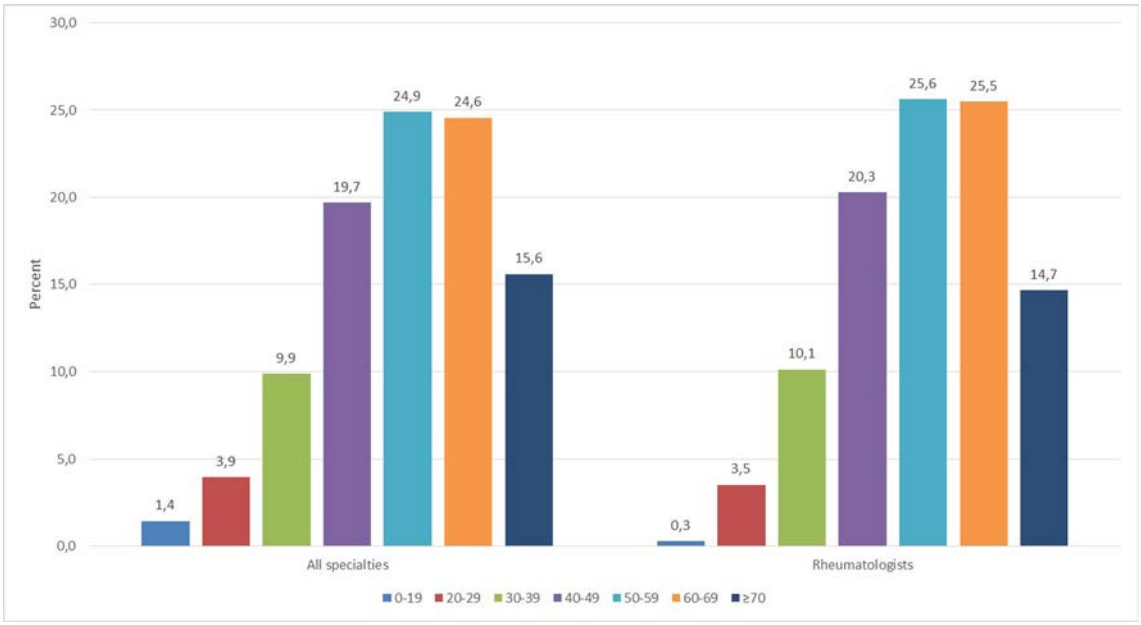


**Figure 3** Distribution of prevalent psoriatic arthritis, stratified by age.

made by rheumatologists. Two-thirds of patients received treatment with systemic non-biologic therapy, and one-fifth of patients were at some point treated with biologics.

While the aim of the present study was to examine the incidence and prevalence of PsA in the general population, studies have reported that most patients with PsA develop cutaneous psoriasis prior to the development of arthritic symptoms.[17 18] Notably, however, data suggest that the risk of PsA remains constant following diagnosis of cutaneous psoriasis.[19] Several studies have examined the incidence and prevalence of PsA in patients with cutaneous psoriasis, yet general population studies of PsA incidence remain conflicting, although a recent meta-analysis reported a PsA prevalence of 0.19% in Europe.[20] Based on self-reported questionnaire data from 2006 to 2008, a Norwegian study of 50 806 citizens found a PsA incidence rate of 41.3 per 100 000 person-years and a prevalence of 0.67% among individuals older than 20 years.[21] In one previous study from Denmark, 34 944 Danish twins were surveyed in 2002, and reported an incidence rate of 6 per 100 000 person-years.[22] The observed differences between previous studies and our findings may be due to methodological differences, as well as the time period in which the incidence and prevalence was examined. A fundamental limitation of the aforementioned studies is the lacking assessment of the developments in PsA incidence over time. However, one small study comprising a total of 147 patients with incident PsA reported an increasing incidence from 3.6 to 9.8 per 100 000 person-years between the periods from 1970–1979 to 1990–1999.[23] Similarly, a study from Taiwan reported an increasing PsA prevalence from 1014 in 2003 (prevalence rate: 0.45/10 000 individuals) to 3072 in 2013 (prevalence rate: 1.31/10 000 individuals).[24] Until now, only one prospective study has been published of patients with psoriasis developing PsA. The study reported a constant risk in these patients.[11] Indeed, this finding was corroborated by a cross-sectional observational study of 1560 patients with psoriasis, of which 126 had PsA.[19]

We found a mean age of 47–50 years at time of PsA diagnosis, a somewhat higher estimate compared with certain other studies.[25] Our population-based study also included cases diagnosed by dermatologists only, which together with milder more insidious cases of onset could explain the higher mean age of diagnosis identified in this study. The female predominance observed in our study is supported by some previous publications, including data from biologics registers.[20 21 26] Although the cause remains speculative, it is conceivable that female patients may be more likely to seek medical treatment for arthritic symptoms compared with men. The increasing PsA incidence is in contrast to rheumatoid arthritis, for example, where recent population-based studies have reported a reduction in incidence and prevalence.[27] Although speculative, decreasing number of active smokers together with less air pollution in the major cities over the last decades, together with an increase in obesity may explain these differences. Moreover, we observed an increase in use of systemic therapies, in particular biologics, in newly diagnosed patients with PsA. It is plausible that the increasing PsA incidence may reflect use of screening questionnaires as well as increased targeted educational initiatives provided to patients and physicians. The increasing use of systemic therapies may also be due to emerging data suggesting that early and aggressive treatment results in improved prognosis,[28 29] and the introduction of the classification criteria for psoriatic arthritis (CASPAR) criteria which may have enabled increased focus on specific symptoms, consequently resulting in earlier disease recognition. However, in daily practice few clinicians use the CASPAR criteria outside of clinical trials and computerised databases. Also, the lack of evidence supporting the use of synthetic disease-modifying antirheumatic drugs may have influenced the use of biologics. For example, studies have suggested that methotrexate does not significantly modify relevant disease outcome measures in PsA,[30] whereas a tight control of disease activity was recently shown to significantly improve PsA.[31]

## Strengths and limitations

Because of its nationwide and population-based design within a setting with equal access to healthcare for the entire population, our study is virtually unaffected by referral and selection biases and is likely to provide highly generalisable results. Nonetheless, a few limitations need to be addressed. Although we find it unlikely that misclassification can explain our findings, we acknowledge that an increase in the completeness of PsA coding over time might play a role in the observed increase in PsA incidence. The ICD-10 classification has been

used in Denmark since 1995. Selection of patients with PsA in this study was based on ICD codes recorded, which may introduce a selection bias towards more severe cases being included while failing to capture patients with mild disease who are managed entirely at primary care units. However, according to a previous study in Sweden (a neighbouring Scandinavian country closely resembling Denmark), this is a minor problem and would only increase the number of cases by less than 4%, at the expense of a larger degree of misclassification.[32]

Regarding the case definitions of PsA used in this study, data and results from another group suggest that rheumatic misclassification occurs in less than 10%.[33] Moreover, our findings were corroborated by analyses limited to diagnoses given by rheumatologists, which yielded similar results as our primary analyses. Nevertheless, we cannot refute that our results may be underestimated. Indeed, a recent meta-analysis found that up to 15.5% of patients with cutaneous psoriasis may have undiagnosed PsA.[34] Since we dealt with confounding by age and gender in stratified analyses, we find it unlikely that confounding plays a substantial role in our findings of an increase in PsA incidence over time. We lacked data on clinical measures of disease severity and used systemic treatment as a measure thereof, which may have biased our results slightly. Lastly, our study was limited by the lack of information on clinical as well as radiographic findings among patients with PsA and we were therefore unable to evaluate the impact of the systemic treatment, for example, on time trends of disease severity and progression.

In conclusion, we found a clear trend of rising PsA incidence on a national level in Denmark. While the cause remains unclear, it is likely that our findings are partly explained by increased attention by patients and physicians, the availability of classification criteria, increased information on disease severity and a need for earlier therapy. Future research is warranted to examine whether early and aggressive treatment of cutaneous psoriasis, for example, with systemic agents such as biologics, may prevent a continuous increase in PsA incidence.

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**Contributors** AE and GHG had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: AE. Acquisition, analysis and interpretation of data: all authors. Drafting of the manuscript: AE. Critical revision of the manuscript for important intellectual content: all authors. Statistical analysis: AE and GHG. Obtained funding: AE and LM. Administrative, technical or material support: AE and GHG. Study supervision: AE and GHG.

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**Competing interests** AE has received research funding from Pfizer and Eli Lilly and honoraria as consultant and/or speaker from Pfizer, Eli Lilly, Novartis, Galderma and Janssen Pharmaceuticals. LEK has received fees for speaking and consultancy from Pfizer, MSD, AbbVie, UCB, Eli Lilly, Novartis, Celgene, Janssen Pharmaceuticals, Roche, Forward Pharma and BMS. JPT is supported by an unrestricted grant from the Lundbeck Foundation and has received speaker honoraria from Galderma and MEDA. GHG is supported by an unrestricted research scholarship from the Novo Nordisk Foundation. ABG has received honoraria as consultant and/or speaker from Amgen Inc.; Astellas, Akros, Centocor (Janssen), Inc.; Celgene Corp., Bristol Myers Squibb Co., Beiersdorf, Inc., Abbott Labs. (Abbvie), TEVA, Actelion, UCB, Novo Nordisk, Novartis, Dermipsor Ltd, Incyte, Pfizer, Canfite, Lilly, Coronado, Vertex, Karyopharm, CSL Behring Biotherapies for Life, Glaxo Smith Kline, Xenoport, Catabasis, Meiji Seika Pharma Co., Ltd, Takeda, Mitsubishi, Tanabe Pharma Development America, Inc., Genentech, Baxalta, Kineta One, KPI Therapeutics, Crescendo Bioscience, Aclaris, Amicus and Reddy Labs and received research funding (paid to Tuft Medical Center) from Centocor (Janssen), Amgen, Abbott (Abbvie), Novartis, Celgene, Pfizer, Lilly, Levia, Merck, Xenoport, Dermira and Baxalta. LCC has reported no conflicts of interest. DJ has received research funding from Pfizer and honoraria as consultant and/or speaker from Abbvie, Amgen, Celgene, Eli Lilly, Janssen Pharmaceuticals, MSD, Novartis and Pfizer. PG has received honoraria as consultant and/or speaker from AbbVie, Celgene, Eli Lilly, Janssen, Leo Pharma, MSD, Novartis, Pfizer and UCB. DDG has received consultancy fees and/or grant support from AbbVie, Amgen, BMS, Celgene, Eli Lilly, Janssen, Pfizer, Novartis and UCB. LS has received consultancy and/or speaker honoraria from Abbvie, Pfizer, Janssen-Cilag, Merck Sharp & Dohme and Leo Pharma and is a member of the advisory boards of Abbvie, Pfizer, Leo Pharma, Janssen-Cilag, Merck Sharp & Dohme, Eli Lilly, Celgene and Novartis. LM is currently employed by Eli Lilly and Company.

**Patient consent** Patient consent is not required for register studies per Danish law.

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CONCISE REPORT

# A randomised, double-blind trial to demonstrate bioequivalence of GP2013 and reference rituximab combined with methotrexate in patients with active rheumatoid arthritis

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## ABSTRACT

**Objectives** The aim of this report is to demonstrate pharmacokinetic (PK) and pharmacodynamic (PD) equivalence as well as similar efficacy, safety and immunogenicity between GP2013, a biosimilar rituximab, and innovator rituximab (RTX) in patients with rheumatoid arthritis (RA) with inadequate response or intolerance to tumour necrosis factor inhibitor (TNFi) treatment.

**Methods** In this multinational, randomised, double-blind, parallel-group study, 312 patients with active disease despite prior TNFi therapy were randomised to receive GP2013 or either the EU (RTX-EU) or the US (RTX-US) reference product, along with methotrexate (MTX) and folic acid. The primary endpoint was the area under the serum concentration–time curve from study drug infusion to infinity ($AUC_{0\text{-}inf}$). Additional PK and PD parameters, along with efficacy, immunogenicity and safety outcomes were also assessed up to week 24.

**Results** The 90% CI of the geometric mean ratio of the AUCs were within the bioequivalence limits of 80% to 125% for all three comparisons; GP2013 versus RTX-EU: 1.106 (90% CI 1.010 to 1.210); GP2013 versus RTX-US: 1.012 (90% CI 0.925 to 1.108); and RTX-EU versus RTX-US: 1.093 (90% CI 0.989 to 1.208). Three-way PD equivalence of B cell depletion was also demonstrated. Efficacy, safety and immunogenicity profiles were similar between GP2013 and RTX.

**Conclusions** Three-way PK/PD equivalence of GP2013, RTX-EU and RTX-US was demonstrated. Efficacy, safety and immunogenicity profiles were similar between GP2013 and RTX.

**Trial registration number** NCT01274182; Results.

## INTRODUCTION

Rituximab (RTX) is a chimeric monoclonal IgG-1 antibody against the CD20 antigen expressed by B cells. RTX is indicated for the treatment of rheumatoid arthritis (RA) in combination with methotrexate (MTX) in patients with inadequate response to tumour necrosis factor alpha inhibitor (TNFi) therapy.[1 2] The current study compares the biosimilar GP2013 (Rixathon, Sandoz, Holzkirchen, Germany) with the reference product approved in Europe (RTX-EU; MabThera; Hoffmann-La Roche, Basel, Switzerland) and the reference product approved in the US (RTX-US; Rituxan; Genentech, San Francisco, California, USA). Comparability of GP2013 and RTX was established by extensive physicochemical and functional characterisation,[3–5] by in vitro assays as well as in vivo non-clinical studies.[6] The current study demonstrates pharmacokinetic (PK) and pharmacodynamic (PD) equivalence between GP2013, RTX-EU and RTX-US, as well as similar efficacy, safety and immunogenicity between the biosimilar and the reference product in patients with active RA.

## METHODS

The study was approved by Competent Authorities and Ethics Committees. Written informed consent was obtained from all patients.

### Patients

The study population consisted of adult patients with active RA refractory or intolerant to conventional synthetic disease modifying antirheumatic drugs and at least one TNFi. Main inclusion and exclusion criteria are detailed in the online supplementary table S1.

### Study design and treatment

This international, randomised, double-blind study was sponsored by Sandoz, a division of Novartis. The study was conducted in 16 countries and 87 centres in Europe, USA, South-America and Asia. Eligible patients were randomised to receive a 1000 mg intravenous infusion of GP2013, RTX-EU or RTX-US on day 1 and day 15. In study part 1, patients were randomised (ratio 1:1) to receive GP2013 or RTX-EU, and in part 2 to receive GP2013 or RTX-US (ratio 1:2). Intravenous methylprednisolone 100 mg or equivalent was administered 30 min prior to each infusion. Patients also received antipyretic and antihistaminic premedication before each infusion. All patients received a stable dose of MTX (7.5 to 25 mg/week) and folic acid during the study.

### Statistical analyses

The primary PK variable was area under the curve of the serum drug concentration time profiles from study drug infusion to infinity ($AUC_{0\text{-}inf}$). To claim

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bioequivalence, the 90% CI of the ratio of the geometric mean AUCs had to be within the predefined range of 80%–125%.[7]

The main efficacy objective was to show non-inferiority of GP2013 versus RTX in terms of change from baseline in disease activity score DAS28(C reactive protein (CRP)) at week 24. Other secondary efficacy objectives included American College of Rheumatology (ACR) response rates, Clinical Disease Activity Index (CDAI), Simplified Disease Activity Index (SDAI) and Health Assessment Questionnaire Disability Index (HAQ-DI).[8–11] The main PD parameter was peripheral CD19 positive B-cell count relative to baseline, up to the second infusion ($AUEC_{0\text{-}14d}$). CD19-positive cells were used to identify CD20-positive cells as CD20 epitopes are covered by rituximab after study drug administration. Further details of statistical methodology can be found in the online supplementary text S1.

### Immunogenicity assessment

A validated affinity capture elution ELISA was used for the determination of antidrug antibodies (ADAs).

Further details regarding the methods are provided in the online supplementary text S1.

## RESULTS

### Patient disposition and baseline characteristics

A total of 312 patients (262 female and 50 male) were randomised. Demographics and baseline clinical characteristics were comparable between the treatment arms (table 1). Patient disposition and recruitment by region are displayed in the online supplementary figure S1 and table S2.

### Pharmacokinetics/pharmacodynamics

Three-way bioequivalence of GP2013, RTX-EU and RTX-US was demonstrated. The 90% CI of the ratio of the geometric mean AUCs were maintained within the predefined range of 80% to 125% for all three comparisons (table 2). Secondary PK parameters were also similar between the treatment arms. Mean AUCs were lower in all three treatment arms in ADA-positive patients (see online supplementary figure S2 and table S3).

**Table 1** Demographics and baseline disease characteristics (full analysis set)

| | GP2013 | RTX-EU | RTX-US |
|---|---|---|---|
| | n=133 | n=87 | n=92 |
| Age (years) | 54.4±11.8 | 52.7±12.5 | 55.0±10.8 |
| Age groups | | | |
| 18–44 (%) | 25 (18.8) | 21 (24.1) | 17 (18.5) |
| 45–64 (%) | 80 (60.2) | 50 (57.5) | 53 (57.6) |
| 65 or more (%) | 28 (21.1) | 16 (18.4) | 22 (23.9) |
| Sex, no (%) of patients | | | |
| Female (%) | 111 (83.5) | 73 (83.9) | 78 (84.8) |
| Male (%) | 22 (16.5) | 14 (16.1) | 14 (15.2) |
| Weight (kg) | 73.2±17.0 | 72.5±17.2 | 79.5±16.5 |
| BMI ($kg/m^2$) | 27.4±6.2 | 27.3±6.0 | 29.7±6.6 |
| Duration of RA (years) | 10.5±8.1 | 10.8±7.1 | 11.0±8.3 |
| Prior csDMARDs | 2.3±1.7 | 2.1±1.1 | 1.9±1.2 |
| Number of prior TNFi therapies (%) | | | |
| 1 (%) | 109 (82.0) | 70 (80.5) | 73 (79.3) |
| 2 (%) | 18 (13.5) | 16 (18.4) | 13 (14.1) |
| >2 (%) | 6 (4.5) | 1 (1.1) | 6 (6.5) |
| Dose of MTX (mg/week) | 15.1±4.9 | 14.7±5.2 | 15.2±5.0 |
| Prednisolone (mg/day) | 6.5±2.7 | 6.7±2.6 | 6.5±3.1 |
| CRP (mg/L) | 17.9±19.9 | 19.5±20.9 | 22.3±29.5 |
| Erythrocyte sedimentation rate (mm/hour) | 48.3±19.1 | 46.4±18.4 | 50.0±22.2 |
| B cell count (CD19+ cells/µL)* | 243±148 | 275±148 | 224±126 |
| Serum IgG (g/L) | 12.4±2.9 | 12.7±3.0 | 11.6±3.3 |
| Serum IgM (g/L) | 1.6±0.9 | 1.6±0.9 | 1.5±0.9 |
| Serum IgA (g/L) | 3.2±2.0 | 3.6±1.5 | 3.0±1.3 |
| DAS28 (CRP) | 5.8±0.9 | 5.9±0.9 | 5.9±1.0 |
| DAS28 (ESR) | 6.7±0.9 | 6.6±0.9 | 6.7±0.9 |
| Anti-CCP antibodies (ACPA) positive (%) | 120 (90.2) | 75 (86.2) | 86 (93.5) |
| RF positive (%) | 126 (94.7) | 81 (93.1) | 86 (93.5) |
| Positive RF and/or anti-CCP (%) | 131 (98.5) | 85 (97.7) | 90 (97.8) |
| Swollen joint count (SD) | 16.0±9.1 | 14.8±9.2 | 15.0±8.1 |
| Tender joint count (SD) | 23.9±13.3 | 22.1±12.5 | 23.5±14.3 |
| HAQ Disability Index | 1.9±0.5 | 1.8±0.6 | 1.9±0.6 |

*In the PK set. Except where indicated otherwise, values in the table represent the mean±SD.
BMI, body mass index; CCP, cyclic citrullinated peptide; CRP, C reactive protein; csDMARDs, conventional synthetic disease modifying antirheumatic drugs; DAS28, Disease Activity Score in 28 joints; ESR, erythrocyte sedimentation rate; HAQ, Health Assessment Questionnaire; MTX, methotrexate; RA, rheumatoid arthritis; RF, rheumatoid factor; RTX, rituximab; TNFi, tumour necrosis factor inhibitor.

**Table 2** Summary of primary and key secondary PK/PD results (PK analysis set)

| | | | | | Treatment comparison | |
|---|---|---|---|---|---|---|
| PK parameter (unit) | Treatment | n | Adjusted geometric mean | Comparison | Geometric mean ratio | 90% CI of mean ratio* |
| Primary PK endpoints | | | | | | |
| $AUC_{0\text{-}inf}$ (day*µg/mL) | GP2013 | 124 | 7627.44 | RTX-US vs RTX-EU | 1.093 | (0.989 to 1.208) |
| | RTX-US | 80 | 7536.89 | GP2013 vs RTX-US | 1.012 | (0.925 to 1.108) |
| | RTX-EU | 79 | 6896.97 | GP2013 vs RTX-EU | 1.106 | (1.010 to 1.210) |
| Key secondary PK endpoints | | | | | | |
| $C_{max}$ first inf. (µg/mL) | GP2013 | 120 | 361.53 | RTX-US vs RTX-EU | 1.050 | (0.946 to 1.167) |
| | RTX-US | 82 | 335.88 | GP2013 vs RTX-US | 1.076 | (0.979 to 1.184) |
| | RTX-EU | 78 | 319.80 | GP2013 vs RTX-EU | 1.131 | (1.027 to 1.244) |
| Main PD endpoint (B cell depletion) | | | | | | |
| $AUEC_{0\text{-}14d}$ (%*day) | GP2013 | 110 | 1226.53 | RTX-US vs RTX-EU | 1.033 | (1.016 to 1.050) |
| | RTX-US | 80 | 1240.57 | GP2013 vs RTX-US | 0.989 | (0.974 to 1.004) |
| | RTX-EU | 76 | 1201.15 | GP2013 vs RTX-EU | 1.021 | (1.003 to 1.040) |

$AUC_{0\text{-}inf}$, area under the serum concentration–time curve from study drug infusion to infinity; AUEC, area under the effect curve; PD, pharmacodynamic; PK, pharmacokinetic; RTX, rituximab.

A similar, rapid depletion of CD19+ peripheral B-cells was observed in all three treatment arms (figure 1). The main PD objective, demonstration of three-way equivalence of GP2013, RTX-EU and RTX-US was met, as the 90% CI of the geometric mean ratios were maintained within the standard range of 80% to 125% (table 2). Further PD data are provided in the online supplementary tables S4 and S5. Reasons of exclusion from the PK set are displayed in the online supplementary table S6. All PK/PD results are provided in the online supplementary table S7.

## Efficacy

Change from baseline in DAS28(CRP) at week 24 was −2.07 (SE=0.108) and −2.11 (SE=0.095) in the GP2013 and the RTX treatment arms, respectively. The difference of 0.04 (95% CI −0.241 to 0.323) was below the predefined non-inferiority margin of 0.6. ACR20 response rate was 72.3% (95% CI 64.2% to 80.3%) and 67.3% (95% CI 59.9% to 74.7%), ACR50 response rate was 34.5% (95% CI 25.9% to 43.0%) and 40.4% (95%CI 32.7% to 48.1%), ACR70 response rate was 15.1% (95% CI 8.7% to 21.6%) and 17.3% (95% CI 11.4% to 23.2%) in the GP2013 and RTX treatment arms at week 24, respectively. Absolute improvements in the HAQ Disability Index were −0.48 and −0.45 in the GP2013 and the RTX treatment arms at week 24. Main efficacy outcomes are shown in figure 2. Outcomes are shown separately for RTX-EU and RTX-US in the online supplementary figure S3. Low disease activity (including remission) was achieved by 40.4% and 41.8% of patients in the GP2013 arm according to CDAI and SDAI, respectively, versus 38.1% and 38.4% of patients in the RTX arm. Rheumatoid factor profiles are displayed in the online supplementary figure S4.

## Safety

Three patients died during the study. One patient died of breast cancer during the screening period and was not included in the safety analysis set. One patient in the GP2013 arm died of multiorgan failure, suspected by the investigator to be related to an accidental MTX overdose by the patient (daily intake instead of weekly). A 34-year-old female patient in the RTX-US arm

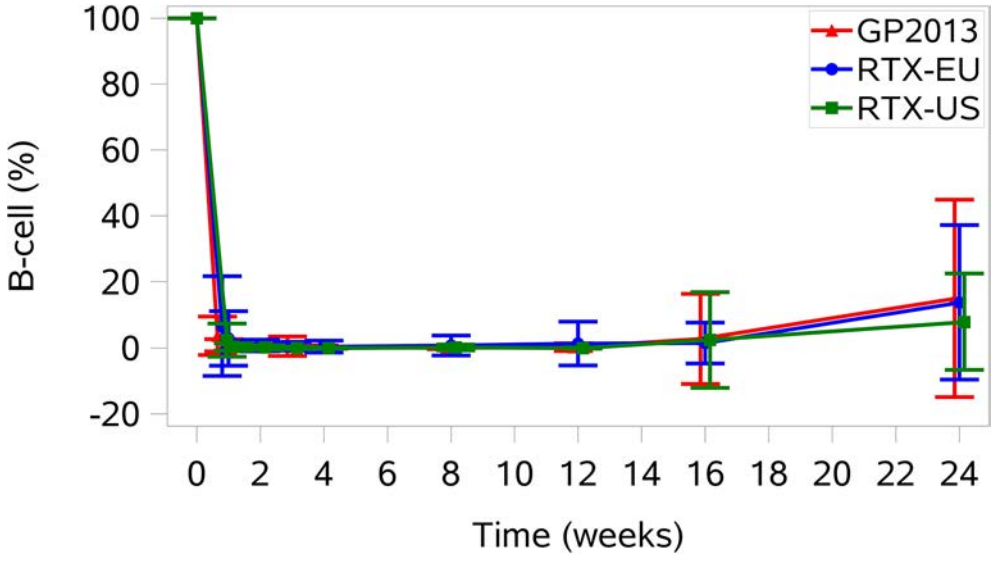


**Figure 1** Arithmetic mean of B cell count relative to baseline up to week 24 (pharmacokinetic set).

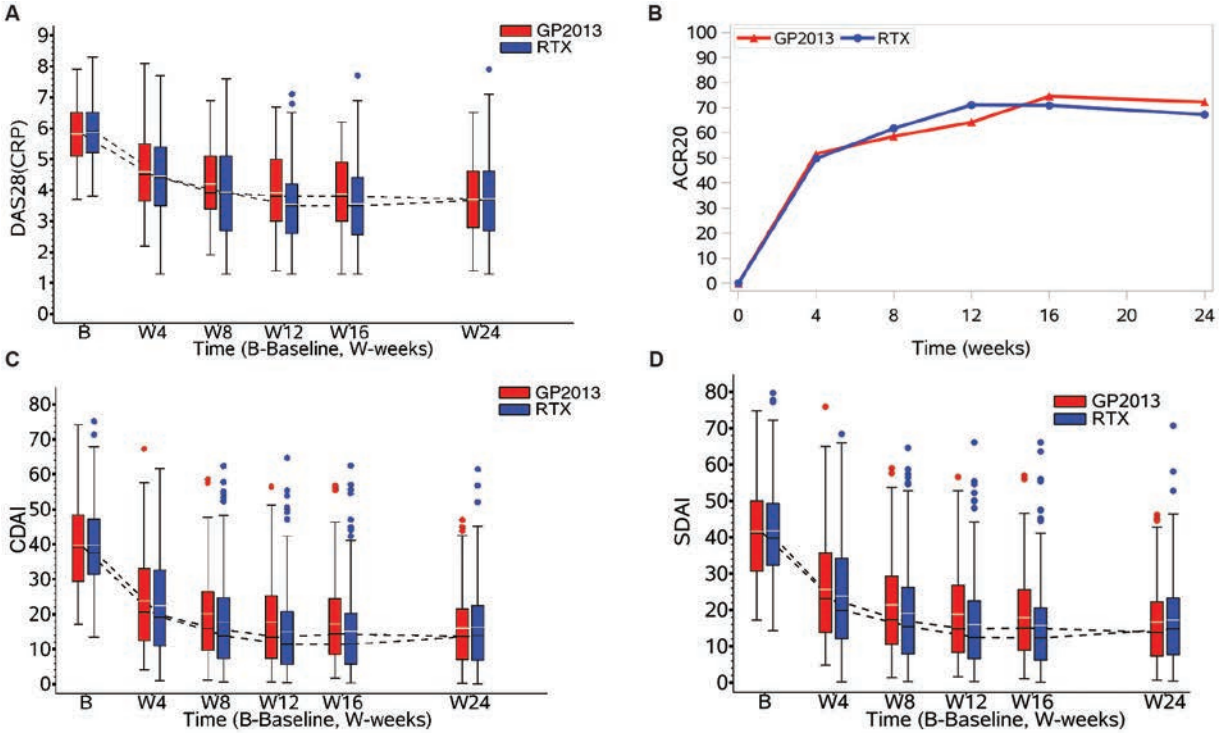


**Figure 2** (A) Box whiskers plot of DAS28(CRP) up to week 24 (per protocol set). (B) ACR20 response rate up to week 24 (per protocol set). (C) Box whiskers plot of CDAI up to week 24 (per protocol set). (D) Box whiskers plot of SDAI up to week 24 (per protocol set). ACR, American College of Rheumatology; CDAI, Clinical Disease Activity Index; CRP, C reactive protein; DAS, Disease Activity Score in 28; RTX, rituximab; SDAI, Simplified Disease Activity Index.

died of purulent pericarditis on day 20. IgG level was 11.7 g/L (normal range: 7 to 16 g/L) on day 18.

The rate of all adverse events (AEs), AEs related to the study medication, AEs leading to study drug discontinuation, serious adverse events and infusion-related reactions were similar between the treatment arms and are displayed in the online supplementary table S8. The rate of binding ADAs was 16.5% in the GP2013 versus 15.1% in the RTX group up to last patient last visit in the study. The majority of ADAs (7.1% and 9.6%, respectively) were transient, meaning that the patient had an ADA-negative sample after having ADA-positive sample(s). Five patients in the GP2013 arm, one patient in the RTX arm had neutralising ADAs. Further details regarding immunogenicity are shown in the online supplementary table S9. Changes in immunoglobulin levels were small and similar between the treatment arms (see online supplementary figure S5).

## DISCUSSION

The current study is part of the stepwise demonstration of similarity of the proposed biosimilar, GP2013 and RTX. The primary objective of the study was met by demonstrating three-way PK bioequivalence of GP2013, RTX-EU and RTX-US. The data are in line with previously published RTX data.[12–20]

The study met its main efficacy objective by demonstrating non-inferiority of GP2013 versus RTX, in terms of DAS28(CRP) change from baseline at week 24. In the historic Randomized Evaluation of Long-Term Efficacy of Rituximab in RA (REFLEX) trial, DAS28 change from baseline at week 24 was −0.34 in the placebo versus −1.83 in the RTX arm,[14] whereas in the current study improvement was −2.07 in the GP2013 and −2.11 in the RTX arm. In a recently published trial comparing the biosimilar CT-P10 and RTX, DAS28(CRP) changes were −1.95 in the CT-P10 and −2.05 in the RTX arm.[15] ACR20 response rates in the historic (REFLEX) trial, were 18% in the placebo versus 51% in the RTX arm at 24 weeks,[14] whereas in the current study, ACR20 response rate was 72.3% in the GP2013 and 67.3% in the RTX arm. In the trial with CT-P10, ACR20 response rates were 63% in the CT-P10 and 66.7% in the RTX arm.[15]

Overall, treatment effect was numerically greater in the current study as compared with the historic data, while efficacy was similar among the treatment arms in the current study. The difference observed between trials may be attributed to differences among patient populations (e.g. in disease activity, RF positivity, number of prior TNFi). Further, in studies with active comparator, patients and investigators are aware that participants would all receive active medication.[14–16]

There were no relevant differences observed between the treatment arms in the rate or severity of adverse events. The comparison of the rate of ADAs between studies is challenging due to the differences of assay methodology but data observed was generally consistent with literature data,[14 15 20] and the rate of ADAs in the current study was shown to be similar between the GP2013 and RTX.

In summary, GP2013, a proposed rituximab biosimilar, was compared with the originator RTX. The study met its primary objective by demonstrating three-way bioequivalence of GP2013, RTX-EU and RTX-US. The study also demonstrated three-way PD equivalence, as measured by the depletion of peripheral B cells. GP2013 and RTX were shown to be similar in terms of efficacy, safety and immunogenicity.'

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**Patient consent** Obtained.

**Ethics approval** The study involved human subjects. Ethics Committee/Institutional Review Board approval was obtained. The following bodies approved the study: National Ethics Committees/Institutional Review Boards.

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OPEN ACCESS

CONCISE REPORT

# The efficacy of motivational counselling and SMS reminders on daily sitting time in patients with rheumatoid arthritis: a randomised controlled trial

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## ABSTRACT

**Objectives** The aim of this report is to investigate the efficacy of an individually tailored, theory-based behavioural intervention for reducing daily sitting time, pain and fatigue, as well as improving health-related quality of life, general self-efficacy, physical function and cardiometabolic biomarkers in patients with rheumatoid arthritis (RA).

**Methods** In this randomised controlled trial 150 patients with RA were randomised to an intervention or a no-intervention control group. The intervention group received three individual motivational counselling sessions and short message service or text messages aimed at reduction of sedentary behaviour during the 16-week intervention period. Primary outcome was change in daily sitting time measured objectively by ActivPAL. Secondary outcomes included change in pain, fatigue, physical function, general self-efficacy, quality of life, blood pressure, blood lipids, haemoglobin A1c, body weight, body mass index, waist circumference and waist–hip ratio.

**Results** 75 patients were allocated to each group. Mean reduction in daily sitting time was −1.61 hours/day in the intervention versus 0.59 hours/day increase in the control group between-group difference −2.20 (95% CI −2.72 to −1.69; p<0.0001) hours/day in favour of the intervention group. Most of the secondary outcomes were also in favour of the intervention.

**Conclusion** An individually tailored, behavioural intervention reduced daily sitting time in patients with RA and improved patient-reported outcomes and cholesterol levels.

**Trial registration number** NCT01969604; Results.

## INTRODUCTION

Rheumatoid arthritis (RA) causes disability[1] and barriers for exercise.[2] Patients with RA have a 50%–60% increased risk of premature death from cardiovascular disease.[3] Supplementary to the pharmacological treatment, patients are recommended to engage in moderate-to-high intensity aerobic and resistance training.[4 5] Most patients do not meet recommended levels of moderate-to-vigorous physical activity[6] and 71%–92% of waking hours are spent sedentarily.[7] Sedentary behaviour is defined as sitting or reclining while awake and with low-energy expenditure.[8] In patients with chronic disease and mobility limitations, replacing sedentary behaviour with light intensity activities may prove more achievable than solely focusing on increasing moderate-to-vigorous physical activity.[9 10] Studies have shown that reduction of daily sitting time through intervention is possible.[11 12] Also, improved resting blood pressure, insulin levels and plasma glucose following regular interruptions of prolonged sitting have been reported.[13] We aimed to investigate the efficacy of an individually tailored, theory-based behavioural intervention for reducing daily sitting time, pain and fatigue, as well as improving quality of life, general self-efficacy, physical function and cardiometabolic biomarkers in patients with RA.

## METHODS

We performed an observer-blinded randomised controlled trial. The protocol was reported to the Danish Data Protection Agency (711-1-08), approved by the Ethics Committee of the Capital Region of Denmark (H-2-2012-112) and registered at www.clinicaltrials.gov (NCT01969604). The Danish National Board of Health Biological Therapies (DANBIO) database[14] was searched for potential participants. A detailed description of the methods of the trial has previously been published. See protocol and feasibility paper.[15 16]

Patients were randomised 1:1 to intervention (n=75) or control group (n=75) by computer generated random numbers in blocks of 10. Participants and project staff delivering the intervention were unblinded to the participants' allocation status, whereas outcome assessors and the statistician were blinded to allocation. The 16-week individually tailored, behavioural intervention consisted of three motivational counselling sessions conducted by health professionals and individual short message service (SMS) or text messages aiming to increase light intensity physical activity through reduction of sedentary behaviour. Participants randomised to the control group were instructed to maintain their usual lifestyles.

The primary outcome measure was change in daily sitting time measured by an ActivPAL 3TM V.7.2.32 Activity Monitor (PAL Technologies, Glasgow, UK). The ActivPAL uses accelerometer-derived information to determine time spent sitting/lying, standing and stepping and is validated in patients with RA.[17] The participants wore the

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**Table 1** Mean changes in behavioural and patient-reported outcomes after 16 weeks

| Variable | Mean change from baseline mean (95% CI) | | Difference in change between groups mean (95% CI) | p Value |
|---|---|---|---|---|
| | Intervention group | Control group | | |
| Daily sitting time (ActivPAL) hours/day | −1.61 (−1.97 to −1.25) | 0.59 (0.24 to 0.95) | −2.20 (−2.72 to −1.69) | <0.0001 |
| Daily standing time* (ActivPAL) hours/day | 1.25 (0.82 to 1.68) | −0.27 (−0.45 to 0.78) | 1.52 (1.10 to 1.95) | <0.001 |
| Daily stepping time* (ActivPAL) hours/day | 0.50 (0.26 to 0.95) | −0.05 (−0.32 to 0.64) | 0.55 (0.35 to 0.74) | <0.001 |
| Breaks up of daily sitting (ActivPAL) (number/day) | −0.47 (−3.52 to 2.57) | −1.97 (−5.02 to 1.07) | 1.50 (−2.81 to 5.81) | 0.49 |
| Self-reported sitting time at work (hour/day) | −1.12 (−1.68 to −0.57) | 0.005 (0.54 to 0.55) | −1.13 (−1.90 to −0.35) | 0.005 |
| Self-reported sitting time in leisure (hour/day) | −1.30 (−1.68 to −0.93) | 0.15 (−0.22 to 0.53) | −1.46 (−2.00 to −0.92) | <0.0001 |
| Physical function (HAQ) | −0.28 (−0.36 to −0.19) | 0.14 (0.06 to 0.22) | −0.42 (−0.54 to −0.30) | <0.0001 |
| Fatigue (VAS)/mm | −19.04 (−24.22 to −13.86) | 7.77 (2.59 to 12.95) | −26.80 (−34.32 to −19.30) | <0.0001 |
| Fatigue (MFI) | | | | |
| General fatigue | −2.17 (−3.00 to −1.35) | 1.25 (0.44 to 2.07) | −3.43 (−4.59 to −2.26) | <0.0001 |
| Physical fatigue | −3.18 (−4.02 to −2.34) | 1.34 (0.50 to 2.18) | −4.52 (−5.73 to −3.30) | <0.0001 |
| Mental fatigue | −1.80 (−2.50 to −1.10) | 0.65 (−0.05 to 1.35) | −2.46 (−3.46 to −1.46) | <0.0001 |
| Reduced activity | −3.28 (−4.05 to −2.50) | 1.60 (0.83 to 2.37) | −4.88 (−5.99 to −3.77) | <0.0001 |
| Reduced motivation | −1.35 (−2.00 to −0.69) | 1.26 (0.60 to 1.91) | −2.60 (−3.54 to −1.67) | <0.0001 |
| Pain (VAS)/mm | −14.77 (−19.50 to −10.04) | 7.59 (2.58 to 12.32) | −22.36 (−29.27 to −15.44) | <0.0001 |
| Self-efficacy (GSES) | 3.96 (2.80 to 5.12) | −2.25 (−3.41 to −1.09) | 6.21 (4.54 to 7.88) | <0.0001 |
| HR-QoL (SF-36) | | | | |
| SF36-PCS | 6.30 (4.33 to 8.26) | −2.58 (−4.54 to −0.61) | 8.88 (6.06 to 11.69) | <0.0001 |
| SF36-MCS | 4.94 (3.42 to 6.46) | −1.83 (−3.34 to −0.32) | 6.77 (4.62 to 8.92) | <0.0001 |

*Not an outcome measure, however, changes in daily sitting, standing and/or stepping time are interdependent, and reduced sitting time may be replaced by either standing or stepping time.
GSES, General Self-efficacy Scale; HAQ, Health Assessment Questionnaire; HR-QoL, Health-Related Quality of Life; MCS, Mental Component Scale; MFI, Multidimensional Fatigue Inventory; PCS, Physical Component Scale; SF36, 36-Item Short Form Survey Instrument; VAS, Visual Analogue Scale.

monitor 24 hours per day for 7 days at baseline and by end of intervention, and recorded their daily sleeping time to separate sleep from waking sitting/lying time.

Secondary outcomes were changes from baseline to 16 weeks in self-reported daily sitting time at work and during leisure time and number of interruptions ('breaks') in daily sitting time, pain, fatigue physical function, quality of life (QoL) and general self-efficacy.[15] Height was measured at baseline. Body weight, hip and waist circumference were additionally measured after 16-week intervention and body mass index (BMI, kg/m$^2$) and waist–hip ratio were calculated. Venous blood sample were drawn. Total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, haemoglobin A1c and resting blood pressure were measured. Pharmacological treatment, duration of RA, C reactive protein, disease activity (Disease Activity Score 28), IgM rheumatoid factor and anti-cyclic citrullinated peptide (CCP) status were retrieved from DANBIO. Additional characteristics were obtained from a self-report questionnaire.[15]

Data analyses were based on the intention-to-treat population and carried out using SAS V.9.3 (SAS Institute) according to the protocol.[15] Missing data were replaced with the value at baseline carried forward. All reported p values and 95% CIs were two sided. Unless stated otherwise, results are expressed as the difference between the group (least-squares) means and 95% CI, based on a general linear model: data were analysed using analysis of covariance with a factor for group and baseline values as covariates in the model. For dichotomous outcomes, proportions were compared based on the risk difference with 95% CIs, as well as including a Wald z test.

The trial was powered for a comparison between the participants allocated to intervention and control group, assuming that the intervention group condition would produce a reduction in daily sitting time of 50 min. Enrolling 75 patients in each group had a reasonable power (84.7%) to detect a mean difference of 50 min.[15 16] A patient with RA from the Danish Rheumatism Association was involved in designing of the trial, including intervention and patient information.[18]

## RESULTS

### Participants

One thousand and eight patients were screened via DANBIO, hereof 801 (79%) were invited. Telephone-based screening was conducted with 722 of these, hereof 617 (85%) were eligible. Of these, 467 declined to participate (online supplementary figure S1). Compared with those declining participation, the included patients were older (60 vs 52 years), had longer disease duration (15 vs 12 years), lower Health Assessment Questionnaire (HAQ) (0.7 vs 1.1) and more were women (81% vs 69%). Outcomes were obtained for 147 (98%) of the randomised patients.

The intervention group had higher scores on fatigue, pain, had more daily sitting time and self-reported leisure-time sitting than the control group (9.8 vs 8.8 hours and 5.3 vs 4.3 hours, respectively) (online supplementary table S1). All in the intervention group completed the counselling sessions (30–90 min) and had SMS reminders.

### Primary outcome

Reductions in daily sitting time favoured the intervention group (online supplementary figure S2). Estimates of intervention effect for behavioural and patient-reported outcomes are presented in table 1. Objectively measured daily sitting time decreased in intervention group by on average 1.61 hours/day and increased in control group by 0.59 hours/day. The difference in change between groups was statistically significant in favour of intervention group (−2.20 hours/day (95% CI −2.72 to −1.69)). The decrease in daily sitting time was replaced by increased standing

**Table 2** Proportions of participants achieving clinically important improvements in physical function, fatigue and pain with corresponding risk differences

| Variable | Number (%) | | Risk difference (95% CI) | p Value |
|---|---|---|---|---|
| | Intervention group | Control group | | |
| Achieved 0.22 improvement in HAQ scores | 38 (51) | 4 (5) | 46% (33% to 58%) | 0.0001 |
| Achieved 10 mm improvement on VAS for fatigue | 46 (62) | 10 (14) | 48% (35% to 62%) | 0.0001 |
| Achieved 10 mm improvement on VAS for pain | 47 (64) | 9 (12) | 51% (38% to 64%) | 0.0001 |

HAQ, Health Assessment Questionnaire; VAS, Visual Analogue Scale.

and stepping time with between-group differences in change of 1.52 hours/day and 0.55 hours/day, respectively.

### Secondary outcomes

Statistically significant differences in favour of the intervention group were found in self-reported daily sitting time at work and during leisure time, for fatigue, pain, physical function, QoL, general self-efficacy and in total cholesterol (tables 1 and 2); also significantly greater proportions achieved clinically meaningful improvements in physical function (HAQ) (minimal clinically important difference (MCID)=0.22), fatigue (Visual Analogue Scale) (MCID=10 mm) and pain (MCID=10 mm) (table 2).[19]

For anthropometric and cardiometabolic measures, no statistically significant differences were found, but numerical differences in change were all in favour of intervention group (table 3).

## DISCUSSION

Individual motivational counselling sessions during a 16-week period accompanied by individual SMS reminders reduced daily sitting time by more than 2 hours compared with the control group. Patient-reported outcomes also improved and, to a lesser extent, cardiometabolic biomarkers. Patients with RA need to manage consequences of an unpredictable disease every day, why the intervention was individualised and targeted sedentary behaviour. This whole-day approach was also targeted in a similar individually tailored, behavioural intervention aiming to reduce daily sitting time in healthy adults.[12] That study showed a non-significant between-group difference in daily sitting time of −0.32 hours, but a statistically significant difference in waist circumference and fasting insulin levels in favour of the intervention group after 6-month intervention. Our 16-week intervention period may not be long enough to detect significant changes in other cardiometabolic biomarkers than total cholesterol. Additionally, the changes in waist circumference almost reached statistical significance, which supports the call for a longer intervention period.

The magnitude of changes in physical function, fatigue and pain was also assessed by looking at a clinical impact of the intervention on RA-related outcomes. Achievement of the MCID was consistently reached for greater proportions in the intervention group. Without neglecting the important and well-established health benefits of engaging in moderate-to-vigorous physical activity, our results indicate that patients with RA can achieve substantial health benefits by reducing sitting time. This would have implications for clinical practice and physical activity recommendations.

Strengths include the randomised controlled design, blinding of outcome assessors and objective measurements. The two groups differed in their baseline measures with respect to daily sitting time, pain and fatigue. However, we regard these differences as random occurrences.

We cannot rule out that the significant changes in cholesterol levels and self-reported clinical outcomes were reached by other pathways than through increases in low-intensity, non-exercise physical activity, for example, through healthy dietary habits.

The results may not be generalisable to all patients with RA, since those how declined participation were younger and the proportion of men was higher. The intervention may have been more appealing to women, since 81% of the included patients were women; however, up to 75% of patients with RA are women.[20] It is also noteworthy that participants were older and had longer disease duration than non-participants. Focus on light everyday activities and individual tailoring may be particular appealing to this group of patients. Only three participants

**Table 3** Mean changes in anthropometric and cardiometabolic biomarkers after 16 weeks

| Variable | Mean change from baseline, mean (95% CI) | | Difference in change between groups, mean (95% CI) | p Value |
|---|---|---|---|---|
| | Intervention group | Control group | | |
| Weight (kg) | 0.00 (−0.91 to 0.92) | 0.58 (−0.34 to 1.49) | −0.58 (−1.87 to 0.72) | 0.38 |
| Waist circumference (cm) | −0.80 (−1.90 to 0.30) | 0.71 (−0.39 to 1.81) | −1.51 (−3.07 to 0.05) | 0.056 |
| Waist–hip ratio | −3.03 (−4.72 to 1.35) | −1.81 (−3.50 to −0.13) | −1.22 (−3.60 to 1.16) | 0.31 |
| Body mass index (kg/m$^2$) | 0.02 (−0.30 to 0.32) | 0.16 (−0.17 to 0.49) | −0.14 (−0.60 to 0.28) | 0.46 |
| Blood pressure (mm Hg) | | | | |
| Systolic | −3.06 (−5.98 to −0.14) | −1.57 (−4.49 to 1.34) | −1.49 (−5.61 to 2.64) | 0.47 |
| Diastolic | −0.85 (−2.38 to 0.69) | −0.08 (−1.62 to 1.45) | −0.77 (−2.94 to 1.40) | 0.49 |
| Lipids (mmol/L) | | | | |
| Cholesterol | −0.24 (−0.33 to −0.14) | 0.13 (0.04 to 0.23) | −0.37 (−0.50 to −0.24) | <0.0001 |
| HDL | 0.06 (0.00 to 0.12) | 0.00 (−0.06 to 0.05) | 0.07 (−0.01 to 0.14) | 0.10 |
| LDL | −0.07 (−0.18 to 0.04) | −0.03 (−0.13 to 0.08) | −0.04 (−0.20 to 0.11) | 0.61 |
| Triglyceride | 0.06 (−0.06 to 0.18) | 0.06 (−0.06 to 0.17) | 0.00 (−0.16 to 0.17) | 0.97 |
| HbA1c (mmol/mol) | −0.05 (−0.22 to 0.12) | 0.10 (−0.07 to 0.27) | −0.15 (−0.40 to 0.09) | 0.22 |

HbA1c, haemoglobin A1c; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol.

(2%) dropped out at end of intervention, which underlines the acceptability of the individually tailored intervention allowing them to set achievable goals for change in everyday activities.

In conclusion, a randomised, observer-blinded 16-week individually tailored, theory-based behavioural intervention with motivational counselling and SMS reminders reduced daily sitting time by an average of 2 hours, improved general self-efficacy, QoL, physical function, total cholesterol and reduced levels of pain and fatigue in sedentary patients with RA.

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**Contributors** BAE, JM, MA, NB, MLH, KL, MØ, PJJ and TT designed and planned the trial in collaboration with the trial steering committee. BAE, KL and TT obtained funding. TT managed the day-to-day running of the trial. RC designed the statistical analysis plan and carried out the data analyses in collaboration with TT. MA advised on the analyses and interpretations of ActivPAL data. All authors had full access to the data and take responsibility for the integrity of the data and for the accuracy of the data analysis. Additionally, all authors contributed to the interpretation of the results and reviewed and approved the final manuscript. TT drafted this article and is the guarantor.

**Competing interests** None declared.

**Patient consent** Obtained.

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**Data sharing statement** BAE is willing to examine all requests for the full dataset after a period of 5 years from the date of this publication. The trial steering committee will be involved in the case of query about access to data. Participants did not give consent for data sharing but the presented data are anonymised and risk of identification is low.



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EXTENDED REPORT

# A single nucleotide polymorphism in the *NCF1* gene leading to reduced oxidative burst is associated with systemic lupus erythematosus

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## ABSTRACT

**Objectives** *Ncf1* polymorphisms leading to low production of reactive oxygen species (ROS) are strongly associated with autoimmune diseases in animal models. The human *NCF1* gene is very complex with both functional and non-functional gene copies and genotyping requires assays specific for functional *NCF1* genes. We aimed at investigating association and function of the missense single nucleotide polymorphism (SNP), rs201802880 (here denoted NCF1-339) in *NCF1* with systemic lupus erythematosus (SLE).

**Methods** We genotyped the NCF1-339 SNP in 973 Swedish patients with SLE and 1301 controls, using nested PCR and pyrosequencing. ROS production and gene expression of type 1 interferon-regulated genes were measured in isolated cells from subjects with different NCF1-339 genotypes.

**Results** We found an increased frequency of the NCF1-339 T allele in patients with SLE, 11% compared with 4% in controls, OR 3.0, 95% CI 2.4 to 3.9, $p=7.0\times10^{-20}$. The NCF1-339 T allele reduced extracellular ROS production in neutrophils (p=0.004) and led to an increase expression of type 1 interferon-regulated genes. In addition, the NCF1-339 T allele was associated with a younger age at diagnosis of SLE; mean age 30.3 compared with 35.9, $p=2.0\times1^{-6}$.

**Conclusions** These results clearly demonstrate that a genetically controlled reduced production of ROS increases the risk of developing SLE and confirm the hypothesis that ROS regulate chronic autoimmune inflammatory diseases.

## INTRODUCTION

Genetic mapping and positional cloning of genetic polymorphisms associated with chronic autoimmune diseases in animal models have revealed the *Ncf1* gene to be of major importance.[1 2] The *Ncf1* gene encodes the $p47^{phox}$/Ncf1 protein of the NADPH oxidase (NOX2) complex, which is critical for the induction of reactive oxygen species (ROS).[3] ROS were at the time believed to mainly contribute to the chronic inflammation in autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). However, it is now clear that ROS also have important regulatory functions in the immune system (reviewed in ref 4). The finding that genetically controlled low capacity of ROS production by NOX2 leads to autoimmunity was also supported by the observation that chronic granulomatous disease (CGD), caused by mutations in the subunits of NOX2, has autoimmune and lupus-like symptoms.[5 6] The question arose whether polymorphisms in the NOX2 genes, leading to decreased ROS production, also are associated with autoimmune diseases. There are now several reports suggesting associations of NOX2 genes with autoimmune diseases such as SLE and RA.[7 8] Interestingly, Jacob *et al* reported an association with SLE to a single nucleotide polymorphism (SNP) in the NOX2 component $p67^{phox}$/NCF2, where the causal allele was shown to reduce ROS production.[9]

In the human genome, *NCF1* is located in a structurally complex region,[10 11] which complicates genotyping and has excluded *NCF1* from genome-wide association studies. Close to *NCF1* are two *NCF1* pseudogenes that encode truncated, non-functional proteins.[12] In addition, the *NCF1* gene exists in a varying number of copies, which seem to consist of parts of *NCF1* and parts of a pseudogene.[8 13] We have previously investigated the functional effects of three SNPs in *NCF1* and found that the minor allele (T) of an SNP in exon 4 (rs201802880, here denoted *NCF1*-339) reduced ROS production in transfected cell constructs.[8] The nucleotide shift from C to T alters the amino acid from arginine to histidine at a membrane binding site of the Ncf1 protein.[14] In light of the findings that genetically encoded low ROS contribute to SLE, we asked if the NCF1-339 SNP was associated with SLE.

## METHODS

### Study populations

Patient characteristics are outlined in online supplementary table 1. All patients included met at least 4 of 11 American College of Rheumatology (ACR) classification criteria.[15] The discovery population comprised the Linköping, Uppsala and Lund patients with SLE and 1016 controls from the epidemiological investigation of rheumatoid arthritis (EIRA) case–control study on incidence (72% women, all Caucasians).[16] About 480 of the EIRA control genotypes are previously published,[8] but an additional 536 EIRA controls were included in this study. The EIRA controls and patients with

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SLE are sampled from the same area of Sweden and should represent comparable populations. The replication population comprised the Karolinska patients with SLE and 303 controls, matched by age and sex to 303 of the patients with SLE.

### Genotyping

The genotyping of NCF1-339 and copy number analysis of *NCF1* were performed using a nested PCR strategy and pyrosequencing[8 17] with modifications detailed in supplementary methods.

### Functional analyses

Patients with RA and SLE with specific NCF1-339 genotypes were recruited at the rheumatology clinics at Karolinska University Hospital and Skåne University Hospital, respectively. Blood was sampled from 2 TT, 4 CT, 8 CC and 3 CCT patients with RA and 5 TT, 12 CT and 13 CC patients with SLE. Polymorphonuclear cells (PMN) and peripheral blood mononuclear cells (PBMC) were isolated according to ref 18 and described in supplementary methods.

#### ROS production

ROS production by blood cells was analysed by isoluminol or luminol-enhanced chemiluminescence (CL)[19] and the Phagoburst assay (Glycotope), detailed in supplementary methods.

#### Gene expression

Blood was collected in PAXgene blood RNA collection tubes and RNA was isolated according to instructions in the PAXgene blood RNA kit (PreAnalytix, Qiagen). mRNA expression was measured using TaqMan assays (Thermo Fisher Scientific) described in supplementary methods.

### Statistical analyses

Statistical analysis was done in the software JMP V.12 (SAS Institute), Prism V.7 (GraphPad), Excel (Microsoft) and StatsDirect V.3 (StatsDirect). Genetic association of genotype, allele and genotype group frequencies was analysed in contingency tables with $\chi^2$ tests or Fisher's exact test. The genetic analyses were stratified according to ancestry, and presented results only include Caucasians. Meta-analyses were performed with Mantel-Haenszel $\chi^2$ tests. Mann-Whitney U test and Kruskal-Wallis non-parametric test were used to compare differences in ROS production and gene expression. Distributions of age at diagnosis, ACR classification criteria and Systemic Lupus International Collaborating Clinics/ACR disease damage index (SDI) scores were analysed with histograms and comparisons of frequencies and mean values were analysed using $\chi^2$ statistics or Fisher's exact tests and Mann-Whitney U test and Kruskal-Wallis statistics.

**Table 1** Allele frequencies of NCF1-339

| Study populations | T allele Frequency (n) | C allele Frequency (n) | p Value | OR (95% CI) |
|---|---|---|---|---|
| Discovery population | | | | |
| Patients with SLE | 0.11 (132) | 0.89 (1074) | $3.2\times10^{-12}$ | 2.63 (2.00 to 3.45) |
| EIRA controls | 0.04 (96) | 0.96 (2052) | | |
| Replication population | | | | |
| Karolinska SLE | 0.10 (88) | 0.90 (764) | $1.3\times10^{-9}$ | 4.59 (2.63 to 8.02) |
| Karolinska controls | 0.02 (15) | 0.98 (598) | | |
| Meta-analysis | | | | |
| Patients with SLE | 0.11 (220) | 0.89 (1838) | $7.0\times10^{-20}$ | 3.03 (2.37 to 3.86) |
| Controls | 0.04 (111) | 0.96 (2650) | | |

EIRA, epidemiological investigation of rheumatoid arthritis; SLE, systemic lupus erythematosus.

### Study approval

Oral and written informed consent was obtained from all subjects. The study protocol for the genetic analyses was approved by the regional ethics review boards in Lund, Linköping, Uppsala and Stockholm and the functional analyses by the regional ethics review boards in Lund and Stockholm.

## RESULTS

### The T allele of the SNP NCF1-339 is highly enriched in patients with SLE

To investigate if the T allele of NCF1-339 is associated with SLE, we genotyped a discovery population of 570 Swedish Caucasian patients with SLE and 1016 controls from the Swedish EIRA cohort. The T allele was enriched in patients with SLE, with a frequency of 0.11 compared with 0.04 in the controls with an OR of 2.6, 95% CI 2.0 to 3.5 (table 1). As replication, we genotyped a Swedish case–control cohort comprising 403 patients with SLE and 285 controls (Karolinska). The T allele was associated with SLE also in this cohort, with a frequency of 0.10 in patients with SLE compared with 0.02 in the controls, OR 4.6, 95% CI 2.6 to 8.0 (table 1). Meta-analysis of the two study populations gives a T allele frequency of 0.11 in patients with SLE compared with 0.04 in the controls, OR 3.0, 95% CI 2.4 to 3.9, $p=7.0\times10^{-20}$. The complete genotype results are presented in online supplementary table 2.

### A higher frequency of patients with SLE have only one *NCF1* gene

Carriers of *NCF1*-CGD have only one *NCF1* gene, due to non-allelic homologous recombination between *NCF1* and the pseudogenes.[11] We analysed *NCF1* gene copy number in the SLE study populations and found that a higher frequency of patients with SLE have only one *NCF1* gene compared with controls, 1.1% compared with 0.2%, in all cohorts combined, OR 5.0, 95% CI 1.4 to 17.6 (online supplementary table 3). There were no significant differences in frequency of more than two *NCF1* genes in patients with SLE compared with controls.

### T-type genotypes have a higher frequency in patients with SLE

The effect of the T allele needs to be considered in relation to the number of *NCF1* gene copies. The CT, TT and CTT genotypes are enriched in patients with SLE, but CCT is not, indicating that additional C alleles can compensate the functional effects mediated by the T allele (online supplementary table 2). To estimate the total genetic effects of the NCF1-339 association, we grouped the genotypes with less than two C alleles: C, T, TT, CT, CTT (denoted T type) and the remaining genotypes: CC, CCT, CCCC, CCCT and CCTT (denoted C type) and compared the frequency in SLE cases and controls. The T-type group had a higher frequency in patients with SLE compared with controls (table 2). Meta-analysis of the combined study populations gives an OR of 3.7, 95% CI 2.7 to 4.9, $p=1.8\times10^{-18}$.

### The SLE-associated T allele of NCF1-339 reduces extracellular ROS production

To investigate if the NCF1-339 T allele has an effect on ROS production, we measured extracellular and intracellular ROS production in primary cells from patients with SLE with two *NCF1* genes and CC, CT and TT NCF1-339 genotypes. To capture the effect of the T allele on different NOX2 activation pathways, three stimuli were used: phorbol 12-myristate 13-acetate (PMA), the chemotactic peptide N-Formyl-Met-Leu-Phe (fMLF) and phagocytosis inducing serum-opsonised zymosan (SOZ). The PMA-stimulated extracellular ROS were delayed and reduced in PMN from patients with TT genotypes compared with CC (figure 1A–C). There was also a T allele-dependent reduction in extracellular ROS in fMLF and non-stimulated PMNs (figure 1D,F). No genotype-dependent differences could be observed when cells were stimulated with SOZ (figure 1E), in PBMCs (online supplementary figure 1), or in intracellular ROS production for any stimuli (online supplementary figure 2). In order to verify these results and to investigate ROS production from different types of cells, we used a flow-cytometry assay to measure ROS accumulated intracellular, after stimulation with PMA or opsonised *Escherichia coli*. The T allele significantly reduced PMA-stimulated ROS in PMN, but not in monocytes or when stimulated with opsonised *E. coli* (figure 2). The T allele also reduced the responsiveness of PMNs to stimulation with PMA.

**Table 2** Frequency of NCF1-339 genotype groups

| Study populations | T-type genotypes Frequency (n) | C-type genotypes Frequency (n) | p Value | Odds ratio (95% CI) |
|---|---|---|---|---|
| Discovery population | | | | |
| Patients with SLE | 0.18 (101) | 0.82 (469) | $5.7\times10^{-12}$ | 3.15 (2.26 to 4.39) |
| EIRA controls | 0.06 (65) | 0.94 (951) | | |
| Replication population | | | | |
| Karolinska patients with SLE | 0.16 (64) | 0.84 (339) | $2.5\times10^{-8}$ | 5.79 (2.91 to 11.55) |
| Karolinska controls | 0.03 (9) | 0.97 (276) | | |
| Meta-analysis | | | | |
| Patients with SLE | 0.17 (165) | 0.83 (808) | $1.8\times10^{-18}$ | 3.65 (2.70 to 4.91) |
| Controls | 0.06 (74) | 0.94 (1227) | | |

EIRA, epidemiological investigation of rheumatoid arthritis; SLE, systemic lupus erythematosus.

There was no difference in age, disease activity, as determined by the SLE Disease Activity Index,[20] or corticosteroid use that could explain the observed differences in ROS production

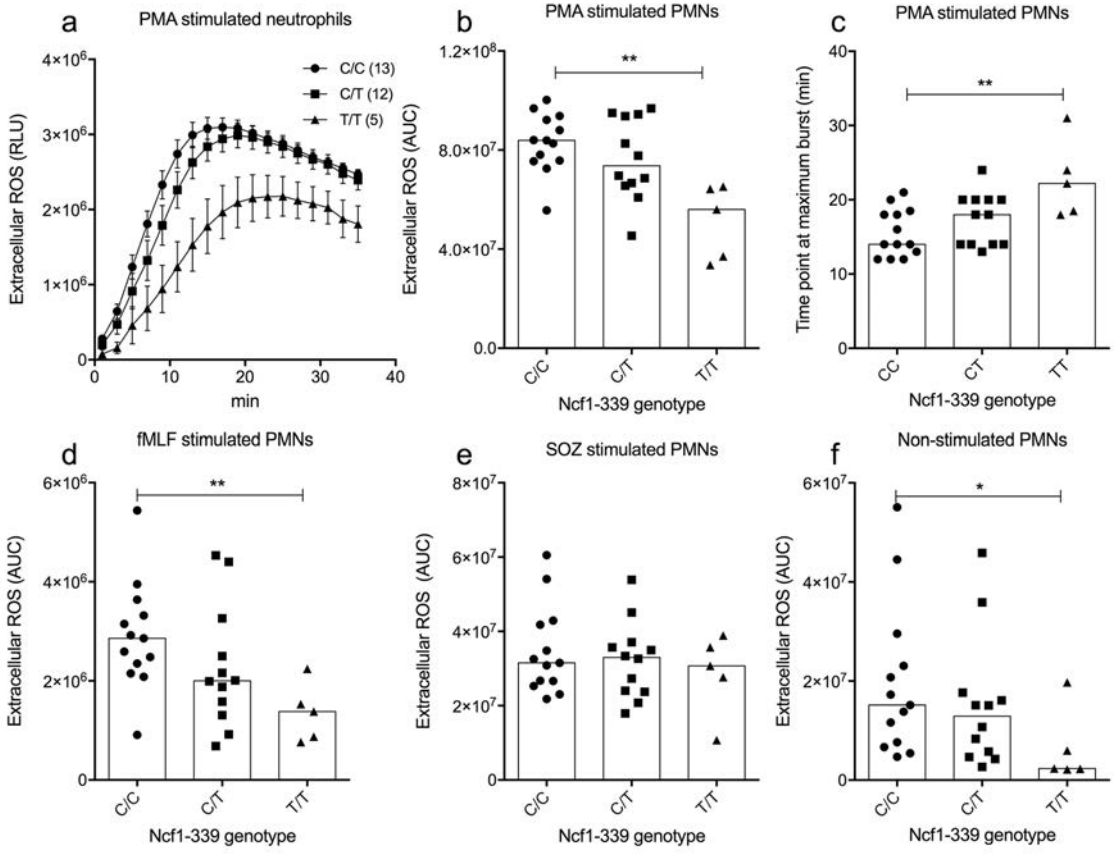


**Figure 1** The NCF1-339 T allele reduces extracellular ROS. Extracellular ROS in PMN grouped by NCF1-339 genotype stimulated by (A–C) PMA, (D) fMLF, (E) SOZ or (F) non-stimulated were measured using isoluminol-enhanced chemiluminescence. (A) Extracellular ROS production over time as RLUs in PMA stimulated PMNs from patients with SLE (n=30), grouped by NCF1-339 genotype (mean ± SEM at each time point), (B) AUC calculated from RLU values for PMA-stimulated extracellular ROS, (C) time point in minutes, at highest RLU value for PMA-stimulated extracellular ROS. AUC calculated from RLU values for (D) fMLF (AUC for 1–5 min), (E) SOZ and (F) non-stimulated PMN cells. Bar heights represent median values. *p<0.05; **p<0.01. AUC, area under the curve; fMLF, N-Formyl-Met-Leu-Phe; PMA, phorbol 12-myristate 13-acetate; PMN, polymorphonuclear cells; RLU, relative luminescence units; ROS, reactive oxygen species; SLE, systemic lupus erythematosus; SOZ, serum-opsonised zymosan.

(online supplementary table 4 and supplementary figure 3). There was a difference in hydroxychloroquine (HCQ) usage between the genotype groups and ROS were higher in patients on HCQ treatment compared with patients without. However, there was no difference when HCQ treatment was analysed within the CT genotype group (online supplementary figure 3).

To investigate the ROS-reducing effect of the T allele in a different autoimmune patient group, and to see if additional *NCF1* genes can restore the reduction of ROS caused by the T allele, we measured extracellular and intracellular ROS in PMN and PBMCs from patients with RA with CC, CT, TT and CCT NCF1-339 genotypes. The T allele reduced PMA-stimulated extracellular ROS in both PMNs and PBMCs, but no difference in intracellular ROS was detected (online supplementary figure 4). There was also no significant difference in ROS production in patients with CCT genotypes compared with patients with CC genotypes.

### The T allele of Ncf1-339 increases the expression of type 1 interferon-regulated genes

We have previously shown that genetically encoded NOX2 deficiency, in both mice and patients with CGD, increased the expression of type 1 interferon-regulated genes (IRG).[21] To investigate if the same is seen for the NCF1-339 T allele, we measured the expression of selected IRGs in whole blood from patients with SLE and RA included in the ROS study. Patients with RA with CT genotypes had a significantly increased expression of five IRGs, *IFI44L*, *ISG15*, *OAS1*, *IRF7* and *STAT1*, compared with the patients with CC genotypes (figure 3). In the SLE cases, however, there were no expression differences between the genotype groups for *IRF7*, *ISG15* and *IFI44L*. The difference in fold change between patients with SLE was much larger compared with patients with RA, reflective of a stronger interferon (IFN) signature in patients with SLE.

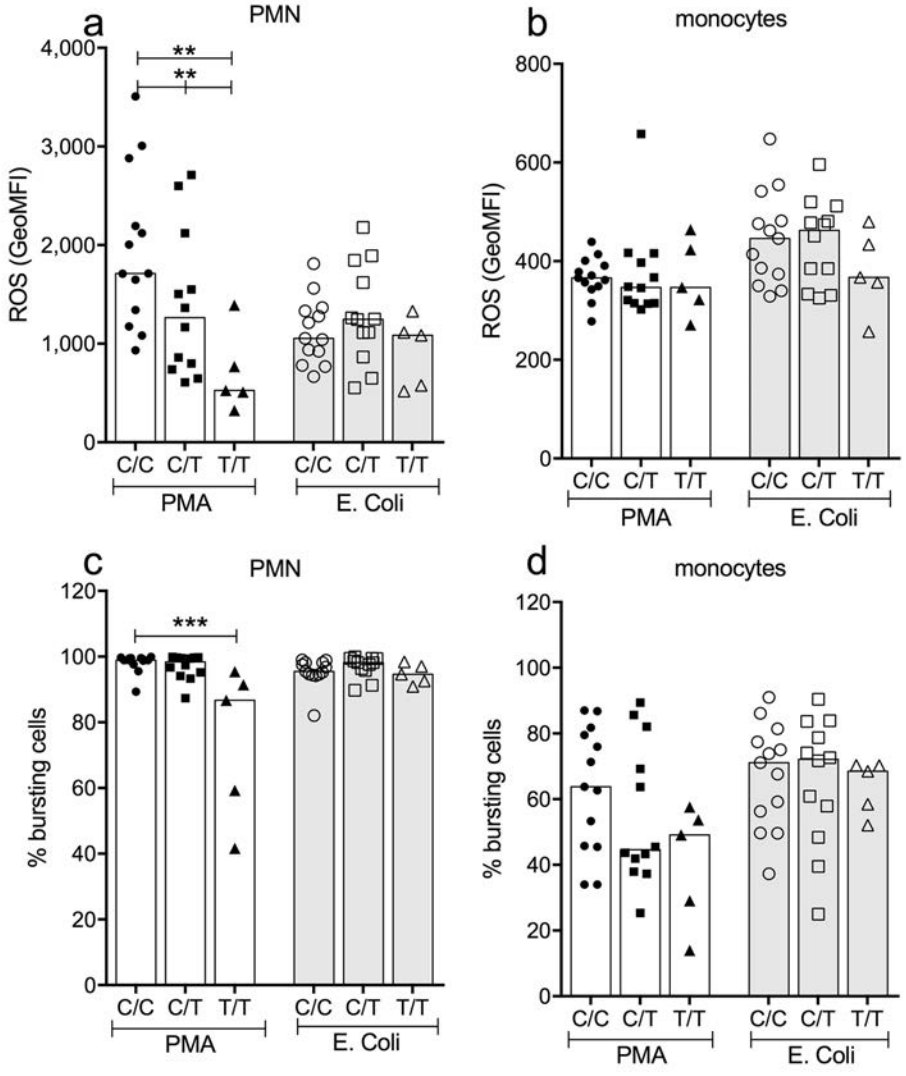


**Figure 2** The NCF1-339 T allele reduces ROS in PMN cells from patients with SLE. ROS measured as geometric mean fluorescence intensity (geoMFI) in (A) PMN and (B) monocytes from patients with SLE (n=30), grouped by NCF1-339 genotype, stimulated with PMA or *Escherichia coli* measured with the Phagoburst assay. % bursting cells for PMA or *E. coli* stimulated (C) PMN or (D) monocytes, grouped by NCF1-339 genotype. Bar heights represent median values. **p<0.01; ***p<0.001. geoMFI, geometric mean fluorescence intensity; PMA, phorbol 12-myristate 13-acetate; PMN, polymorphonuclear cells; ROS, reactive oxygen species; SLE, systemic lupus erythematosus.

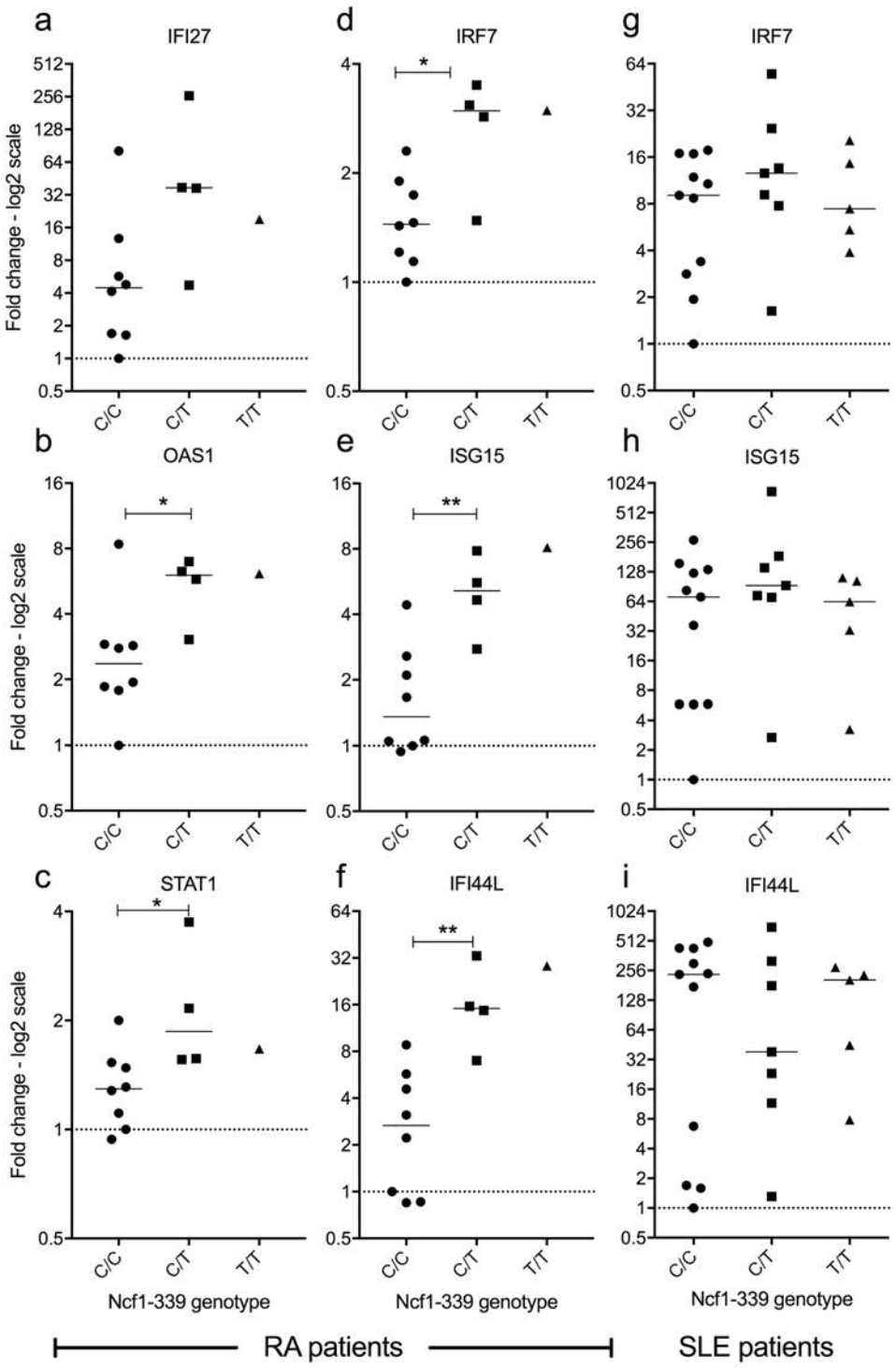


**Figure 3** The T allele of NCF1-339 increases expression of type 1 IRG. Plotted FC values for IRGs grouped by NCF1-339 genotype shown for (A–F) patients with RA and (G–I) patients with SLE. FC values are calculated against the lowest delta CT value. Y-axis are in log2 scale and lines representing median are shown. A dotted line at FC=1 represents no increase in expression. *p<0.05; **p<0.01. FC, fold change; IRG, interferon-regulated genes; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.

### NCF1-339 T allele is associated with a lower age at diagnosis

We analysed the clinical data from the SLE study populations to investigate if the NCF1-339 T-type genotypes are associated with clinical phenotypes or disease severity. Patients with T-type genotypes had a younger age at diagnosis compared with patients with C-type genotypes, mean age 30.3 compared with 35.9, $p=2.0\times10^{-6}$ (table 3). There were no consistent differences in the distribution of ACR criteria or in SDI score between C-type and T-type genotypes, although renal disorder was more common in the T-type group in the Karolinska patients with SLE (p=0.03), with the same tendency seen in Lund and Linköping (online supplementary table 5).

**Table 3** Age at diagnosis in the study populations

| | Age at diagnosis mean (95% CI) | | |
|---|---|---|---|
| Study populations | T-type genotypes | C-type genotypes | p Value |
| Linköping SLE | 32.6 (25.7 to 39.5) | 38.8 (35.7 to 41.9) | 0.03 |
| Uppsala SLE | 30.2 (25.2 to 35.3) | 31.3 (28.4 to 34.1) | 0.70 |
| Lund SLE | 29.8 (26.0 to 33.6) | 38.9 (36.9 to 40.8) | 0.0003 |
| Karolinska SLE | 29.7 (26.5 to 33.0) | 34.6 (33.0 to 36.1) | 0.006 |
| All patients with SLE | 30.3 (28.1 to 32.4) | 35.9 (34.9 to 37.0) | $2.0\times10^{-6}$ |

SLE, systemic lupus erythematosus.

## DISCUSSION

We report that an amino acid replacement in *NCF1*, leading to a lower capacity of inducing oxidative burst, is strongly associated with SLE. The OR is 3.7, which makes it one of the strongest identified genetic associations with SLE.[22 23] The strength of the association is indicated by a 6-year earlier disease onset in patients with T-type genotypes. The *NCF1* gene is highly complex, which has excluded SNPs in *NCF1* in genome-wide association studies. *NCF1* genotyping requires specialised methods to exclude the non-functional *NCF1*-pseudo genes, and capture all functional gene copies. During the finalisation of this paper, a similar study was published confirming a strong effect of the NCF1-339 T allele on SLE and also on Sjögren's syndrome and RA,[24] thus two independently performed reports show that NCF1-339 is one of the strongest SNPs, outside the HLA region, associated with autoimmune diseases.

The NCF1-339 T allele leads to a shift from Arg to His at position 90, which is located in the phox domain of NCF1 and mediate binding to the cellular membrane. We and others have previously shown that mutating position 90 reduces the ROS response[8] and the binding efficiency of NCF1 to the membrane.[25] Here we wanted to investigate the functional effects of the NCF1-339 T allele on primary cells relevant for the pathogenesis of SLE. We demonstrate that the NCF1-339 T allele reduced the capacity of the NOX2 complex to produce ROS in PMNs from both patients with SLE and RA. In patients with RA, a significant reduction is seen also in PBMCs.

The high frequency of a ROS-reducing allele in patients with SLE, found in this study, goes in line with the previously reported association of a ROS-reducing SNP in the *NCF2* gene with SLE,[9] as well as with data from animal models. Mice with a loss-of-function mutation in *Ncf1* develop spontaneous SLE-like disease, including production of autoantibodies and increased expression of type 1 IRGs.[21] Furthermore, patients with CGD have an increased expression of type 1 IRG in whole blood.[21] In this study, we found that the NCF1-339 T allele increased the expression of type 1 IRG in whole blood from patients with RA. Taken together, these findings show that ROS are important for regulation of this pathway. However, we were not able to detect an increased type 1 IRG expression in patients with SLE with the NCF1-339 T allele, instead we saw a dramatic increase in expression of IRG in most of the patients, reflective of the IFN signature. A likely explanation could be that ROS are involved in regulating the initiation of the type 1 IFN pathway, but once the pathway is activated the strong IFN signature in SLE overshadows the genetic effect of the T allele. However, it is also possible that the IFN effect caused by the NCF1-339 T allele seen in patients with RA and CGD is not present in patients with SLE.

How ROS affect the type 1 IFN pathway is not known, but the influence of oxidation is far upstream, affecting STAT1 in monocytes[21] or possibly the release of IFN-α from plasmacytoid dendritic cells.[26] ROS produced by monocytes can also inhibit interacting cells, for example, NK cells or classical αβ T cells during antigen presentation.[27 28] ROS can be released within the immunological synapse or by transfer of exosomes containing NOX2 to CD4 T cells.[29] These findings all point to the crucial role that ROS play in controlling the immune response and preventing excessive activation of immune effector cells, such as T cells and NK cells. Furthermore, we show that the NCF1-339 T allele reduces the burst capacity of PMNs, which mainly consist of neutrophils. This is possibly due to that neutrophils produce large amount of ROS detectable in vitro and does not exclude that the regulatory ROS come from other cell types. Neutrophil-derived ROS are a prerequisite for the formation of neutrophil extracellular traps (NETs), suggested to be pathogenic in SLE. However, NETs have also been reported to protect against chronic inflammation by absorption of inflammatory cytokines.[30] In addition, in patients with CGD, NETs are also formed as a result of mitochondrial-derived ROS, which seem to be more inflammatory compared with nuclear DNA in regular NETs.[31]

The previously reported SLE-associated SNP in *NCF2* only affects intracellular ROS, stimulated by phagocytosis inducing stimuli, via the Fcγ receptor (FcγR).[9] The NCF1-339 T allele, on the other hand, has no effect on ROS induced via the FcγR with opsonised zymosan or *E. coli*, and a strong effect on extracellular ROS. These two SLE-associated SNPs seem to affect two different NOX2 activation pathways. However, it is methodologically difficult to separate extracellular from intracellular ROS production. We used two methods to measure ROS inside the cell with various results, likely explained by the different experimental set-up. In the Phagoburst assay, extracellular $H_2O_2$ produced by NOX2 in the plasma membrane could diffuse across the membrane into the cell and react with the detection probe.[32] In the intracellular CL assay, extracellular ROS are immediately scavenged by added superoxide dismutase and catalase, ensuring that only intracellular ROS are measured. The difficulties to separate intracellular from extracellular ROS are also reflected in the in vivo situation, where ROS produced by NOX2 in the plasma membrane could diffuse or be transferred into the cell and affect intracellular pathways. The fact that we see a genotype-dependent difference in burst using the Phagoburst assay, but not using the intracellular CL assay, suggests that extracellular $H_2O_2$ has the capacity to alter intracellular systems. The reasons why the T allele has no effect on FcγR-stimulated ROS could be because NCF1 is less important for the intracellular ROS induced by particulate stimuli and released during phagocytosis.[25] NCF1 and the NOX2 subunit NCF4 are both keeping the cytosolic part of NOX2 attached to the membrane, which is required for full activation. The two membrane-binding sites of NCF1, including Arg90, mediate stronger binding to the plasma membrane, whereas the binding sites of NCF4 have specificity for phagosomal membranes. Mutations in *Ncf4* and *Ncf1* have in fact been shown to affect autoimmune diseases differentially.[33]

We saw no difference in ROS production in subjects with the NCF1-339 CCT genotype compared with CC, despite the T allele, which demonstrates how important it is to consider *NCF1* copy number when analysing the genetic and functional effects of the NCF1-339 association. The functional effects of the NCF1-339 genotypes with additional *NCF1* genes are harder to predict than the standard two allele genotypes. One additional *NCF1* gene does not increase ROS production because there is no increase in the other NOX2 subunits.[34] The CCT genotype could have a similar functional effect as CC, because of the two functional C alleles; however, the NCF1 protein with

His90 (encoded by the T allele) could also compete with NCF1 proteins with Arg90 for binding to NOX2 subunits, and thus lead to reduced ROS production.

Taken together, the SLE-associated NCF1-339 SNP, leading to amino acid variability in the NCF1 protein and a lower capacity to induce oxidative burst, is so far one of the strongest loci associated with an autoimmune disease and confirm the hypothesis that ROS regulate chronic autoimmune inflammatory diseases.

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**Contributors** LMO and RH designed the research. LMO performed the major part of the experimental work and statistical analyses. ÅCJ performed the Phagoburst assay analysis and BG helped with preparing the patient samples. AJ, SS, LR, DL, JW, CS, ES, IG and AAB contributed patient samples and clinical data. LMO and RH wrote the manuscript. All authors revised and approved the final manuscript.

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EXTENDED REPORT

# Faecal microbiota study reveals specific dysbiosis in spondyloarthritis

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## ABSTRACT

**Objective** Altered microbiota composition or dysbiosis is suspected to be implicated in the pathogenesis of chronic inflammatory diseases, such as spondyloarthritis (SpA) and rheumatoid arthritis (RA).

**Methods** 16S ribosomal RNA gene sequencing was performed on faecal DNA isolated from stool samples in two consecutive cross-sectional cohorts, each comprising three groups of adult volunteers: SpA, RA and healthy controls (HCs). In the second study, HCs comprised a majority of aged-matched siblings of patients with known HLA-B27 status. Alpha and beta diversities were assessed using QIIME, and comparisons were performed using linear discriminant analysis effect size to examine differences between groups.

**Results** In both cohorts, dysbiosis was evidenced in SpA and RA, as compared with HCs, and was disease specific. A restriction of microbiota biodiversity was detected in both disease groups. The most striking change was a twofold to threefold increased abundance of *Ruminococcus gnavus* in SpA, as compared with both RA and HCs that was significant in both studies and positively correlated with disease activity in patients having a history of inflammatory bowel disease (IBD). Among HCs, significant difference in microbiota composition were also detected between HLA-B27+ and HLA-B27 negative siblings, suggesting that genetic background may influence gut microbiota composition.

**Conclusion** Our results suggest that distinctive dysbiosis characterise both SpA and RA and evidence a reproducible increase in *R. gnavus* that appears specific for SpA and a marker of disease activity. This observation is consistent with the known proinflammatory role of this bacteria and its association with IBD. It may provide an explanation for the link that exists between SpA and IBD.

## INTRODUCTION

Spondyloarthritis (SpA) is a multifactorial heterogeneous disorder, which is thought to result from complex interactions between a particular genetic background and environmental factors. The clinical spectrum of SpA is diverse, comprising both axial and peripheral joint inflammation and extra-articular manifestations, including psoriasis, uveitis and inflammatory bowel disease (IBD).[1] Heredity is high in SpA, and several genetic polymorphisms have been shown to influence the risk of this disorder. The most important one is the MHC class I allele HLA-B27.[2] Several other polymorphisms have been discovered in the recent years, through genome-wide association studies, pointing towards putative pathophysiological pathways. Remarkably, a large subset of the responsible genes code for proteins involved in immune response, and particularly in the interleukin (IL)-23/Th17 pathway of T cell differentiation, which is primarily implicated in response against extracellular pathogens, including bacteria and yeasts, and/or in microbial sensing.[3] Moreover, cross-disease genetic association study established that a substantial fraction of SpA heritability is due to genetic factors that also predispose to IBD.[4]

In contrast to genetic factors, the role of external influences on disease development and/or evolution has been far less studied in SpA. This is particularly the case regarding the putative influence of the abundant gut microbiota content. Disequilibrium in such complex realm of microbes that closely interact with the gut mucosal immune system, a situation referred to as dysbiosis, has been associated with several chronic inflammatory disorders and in particular with IBD, including Crohn's disease and ulcerative colitis.[5]

The role of microbiota in animal models of arthritis has been established for decades. It is notably the case of the HLA-B27/human β2-microglobulin transgenic rat model of SpA, in which gut dysbiosis was found and breeding under germ-free conditions prevented the development of both arthritis and the accompanying IBD phenotype mimicking ulcerative colitis.[6] More recently, several pioneering microbiota high-throughput sequencing studies conducted in human arthritides also reported gut dysbiosis either in stool samples from patients affected by rheumatoid arthritis (RA), psoriatic arthritis (PsA) or juvenile enthesitis-related arthritis, a paediatric form of SpA, or in ileal biopsies from adult patients with SpA.[6–8] Despite showing promising results, the studies that concerned PsA and SpA were each of relatively small size (9–27 patients compared with 9–17 healthy controls (HCs)), and the differences between patients and controls were therefore of limited statistical significance with regard to the number of microbial species examined.[9]

In an attempt to further interrogate if specific gut dysbiosis may associate with SpA, we performed metagenomic analysis, comparing stool samples between adult SpA and control groups, that included both healthy individuals and patients with RA. We used a two-stage design that allowed us to test for the reproducibility of our results. Furthermore, the second phase of the study included a large number of healthy sibling of patients with SpA as controls, allowing us to control in as much

as possible for genetic background variability and to interrogate HLA-B27 effect.

## PATIENTS AND METHODS

### Ethics statement

The study was conducted according to the Declaration of Helsinki and French legislation. Before study inclusion, each participant gave written informed consent for research use and publication of their data.

### Population recruitment and study design

The study population was recruited in two consecutive phases. In the first discovery phase, patients having established SpA or RA and consulting in a tertiary-care centre, that is, the rheumatology clinic of Ambroise Paré Hospital, were included, as well as HCs belonging mostly to the hospital staff. In the second confirmatory phase, patients identified as having SpA and belonging to French families collected by Groupe Français d'Etude Génétique des Spondyloarthrites for genetic purposes as previously described,[10] were included as well as aged-matched healthy siblings. Recently diagnosed patients with RA having not yet received corticosteroid, disease-modifying antirheumatic drug (DMARD) or biotherapy were recruited from Ambroise Paré Hospital outpatient clinic.

Altogether, stool samples were collected from 199 volunteers consisting of 96 SpA patients, all fulfilling the Assessment of SpondyloArthritis International Society (ASAS) classification criteria,[11] 32 independent patients with RA fulfilling the American College of Rheumatology/European League Against Rheumatism classification criteria[12] and 71 HCs, including 28 genetically independent controls and 43 who were recruited as siblings of patients with SpA enrolled in the study (22 HLA-B27+ and 21 HLA-B27− siblings).

All participants were required not to have received antibiotics during the preceding month, nor to have undergone gut preparation for colonoscopy during the preceding 6 months. Individuals affected with medical condition other than the diseases under investigation thought to potentially affect results of the study were excluded. This concerned notably other osteoarticular inflammatory disorders and/or autoimmune disorders, obesity, diabetes, cancer and any chronic organ failure. Demographic data, body mass index, clinicobiological data relevant for disease classification or disease activity evaluation (ie, Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) as for SpA and Disease Activity Score 28 (DAS28)-erythrocyte sedimentation rate as for RA, respectively) and current treatments were collected. Active disease was defined as a BASDAI ≥3/10, a cut-off corresponding to the lower limit of the 'patient acceptable symptomatic state' 95% CI, as proposed in an earlier study[13] or a DAS28 ≥3.2/10, as for SpA and RA, respectively.

Fifteen individuals were removed from analysis because of an insufficient yield of bacterial sequences (see below). Therefore, detailed characteristics of the study population are shown for the 184 individuals that were included in the final analysis (table 1).

**Table 1** Characteristics of patients and controls included in the final analysis

| | Discovery cohort | | | Confirmatory cohort | | |
|---|---|---|---|---|---|---|
| Characteristic | SpA (n=49) | HC (n=18) | RA (n=17) | SpA (n=38) | HC (n=51) | RA (n=11) |
| Age in years, mean±SD | 48±13 | 37±10.4 | 65.5±11.6 | 53±9.8 | 54±8.6 | 56±19.5 |
| Disease duration in years, mean±SD | 22.1±12.0 | N/A | 19.9±12.8 | 29.3±11.2 | N/A | 2±2.2 |
| Sex ratio, % of men | 45 | 50 | 18 | 47 | 33 | 27 |
| BMI, mean±SD | ND | ND | ND | 25.2±3.9 | 25.6±4.9 | 24±4.9 |
| HLA-B27 positivity, % | 67 | ND | ND | 97.4 | 45 | ND |
| Disease activity*, mean±SD | 3.9±2.2 | N/A | 3±1.4 | 3.6±2.2 | N/A | 4±1 |
| Radiographic sacroiliitis†, % | 33 | ND | ND | 68 | 3.6 | ND |
| Classification criteria fulfilment | | | | | | |
| Modified New York AS, % | 33 | 0 | 0 | 68 | 0 | 0 |
| ASAS axial SpA, % | 80 | 0 | 0 | 97 | 0 | 0 |
| ASAS peripheral SpA, % | 20 | 0 | 0 | 3 | 0 | 0 |
| ACR/EULAR RA, % | 0 | 0 | 100 | 0 | 0 | 100 |
| Extra-articular manifestations | | | | | | |
| Uveitis, % | 33 | 0 | 6 | 29 | 0 | 0 |
| Psoriasis, % | 49 | 0 | 0 | 16 | 13 | 9 |
| Inflammatory bowel disease, % | 12.2 | 0 | 0 | 5.3 | 0 | 0 |
| Treatments of interest | | | | | | |
| Any, % | 65.3 | 0 | 94.1 | 76.3 | 9.8 | 63.6 |
| NSAIDs, % | 51 | 0 | 35.3 | 60.5 | 7.8 | 45 |
| Corticosteroids % | 12.2 | 0 | 82 .4 | 2.6 | 0 | 0 |
| DMARDs, % | 4 | 0 | 53 | 0 | 0 | 0 |
| Biotherapy, % | 30.6 | 0 | 70.6 | 26 | 0 | 0 |
| Antiacid, % | 30.6 | 0 | 70.6 | 44.7 | 2 | 27.3 |

The registered manifestations correspond to those present at the time of examination, or retrieved from medical history.
*Refers to BASDAI (SpA) or DAS28 (RA).
†Refers to radiographic sacroiliitis ≥grade II bilateral or grade III unilateral.
ACR, American College of Rheumatology; AS, ankylosing spondylitis; ASAS, Assessment of SpondyloArthritis linternational Society; BMI, body mass index; DMARD, disease-modifying drug (ie, methotrexate or sulfasalazine); EULAR, European League Against Rheumatism; HCs, healthy controls; N/A, not applicable; ND, not done; NSAIDs, non-steroidal anti-inflammatory drugs; RA, rheumatoid arthritis; SpA, spondyloarthritis.

### Faecal DNA isolation

Fresh stool samples were either conserved in a close bucket containing Anaerocult A system (Merck, Darmstadt, Germany) for a maximum of 4 hours before being processed and stored frozen or immediately frozen and kept at −80°C before being further processed.

Faecal DNA was extracted from the weighted stool samples as previously described.[14] After the final precipitation, DNA was resuspended in 150 mL of Tris-EDTA (TE) buffer, and stored at −20°C for further analysis.

### 16S ribosomal RNA (16S rRNA) gene sequencing

Microbial diversity was determined for each sample by targeting a portion of the ribosomal genes. A 16S rRNA gene fragment comprising the V3 and V4 hypervariable regions (16S (sense) 5′-TACGGRAGGCAGCAG-3′ and (antisense) 5′-CTACCNGGGTATCTAAT-3′) was amplified using an optimised and standardised 16S-amplicon-library preparation protocol that gives the lowest error rates. Barcode sequences (GsFLX key) TCAG and MIDGsFLX (10 nucleotides) were attached between the 454 GsFLX adaptator sequence and the forward primer V3F. The GsFLX key and the 454 GsFLX adaptator were attached to the reverse primer. The concentration and quality of the PCR products were assessed with Picogreen in order to obtain equal amounts of each of the samples (108 molecules/mL), and then 16S rRNA gene amplicons were sequenced on a Roche GS FLX 454 sequencer (Genoscreen, Lille, France) and processed with standard protocol from manufacturer (http://genoscreen.fr/).

### 16S rRNA gene sequence analysis

The analysis was performed as described previously.[15] The sequences were demultiplexed and quality filtered using the 'quantitative insights into microbial ecology' (QIIME, V.1.8.0) software package.[16] The sequences were assigned to operational taxonomic units (OTUs) using the UCLUST algorithm[17] with a 97% threshold of pairwise identity and classified taxonomically using the Greengenes reference database.[18] Rarefaction was performed (2028–50 076 sequences per sample; 15 samples with less than 2000 sequences were excluded from analysis) and used to compare the abundances of OTUs across samples.

### Statistical plan

Two series of samples, each including patients with SpA and RA and HCs were recruited successively, as described above, and processed in two different runs of sequencing that were analysed separately.

Principal component analyses of the Bray Curtis distance with each sample coloured according to the disease phenotype were built and used to assess the variation between experimental groups (beta diversity). The number of observed species as well as the Shannon, Simpson and Chao1 diversity indexes were calculated using rarefied data (depth=2000 sequences/sample) and used to characterise species diversity in a community. Differential analysis was performed using the linear discriminant analysis effect size (LEfSe) pipeline.[19]

MaAsLin, a multivariate statistical framework, was used to find associations between treatment and microbial community abundance.[20] The following parameters were taken into account

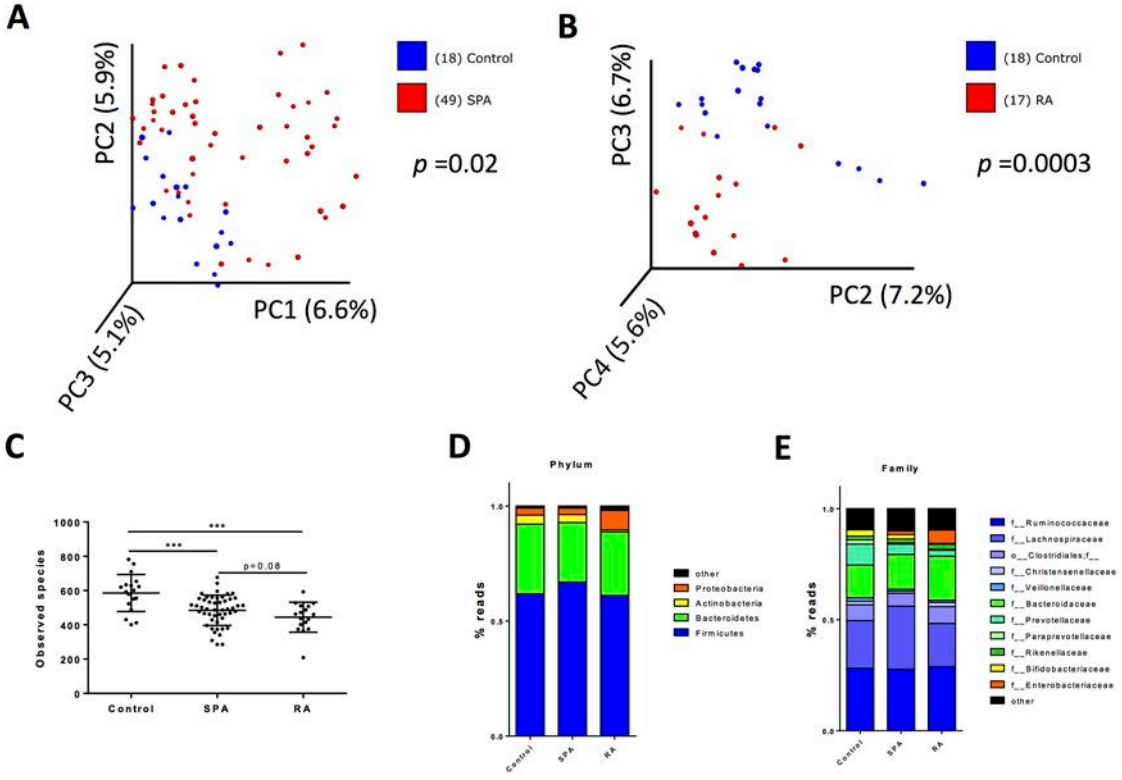


**Figure 1** Bacterial composition of the gut microbiota was significantly different between disease groups and HCs in the discovery cohort. (A and B) Beta diversity according to Bray Curtis index was significantly different between SpA and HCs (A) and between RA and HCs (B). (C) Alpha diversity as assessed by the observed number of bacterial species was significantly reduced both in SpA and RA, as compared with HC. (D and E) Variations in the bacteria phyla (D) and families (E) profiles were apparent between patient groups and HCs. HCs, healthy controls; PC, principal component; RA, rheumatoid arthritis; SpA, spondyloarthritis.

in the analysis: age, gender, disease status (SPA, RA and HCs), definite radiographic sacroiliitis (modified New York criteria), B27 genotype, disease activity, association with IBD and treatments (including biotherapy, non-steroidal anti-inflammatory drug (NSAID), corticosteroid, methotrexate, sulfasalazin and proton pump inhibitor). Associations were considered significant for p value <0.05 and q value <0.1.

Mann-Whitney test was used to compare *Ruminococcus gnavus* abundance between groups, and non-parametric Spearman's test was used to test the correlation between *R. gnavus* relative abundance and BASDAI. A two-way analysis of variance was used to compare *R. gnavus* abundance between SpA patients with and those without a history of IBD, stratifying for disease activity.

## RESULTS

### Discovery study

In the discovery study, faecal microbiota composition was analysed in 49 SpA, 17 RA and 18 HC. Beta diversity analysis showed that the microbiota composition was significantly different between the three groups (Bray Curtis index, Anosim 9999 permutations, p=0.005; see online supplementary figure 1A). Both SpA and RA differed from HCs (p=0.02 and p=0.003, respectively; figure 1A, B) as well as SpA from RA (p=0.03; see online supplementary figure 1B). The alpha diversity assessed by the number of observed species was significantly decreased in both SpA and RA, as compared with HCs (p<0.001; figure 1C). There was a trend towards more reduced diversity in RA than SpA (p=0.08; figure 1C). The reduced alpha diversity was not correlated with disease activity, as shown for SpA (see online supplementary figure 2).

In all groups, the profile of gut microbiota appeared dominated by Firmicutes and Bacteroidetes at the phylum level and by *Lachnospiraceae*, *Ruminococcaceae* and *Bacteroidaceae* families, consistent with the usual composition of the human gut microbiome (figure 1D). However, some variations in the distribution were apparent between groups, including more *Lachnospiraceae* in SpA, less *Prevotellaceae* and *Paraprevotellaceae* in SpA and RA than in HCs, less *Bifidobacteriaceae* in RA and more *Proteobacteria*, including *Enterobacteriace*ae, in RA than in both other groups.

Discriminant analysis using LEfSe identified significant taxa variations distinguishing each sample group from both others (figure 2). Patients with SpA had an increase in Firmicutes belonging to the *Lachnospiraceae* family, including *Ruminococcus*, *Dorea*, *Coprococcus* and *Blautia* genera and in Actinobacteria from the *Coriobacteriaceae* family. Increased species included *R. gnavus*, *Blautia pruducta* and *Bifidobacterium longum*. At the OTU level, we observed significant differences between SpA and controls, including increased amount of several *Blautia*, *Ruminococcus* and *Coprococcus* OTU and decreased amount of several *Roseburia faecis* OTU in SpA (see online supplementary figure 3).

In contrast, patients with RA had an increase in Proteobacteria, including *Klebsiella* genus, *Desulfovibrionaceae* and *Succinivibrionaceae* families, and in the Tenericutes and Synergistetes phyla

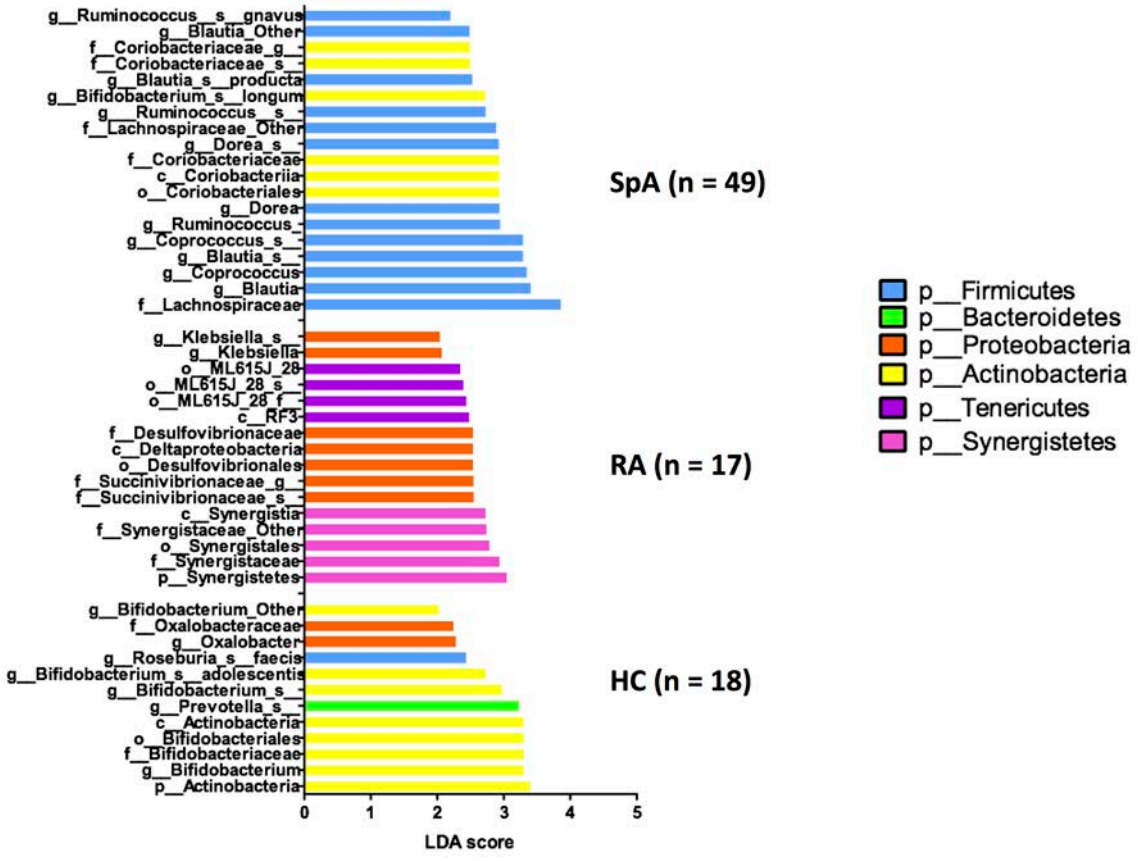


**Figure 2** Bacterial taxa that were differentially represented in the three studied groups, that is, SpA, RA and HCs, in the discovery cohort, with statistical level of significance according to linear discriminant analysis (LDA score >2). The histogram displays all taxa that were increased in each group, as compared with both others, and the corresponding level of significance (LDA score). Taxa were identified at the order, family, gender or species level and colour-coded according to their phylum, and the legend is shown on the right-hand side. HCs, healthy controls; RA, rheumatoid arthritis; SpA, spondyloarthritis.

(figure 2). Finally, HCs were characterised by significantly more Actinobacteria of the *Bifidobacteriaceae* family, Proteobacteria of the *Oxalobacteraceae* family, *R. faecis* and an unidentified *Prevotella* species (figure 2).

Multivariate statistical analysis using MaAsLin identified some treatment effects, mostly related to sulfasalazine intake, that were distinct from disease status-related variations (see online supplementary table 1).

### Confirmatory study

This foregoing discovery study revealed striking differences between the three studied groups, indicative of distinct dysbiosis in both SpA and RA. This prompted us to undertake a replication study in which we included a majority of siblings of patients with SpA as HCs, allowing somehow to control for the genetic background and in particular for the HLA-B27 status shared between patients and half of their siblings. Besides disease status, confounding factors, such as immunomodulatory treatments, could have impacted results of the discovery study, especially in patients with RA, a majority of whom were receiving such treatments. This is why we only included this time patients with early RA having never been treated with corticosteroid, DMARD or biotherapy.

The overall microbiota profile was broadly comparable with that seen in the discovery cohort, although this time, the SpA group appeared more similar to the healthy controls (online supplementary figure 4). The RA had less Firmicutes of the *Ruminococcaceae* family and again less Bacteroidaceae but more Proteobacteria of the *Enterobacteriaceae* family than both other groups (see online supplementary figure 4). In the family-based study, comparison of a group of 29 patients with SpA (all B27+) with their 41 matched siblings showed again that, based on beta diversity index, the composition was significantly different between SpA and HCs (Bray Curtis index, Anosim 9999 permutations, p=0.02; figure 3A), whereas it was not different between the B27+ and B27− healthy siblings (p=0.3; figure 3B). Using LEfSe, the most significantly increased taxa in the SpA group was *R. gnavus*, which had already been identified in the discovery cohort (figure 4A). Besides, a majority of the differentially expressed taxa were decreased in SpA. Among healthy siblings of SpA, the most significant differences between HLA-B27+ and B27− controls consisted of an increase in *Micrococcaceae* and *Rothia mucilaginosa* and a decrease in *Bifidobacterium* genus and *Odoribacter* species in B27+ siblings (figure 4B). At the OTU level, increased amount of several *Blautia*, *Ruminococcus* and of *Eggertthella lenta* and decreased amount of several *Bifidobacterium* characterised HLA-B27+ siblings (see online supplementary figure 5).

In the next step, we analysed together all the samples collected in the replication study, consisting of SpA (n=38), family and unrelated HCs (n=51) and RA of recent onset (n=11). Again, these three groups were distinguishable from each other based on LEfSe and, remarkably, the unique taxon that differentiated SpA from both RA and HCs was *R. gnavus* (figure 4C). This time, RA was characterised by an increase in unidentified *Lactobacillus* species, *Corynebacterium variabile*, *Staphylococcus aureus*, *Facklamia* and *Paraprevotallaceae*. HCs were characterised by a greater abundance of an unidentified *Anaerostipes* species.

### *R. gnavus* abundance correlates with BASDAI in patients with SpA having IBD history

We further analysed the influence of clinical parameters associated with SpA status on the increased abundance of *R. gnavus* in the combined studies. Increased *R. gnavus* was significant only in active SpA, predefined by a BASDAI ≥3/10 (p=0.034). *R. gnavus* was increased in SpA patients without IBD (n=74; p=0.012) as well as in those having a history of IBD (n=12; p=0.007), as compared with HCs (figure 5A). However, stratifying on disease activity, the increase in *R. gnavus* appeared of greater magnitude in patients with IBD history than in those without (mean percentage of *R. gnavus* reads: 0.0267 vs 0.0033, respectively; p=0.001). Moreover, there was a striking correlation between *R. gnavus* abundance and BASDAI in the subgroup of patients having IBD history (p<0.005, r=0.77; figure 5B) but not in the others (data not shown). Interestingly, this was not related to IBD activity, since 8 out of the 10 patients with SpA having a history of IBD, for which the information was available, were in remission of IBD at the time of faecal sampling and the two who had active IBD had low BASDAI activity (1.3 and 2.2, respectively). Noteworthy, there was no correlation between abundance of *R. gnavus* and NSAID intake.

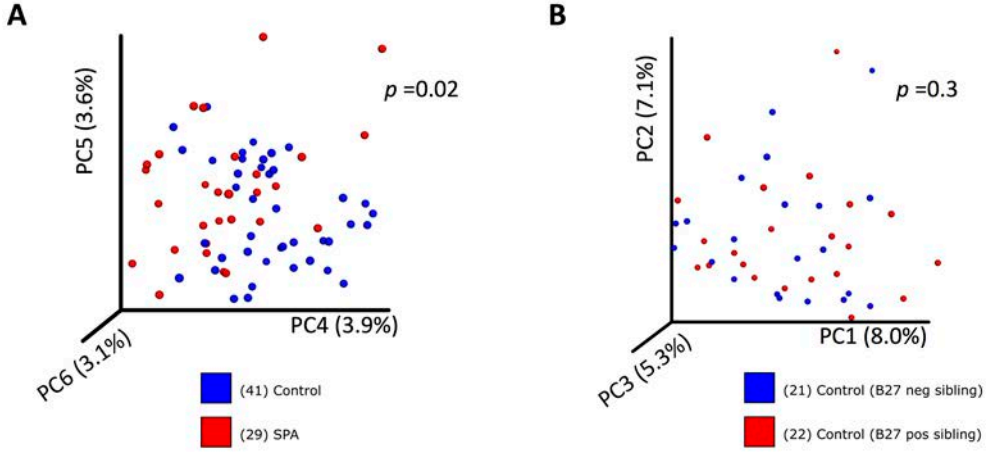


**Figure 3** (A) Bacterial composition of the gut microbiota was significantly different between HLA-B27+ SpA cases and their matched healthy siblings in the replication cohort, according to Bray Curtis index (beta diversity), (B) but not between matched HLA-B27+ and HLA-B27− healthy siblings. PC, principal component.

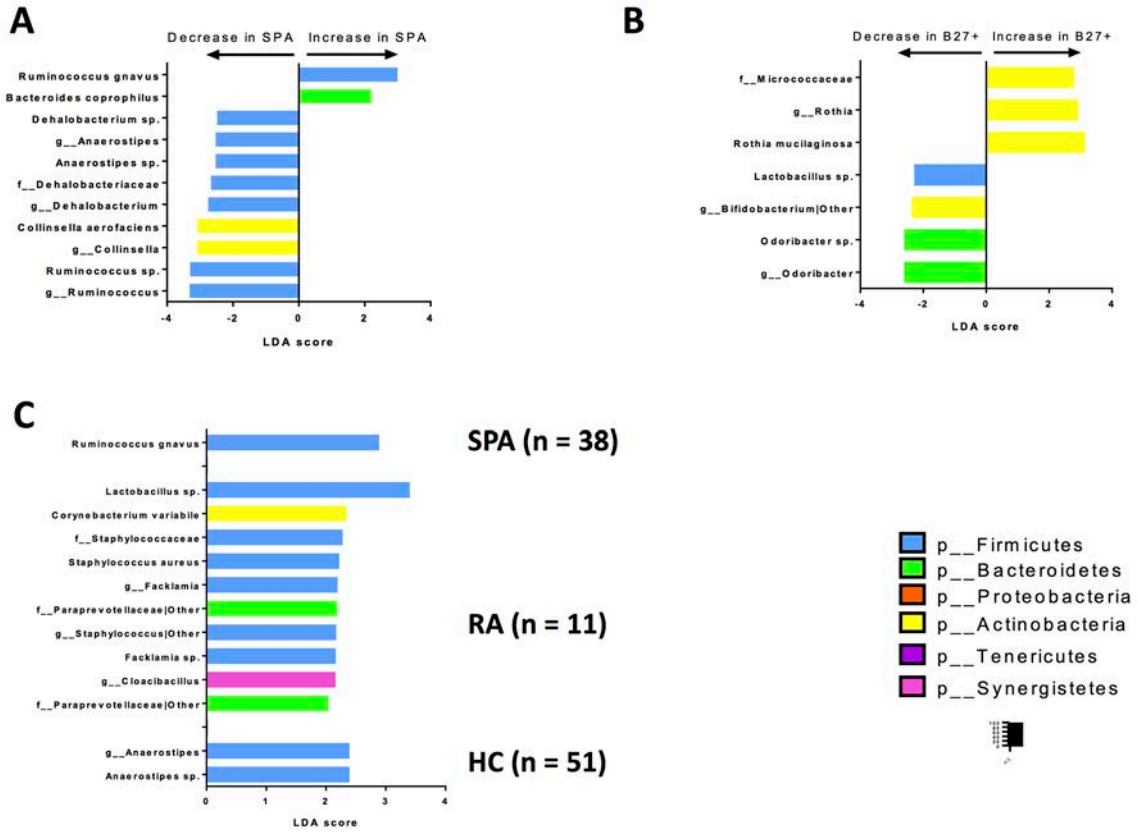


**Figure 4** Bacterial taxa that were differentially represented between groups in the replication cohort, with statistical level of significance according to linear discriminant analysis (LDA score >2). (A) Histogram of the taxa that were significantly different between HLA-B27+ SpA patients (n=29) and matched healthy siblings (n=41). (B) Histogram of the taxa that were significantly different between HLA-B27+ healthy siblings of patients (n=22) and matched HLA-B27– healthy siblings (n=21). (C) Histogram of the taxa that were significantly over-represented in the whole SpA, RA and HC groups, as compared with both other groups. Taxa were identified at the order, family, gender or species level and colour-coded according to their phylum (same colour code as in figure 2).

## DISCUSSION

Altered gut microbiota composition or intestinal dysbiosis is a possible actor in chronic inflammation, even in distant sites, such as the joint.[21] However, until now, only few studies have addressed this question.[6] Here we report a two-stage study, including a discovery and a replication steps that compared for the first time gut microbiota composition between the two most common causes of chronic inflammatory disorders of the joint, that is, SpA and RA, and HCs.

We first identified dysbiosis in both arthritic disorders but with striking differences between them, indicating that it was not a mere consequence of the inflammatory state, but rather bore

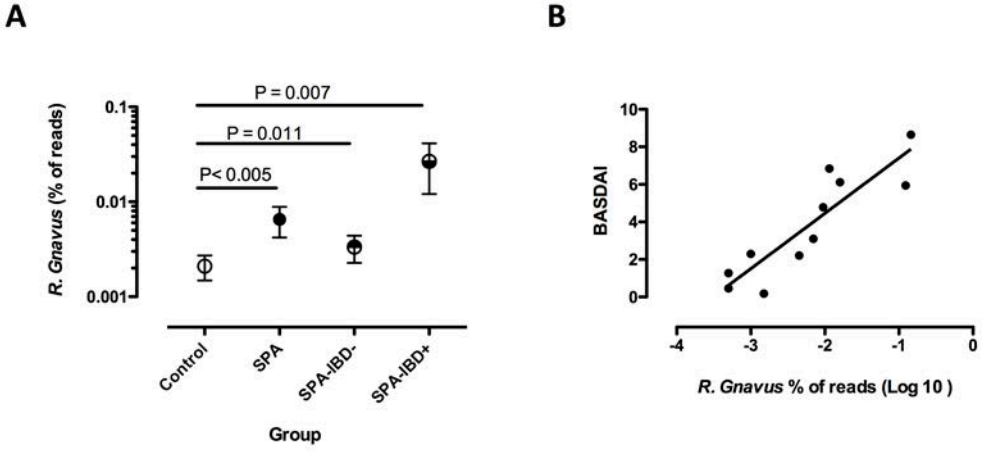


**Figure 5** *Ruminococcus gnavus* was significantly over-represented in the gut microbiota of SpA patients, with (IBD+) or without (IBD–) a positive history of IBD (A). *R. gnavus* abundance was significantly correlated with the BASDAI in SpA patients with a positive history of IBD (B). Symbols in (A) represent the proportion of *R. gnavus* reads among all bacteria in faecal samples, expressed as a mean percentage and SE of the mean of all reads in each group. BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; IBD, inflammatory bowel disease; SpA, spondyloarthritis.

disease specificity. One of the main changes that was observed in both diseases was a reduced microbial diversity, a consistent finding in microbiota studies of chronic inflammatory disorders[20] that was also reported in faecal microbiota of patients with PsA.[22]

However, the most striking finding of our study was a significant enrichment in *R. gnavus*, which specifically distinguished SpA from other groups (RA and HCs) in both cohorts. In the replication cohort, *R. gnavus* was significantly enriched in patients with SpA, even as compared with matched healthy siblings. The latter group was included, since it was expected to have microbiota profile more similar to patients than unrelated HCs, except for disease-related differences, due to a greater share of genetic and environmental factors.[23 24]

To our best knowledge, this is the first metagenomic study to report a significant and reproducible enrichment in a gut-resident bacterial species in SpA. *R. gnavus* is a strict anaerobic Gram-positive non-spore-forming cocci that belongs to the *Lachnospiraceae* family and is a frequent commensal of the gut.[25] It belongs to a limited consortium of bacteria that display mucolytic activity on mucin-2, the principal constituent of intestinal mucus, due to their glycosidases activity.[26] Such property may facilitate their association with the mucosa and close location to the epithelial border. *R. gnavus* also expresses β-glucuronidase activity that can generate toxic metabolites in the colon that might provoke local inflammation.[27] Additionally, it exhibits high bile acids 7α-dehydroxylating activity, leading to the production of secondary bile acids, that is, deoxycholic and lithocholic acids, and 3α-hydroxysteroid dehydrogenase required to convert bile acids into isobile acids, that is, isocholic, isodeoxycholic and isolithocholic acids, which may alter microbiota composition by detoxifying deoxycholic acid.[28]

Its increased abundance in faeces and mucosa has been associated with Crohn's disease, with the recurrence of Crohn's disease after ileocolonic resection and with the risk of ulcerative colitis on anastomosis, that is, pouchitis.[29–33] In IBD, it was preferentially associated with the gut mucosa, which confers to this mucolytic bacteria a possible role in the triggering or maintenance of inflammation.[31 34] This is particularly relevant to our study, since it was mainly over-represented in patients with active SpA. Remarkably, it was most abundant in the subgroup of patients having a history of IBD and its abundance positively correlated with SpA activity in those patients, even though IBD was inactive at the time of sampling in most of them. This suggests that the abundance of *R. gnavus* could be more or less directly involved in osteoarticular inflammation and may provide some insight into the pathogenic link that exists between SpA and IBD.

A direct role for gut microbiota in the triggering of SpA is strongly supported by animal models. In HLA-B27 transgenic rats, the spontaneous development of both arthritis and IBD was prevented by raising animals in germ-free conditions, and those manifestations were triggered by recolonising the germ-free rats with a limited consortium of bacterias and even with single bacterial species.[35] In those experiments, *Bacteroides vulgatus*, which has been shown as increased in the HLA-B27 transgenic rat gut was sufficient to trigger both IBD and arthritis.[35 36] *B. vulgatus* was not increased in our study, but it is interesting to underline that this bacterial species is one of a few having mucolytic activity and that it could degrade porcin but not human mucin, showing that a bacterial species may bear distinct biological consequences, according to the colonised animal species.[34] Remarkably, *Akkermansia muciniphila*, which is another mucolytic bacterial species, was also reported as increased in the gut of HLA-B27 transgenic rat in correlation with local expression of proinflammatory cytokines, including interferon-γ, IL-17A, and IL-23, and with the development of arthritis,[37] as well as in a subgroup of children with enthesitis-related arthritis, an early form of SpA.[38] Altogether, those observations highlight that distinct bacterial species that have been shown as associated with SpA in different settings share mucolytic activity. Such property could be an essential trigger of disease pathogenesis by facilitating access of the gut epithelium to other commensal bacteria and their invasion inside the mucosa that may contribute to distant joint inflammation.[39]

In our study, we examined the bacterial composition of stool samples, whereas two previous studies performed in SpA were focused on the mucosal-associated microbiota.[7 40] The first of those studies compared the terminal ileum-associated microbial community between 10 ankylosing spondylitis patients and nine HCs. Striking differences at the phylum and gender bacterial levels were reported between both groups, some of which were consistent with our findings, such as an enrichment of the Firmicutes phylum and more specifically of the *Lachnospiracae* family, including *Coprococcus* species and *Ruminococcaceae,* in SpA. Also an increase in the secondary bile acid biosynthesis pathway was consistent with an increase in *Ruminococcaceae*. However, the microbial diversity was increased in SpA, as opposed to our results, a finding that is difficult to explain as inflammation have been reported in human and animals to be associated with a decreased biodiversity. The second study included ileal and colonic biopsies from 27 SpA, half of them displaying microscopic bowel inflammation at the time of sampling, and 15 HCs. Overall, there was no significant difference in bacterial composition between both groups but a trend towards an increased richness in the inflamed samples. Besides, there was also an increased abundance of the *Dialister* genus in the inflamed biopsies, which correlated with disease activity index.[7]

Studying mucosal specimens may give access to the bacterial community in close interaction with the intestinal tissue and its immune system, the most likely to be directly involved in disease pathogenesis. However, it requires an invasive procedure and a simultaneous evaluation of the histological aspect of the mucosal biopsies, whereas studying stools allowed us to readily collect large numbers of samples following reproducible protocol. Moreover, we included patients having established disease and, in the context IBD, it was shown that if dysbiosis was more readily detected in the mucosal-associated bacterial community in early disease, it was spreading to the lumen content in advanced disorder, allowing to catch similar variations on stools than mucosal biopsy samples in established disease.[41 42]

Environmental factors known as affecting microbiota, such as breastfeeding, have been shown to influence SpA susceptibility.[39] Given the high heredity in SpA, genetic factors may also contribute to the development of dysbiosis. In support of such hypothesis, the HLA-B27+ healthy siblings of patients with SpA exhibited a microbiota composition distinct from their HLA-B27− healthy siblings, with increased *R. mucilaginosa* and *E. lenta*, two bacterial species that have been associated with IBD[42 43] and low levels of *Bifidobacterium* and *Odoribacter*, similar to what has been reported in patients with ileal Crohn's disease and ulcerative colitis.[20 42]

We evidenced dysbiosis in RA that was distinct from that seen in SpA. RA-associated dysbiosis differed also between both cohorts, a finding that could be attributed to the characteristics of patients with RA recruited in those cohorts. Hence, in the discovery study, patients with RA had long-standing

disease and a majority of them received immunosuppressive treatment, including corticosteroids, disease-modifying anti-rheumatic drugs and biotherapies. Besides, their disease was moderately active in average. This population had increased proportions of aerotolerant Proteobacteria, as frequently reported in other inflammatory disorders, such as IBD, and Synergistetes, which have been associated with periodontal inflammation.[44] In contrast, patients with RA included in the second cohort had short disease duration, no immunosuppressive treatment and more active disease. Their microbiota was enriched in a majority of aerotolerant Gram-positive bacteria belonging to the Firmicutes phylum, including the *Lactobacillus* genus and cocci of the *Facklamia* and *Staphyococcus* genera, and to the Actinobacteria phylum, such as C. *variabile*. Interestingly, Gram-positive bacteria, including *Lactobacillus* sp, have been shown as pathogenic in animal models of RA and increased in the gut microbiota in correlation with disease activity in such patients.[8 9] The *Paraprevotellacea* family was also enriched in our study. Interestingly, *Prevotella copri*, a member of that family, was previously reported as increased in recent onset RA but not in long-standing RA.[45]

In conclusion, specific dysbiosis allowed us to distinguish SpA from HCs and from RA, whatever the stage of the latter disease. Moreover, we found evidence tha *R. gnavus* could be involved in SpA activity and thereby contribute to the link between SpA and IBD. Finally, HLA-B27 may contribute to gut dysbiosis and thereby to SpA predisposition.

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EXTENDED REPORT

# Endothelin-1 promotes vascular smooth muscle cell migration across the artery wall: a mechanism contributing to vascular remodelling and intimal hyperplasia in giant-cell arteritis

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## ABSTRACT

**Background** Giant-cell arteritis (GCA) is an inflammatory disease of large/medium-sized arteries, frequently involving the temporal arteries (TA). Inflammation-induced vascular remodelling leads to vaso-occlusive events. Circulating endothelin-1 (ET-1) is increased in patients with GCA with ischaemic complications suggesting a role for ET-1 in vascular occlusion beyond its vasoactive function.

**Objective** To investigate whether ET-1 induces a migratory myofibroblastic phenotype in human TA-derived vascular smooth muscle cells (VSMC) leading to intimal hyperplasia and vascular occlusion in GCA.

**Methods and results** Immunofluorescence/confocal microscopy showed increased ET-1 expression in GCA lesions compared with control arteries. In inflamed arteries, ET-1 was predominantly expressed by infiltrating mononuclear cells whereas ET receptors, particularly ET-1 receptor B ($ET_BR$), were expressed by both mononuclear cells and VSMC. ET-1 increased TA-derived VSMC migration in vitro and α-smooth muscle actin (αSMA) expression and migration from the media to the intima in cultured TA explants. ET-1 promoted VSMC motility by increasing activation of focal adhesion kinase (FAK), a crucial molecule in the turnover of focal adhesions during cell migration. FAK activation resulted in Y397 autophosphorylation creating binding sites for Src kinases and the p85 subunit of PI3kinases which, upon ET-1 exposure, colocalised with FAK at the focal adhesions of migrating VSMC. Accordingly, FAK or PI3K inhibition abrogated ET-1-induced migration in vitro. Consistently, ET-1 receptor A and $ET_BR$ antagonists reduced αSMA expression and delayed VSMC outgrowth from cultured GCA-involved artery explants.

**Conclusions** ET-1 is upregulated in GCA lesions and, by promoting VSMC migration towards the intimal layer, may contribute to intimal hyperplasia and vascular occlusion in GCA.

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## INTRODUCTION

Giant-cell arteritis (GCA) is a granulomatous vasculitis targeting large and medium-sized arteries in aged individuals.[1 2] Inflammation-induced vascular remodelling results in intimal hyperplasia leading to symptoms of vascular insufficiency or irreversible ischaemic complications in 20%–30% of patients.[3–6] It is generally assumed that vascular smooth muscle cells (VSMC) migrate through disrupted elastic fibres towards the intima where they produce abundant matrix proteins.[6] However, underlying mechanisms remain virtually unexplored. Several growth factors, including PDGF, TGFβ, EGF, NGF or BDNF, are expressed in GCA lesions and may participate in this process based on their ability to stimulate proliferation and/or migration of VSMC in vitro.[7 8]

Visual loss, the most frequent ischaemic complication in GCA, is frequently preceded by transient episodes of blindness (*amaurosis fugax*) suggesting that reversible vasospasm may initially contribute to flow reduction in small arteries supplying the optic nerve.[3–5] Endothelin-1 (ET-1) is a potent vasoactive peptide that might potentially participate in this process.[9 10] ET-1 is mainly synthesised by endothelial cells although VSMC and macrophages may also produce it.[9 11] ET-1 signals through two G-protein coupled receptors (GPCR): E-1 receptors A and B ($ET_AR$ and $ET_BR$). Both $ET_AR$ and $ET_BR$ mediate VSMC contraction. Signalling through $ET_BR$ on endothelial cells may also produce vasodilatation by stimulating nitric oxide and prostacyclin production.[9 10 12]

Although the majority of previous studies on ET-1 functions have focused on VSMC regulation of the vascular tone, in recent years, skin, liver and lung fibroblasts have been identified as important targets of ET-1.[13 14] ET-1 promotes myofibroblast differentiation of fibroblasts, a crucial step in the development of fibrogenic diseases such as systemic sclerosis and cardiac, pulmonary or hepatic fibrosis.[13–15]

The inflammatory milieu of GCA is enriched in cytokines and growth factors able to enhance ET-1 expression such as TGFβ among others.[9 16] We and others have recently shown that ET-1, $ET_AR$ and $ET_BR$ are increased in GCA lesions, although the specific cells expressing the ET-1 system components have not been determined.[17 18] In spite of the short half-life of circulating ET-1, plasma ET-1 concentrations are elevated in patients with GCA-related cranial ischaemic complications.[18]

Since arteries involved by GCA are usually larger than resistance arteries controlling vascular tone, we hypothesised that, in addition to its vasoactive function, ET-1 might contribute to the development of intimal hyperplasia by stimulating a myofibroblast

phenotype in VSMC and promoting their migration towards the intimal layer. Consequently, we investigated the effect of ET-1 on human temporal artery (TA)-derived VSMC migration in vitro and ex vivo as well as the signalling pathways involved.

## METHODS

### Patient samples

TA biopsies were performed to 10 patients with suspected GCA (see online supplementary table S1). Five biopsies disclosed typical GCA histopathological features and were used in the indicated experiments. The remaining five showed no inflammatory lesions and served as controls. Patients with negative biopsies were eventually diagnosed with other conditions (see online supplementary table S2). The study was approved by the local Ethics Committee (Hospital Clinic of Barcelona) and patients signed informed consent.

### Isolation and culture of VSMC derived from human TA

Human TA-derived VSMCs were obtained from explanted TA sections from the above patients cultured on Matrigel and characterised by flow cytometry, as described.[7 18 19] In specific experiments, VSMCs were cocultured with peripheral blood mononuclear cells (PBMC) or purified subsets (CD4+ T cells or CD14+ monocytes) (online supplementary methods).

### Reagents

See online supplementary methods.

### Immunofluorescence

Immunofluorescence staining was performed in cultured VSMC or in fresh-frozen or cultured TA sections. Antibodies used, dilutions and detailed steps are depicted in online supplementary methods.

### Quantitative real-time reverse transcription PCR

RNA was extracted from cultured VSMC using TRIzol Reagent (Life Technologies, Paisley, UK). Prepro-ET-1 and α-smooth muscle actin (αSMA) mRNAs (1 µg) were measured by quantitative reverse transcription PCR with specific TaqMan gene expression assays from Applied Biosystems as reported.[18]

### ET-1 immunoassay

ET-1 in cell supernatants was measured using R&D Quantikine ELISA Kit.

### Migration assay

VSMC migration was assessed using Boyden chambers with 10 $\mu$m pore polyester filters. Further details are exposed in the online supplementary methods.

### Scratch wound-healing assay

VSMCs were seeded at 80% confluence onto 0.1% gelatin-precoated 12-well plates and cultured overnight. One scratch per well was done before adding fresh Dulbecco's modified Eagle medium supplemented with 50 mmol/L of HEPES (Sigma-Aldrich) and BQ123, BQ788 (20 $\mu$mol/L) or combination of both inhibitors. ET-1 ($10^{-9}$ mol/L) or fresh medium was added to each corresponding well. Time-lapse video microscopy was applied to record cell movement and results were analysed as depicted in online supplementary methods. A proliferation assay was also performed to assess the potential impact of ET-1 on cell growth (see online supplementary figure S1).

### Western blot and gelatin zymography

See details in online supplementary methods.[20 21]

### Transient transfection

Focal adhesion kinase (FAK) wild-type cDNA and FAK point mutants Y397F and Y925F, cloned into the pCDNA3 expression vector, were kindly provided by Kazue Matsumoto and Kenneth M Yamada (National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, Maryland) and generated as previously described.[20 22] Lipofectamine 2000 Reagent (Invitrogen) was used for transient transfection of VSMC. Transfection efficiency was about 30% (see online supplementary figure S2).

### Ex vivo-cultured TA sections from patients with GCA

TA sections from four treatment-naive patients with GCA and four controls were cultured on Matrigel as described,[19 23] with or without BQ123 or BQ788 (20 $\mu$mol/L). VSMC outgrowth was scored in three arteries at various time points by two investigators (EPR and MCB) blinded to the conditions tested.[7]

### Statistical analysis

Mann-Whitney U test for independent variables was applied using SPSS software, PASW V.22.0.

## RESULTS

### Distribution of the ET-1 system in GCA lesions compared with controls

In control arteries, slight ET-1 expression was observed in organised VSMC in the media and in the luminal endothelium (figure 1A). In GCA-involved arteries, ET-1 was intensively expressed by clusters of infiltrating inflammatory cells (figure 1B, b.1 and figure 1C, c.1) and by scattered remaining VSMC (figure 1B, b.2). In addition, ET-1 expression by the luminal endothelium was increased (figure 1B,C) compared with control arteries (figure 1A).

To further characterise the cell types responsible for ET-1 production in GCA, primary cultures of VSMC were obtained from normal TA and cocultured with purified CD4+ T lymphocytes or monocytes (CD14+) from healthy donors in order to mimic vascular inflammation.[24] Interestingly, a slight but consistent increase in prepro-ET-1 mRNA expression was observed in CD4+ T lymphocytes and to a lesser extent in CD14+ monocytes, when cocultured with VSMC (figure 1D). VSMC remarkably expressed and secreted mature ET-1 (figure 1E). When in coculture, unprocessed big ET-1 increased in PBMC and decreased in VSMC lysates. Overall, secreted ET-1, mainly produced by VSMC, decreased in coculture supernatants (figure 1F). The increase in prepro-ET-1 mRNA in PBMC cocultured with VSMC was confirmed in three paired experiments performed with PBMC and VSMC from the same GCA donor (figure 1G).

Expression of $ET_AR$ and $ET_BR$ was explored in the same TA specimens. In control arteries, $ET_AR$ was expressed by VSMC in the media whereas $ET_BR$ was hardly detected (figure 1H,I). In GCA, both $ET_AR$ and $ET_BR$ receptors were expressed by αSMA-positive cells at the intima-media border (figure 1J,K). Endothelial cells and inflammatory cells also expressed both ET receptors (figure 1J,K).

### ET-1 promotes VSMC cytoskeleton reorganisation and migration through $ET_AR$ and $ET_BR$

To investigate whether ET-1 promoted a myofibroblast phenotype in VSMC, we explored changes in cytoskeleton organisation induced by ET-1 in cultured TA-derived VSMC. ET-1 elicited

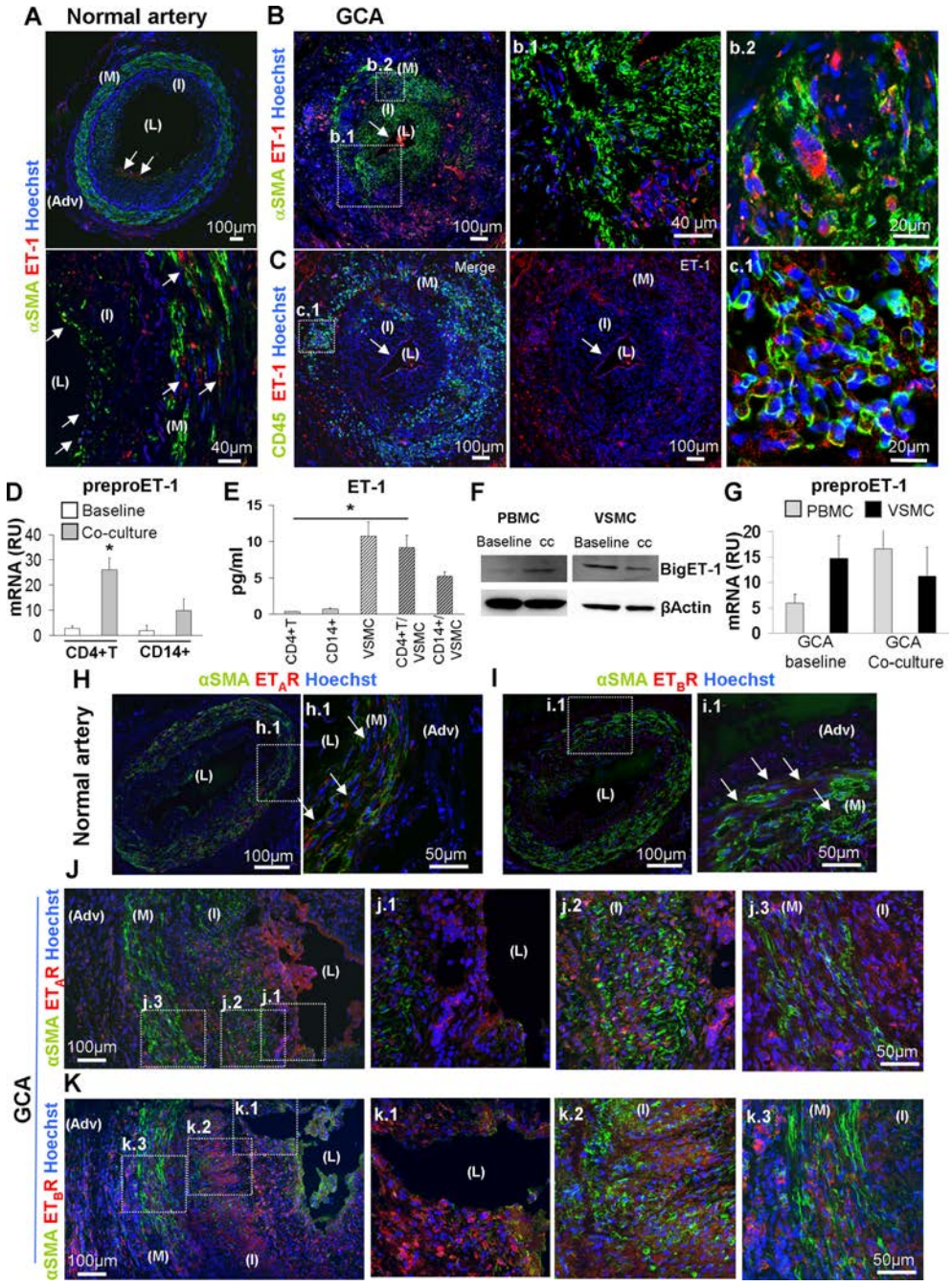


**Figure 1** ET-1, $ET_AR$ and $ET_BR$ expression in GCA lesions compared with control TA. (A) Immunostaining of ET-1 (red), αSMA (green) and nuclei (blue) in a control TA. (B) Immunostaining of ET-1 (red), αSMA (green) and nuclei (blue) in a TA with typical GCA involvement. White arrow highlights ET-1 expression by the endothelium. (b.1 and b.2) Magnifications of 1B showing independent expression or coexpression of αSMA and ET-1, respectively. (C) Immunostaining of ET-1 (red), CD45 (green) and nuclei (blue) in a GCA-involved TA. (c.1) Magnification of 1C showing CD45+ cells expressing ET-1. ET-1 distribution was confirmed in three different GCA and control arteries. L, lumen; I, intima; M, media; Adv, adventitia. (D) Prepro-ET-1 mRNA expression by purified CD4+ T cells or CD14+ monocytes isolated or cocultured with VSMC for 24 hours. Bars represent mean and SEM of triplicates. *p<0.05 cocultured versus isolated. (E) Immunoassay of supernatants from isolated CD4+ T lymphocytes, CD14+ monocytes or VSMC, or supernatants from cocultures of CD4+ T cells or CD14+ monocytes with VSMC for 24 hours. Bars represent mean and SEM of triplicates. *p<0.05 cocultured versus isolated. (F) Big-ET-1 detection by western blot in lysates (20 µg/lane) of isolated PBMC or PBMC cocultured with VSMC, and in lysates of VSMC isolated or cocultured with PBMC for 24 hours. (G) Prepro-ET-1 mRNA expression by PBMC from three patients with GCA and their corresponding VSMC isolated or in coculture for 24 hours. (H, I) Immunofluorescence staining of $ET_AR$ (red) or $ET_BR$ (red) together with αSMA (green) and nuclei (blue) in a control TA and their corresponding magnifications (h.1, i.1). (J, K) Immunofluorescence staining of $ET_AR$ (red) or $ET_BR$ (red) together with αSMA (green) and nuclei (blue) in a GCA-involved TA and their corresponding magnifications of the endothelium (j.1, k.1), neointima (j.2, k.2) and media (j.3, k.3). αSMA, α-smooth muscle actin; ET-1, endothelin-1; $ET_AR$, ET-1 receptor A; $ET_BR$, ET-1 receptor B; GCA, giant-cell arteritis; RU, relative units; PBMC, peripheral blood mononuclear cells; TA, temporal arteries; VSMC, vascular smooth muscle cells.

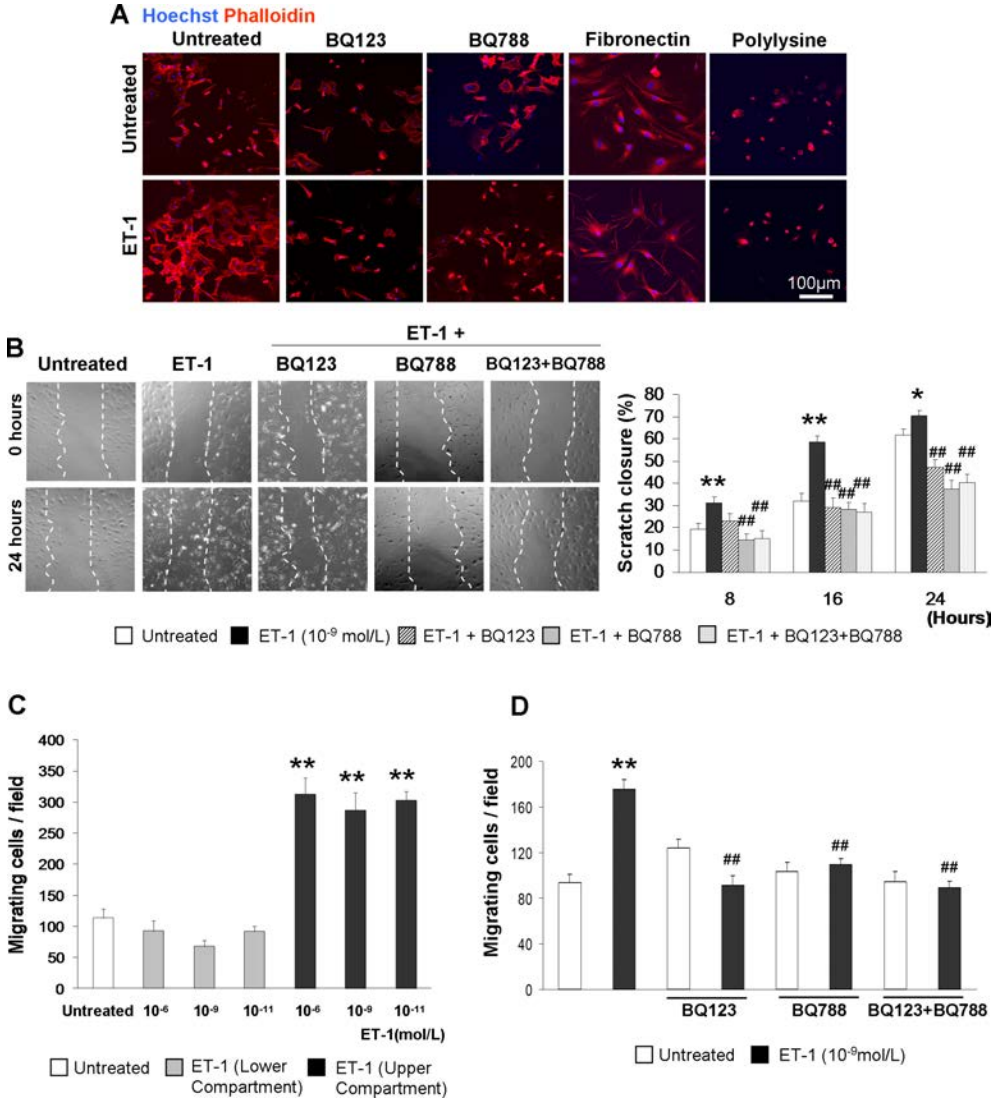


**Figure 2** Effect of ET-1 on TA-derived VSMC cytoskeleton reorganisation and migration. (A) Immunofluorescence of VSMC f-actin with phalloidin-rhodamine (red) and nuclei (blue). VSMCs were preincubated with $ET_AR$ antagonist BQ123 (20 µmol/L), $ET_BR$ antagonist BQ788 (20 µmol/L) or both in suspension for 45 min. ET-1 ($10^{-9}$ mol/L) was added at the time of VSMC seeding on plastic, fibronectin (5 µg/cm$^2$) or polylysine (10 µg/mL) and VSMCs were incubated for 3 hours before fixing and staining. (B) Scratch wound healing assay of VSMC untreated or exposed to ET-1 ($10^{-9}$ mol/L), with or without the presence of BQ123 (20 µmol/L), BQ788 (20 µmol/L) or both. Graph represents percentage of scratch closure over time in three independent experiments. (C) Boyden chamber migration assay. ET-1 was added either to the lower or in the upper compartment at the indicated concentrations. Cells were counted at 4× magnification. **p<0.005 for untreated cells versus ET-1-treated cells. Bars represent number of cells (mean and SEM of quadruplicates). (D) Boyden chamber migration assay where ET-1 was added to the upper compartment with or without preincubation with antagonists BQ123, BQ788 or both. Cells were counted at 10× magnification. Bars represent number of cells (mean and SEM of quadruplicates). (B, D) *p<0.05/**p<0.005 for untreated cells versus ET-1-treated cells. #p<0.05/##p<0.005 comparing ET-1-treated cells versus cells incubated with ET-1 receptor antagonists BQ123 or BQ788. ET-1, endothelin-1; $ET_AR$, ET-1 receptor A; $ET_BR$, ET-1 receptor B; TA, temporal arteries; VSMC, vascular smooth muscle cells.

spreading of VSMC with a striking formation of cytoplasm protrusions (figure 2A). Cell spreading was not induced in VSMC cultured on polylysine and was more remarkable when VSMCs were cultured on fibronectin (figure 2A), suggesting participation of integrin-mediated signalling pathways in this process.[25] Spreading was reverted by blocking ET-1 signalling with $ET_AR$ antagonist BQ123 and $ET_BR$ antagonist BQ788 (figure 2A).

ET-1-induced VSMC morphology changes were associated with increased migratory activity (see online supplementary movie). ET-1 exposure resulted in significantly faster scratch-wound closure (figure 2B). $ET_AR$ and $ET_BR$ antagonists (BQ123 and BQ788, respectively) and combination of both inhibitors significantly abrogated ET-1-induced VSMC migration, indicating implication of both receptors in this process (figure 2B). ET-1 did not accelerate scratch-wound closure by stimulating VSMC growth, since no significant increase in VSMC proliferation was elicited by ET-1 (online supplementary figure S1).

ET-1 induced VSMC migration in Boyden chambers when added to the upper compartment (figure 2C). In contrast, when ET-1 was added to the lower compartment, no differences in migration were observed, indicating that ET-1 has no chemoattractant activity and primarily stimulates motility (figure 2C). ET-1-induced migration was abrogated by BQ788 and BQ123 or the combination of both (figure 2D).

### FAK phosphorylation at Y397 is essential for ET-1 induction of VSMC migration

Based on the relevance of integrin engagement in ET-1-induced cytoskeleton reorganisation, and the seminal role of FAK in integrin-mediated cell motility, we explored the involvement of FAK in ET-induced VSMC migration. FAK is a docking and signalling tyrosine kinase with a seminal role in focal adhesion turnover required for cell migration in response to integrin binding or growth factor signalling.[25–27]

FAK activation results in autophosphorylation of crucial tyrosine residues.[25–27] One of the best characterised is Y397 which provides a binding site for Src-type tyrosine kinases promoting their recruitment to focal adhesions and allowing their phosphorylation. This interaction is essential for cell migration in fibroblasts and malignant cells.[26–29] Src, in turn, phosphorylates additional tyrosine residues, including Y925, located within the focal adhesion targeting sequence at the FAK C-terminal domain.[30] Phosphorylated Y925 may recruit the adaptor protein Grb2, leading to activation of the GTP-binding protein Ras, and to ERK1/2 activation.[30]

ET-1 increased phosphorylation of Y397 and Y925 FAK residues (figure 3A), particularly when cells were cultured on plastic or fibronectin whereas this effect was absent in cells plated on polylysine (figure 3A,B). FAK phosphorylation was reduced by $ET_AR$ or $ET_BR$ antagonists BQ123 and BQ788 (figure 3A). As G-coupled receptors, ET-1 receptors may activate heterotrimeric G proteins which have important roles in integrin inside-out and outside-in signalling.[31] Pertussis toxin induces ADP-ribosylation of several $G\alpha_i$ subunits inhibiting their activity.[32] As shown in figure 3C, ET-1-induced Y397 FAK phosphorylation was abrogated by pertussis toxin confirming the participation of heterotrimeric G proteins in ET-1-induced FAK activation.

To confirm the role of FAK in ET-1-induced VSMC migration, we investigated the effect of PF-573228, an inhibitor of FAK kinase activity. At concentrations able to inhibit FAK phosphorylation (see online supplementary figure S3), PF-573228 significantly decreased ET-1-induced VSMC cytoskeleton organisation and migration in a dose-dependent manner (figure 3D,E). Interestingly, at low concentrations, PF-573228 inhibited ET-1-induced migration whereas at higher concentrations it was also able to reduce baseline VSMC migration.

Consistent with a seminal role of FAK in mediating ET-1-induced migration, transient transfection of VSMC with FAK wild type significantly increased VSMC migration through Boyden chambers overcoming the effect of ET-1 which was not able to increase migration in FAK-overexpressing cells. However, transient transfection with an expression vector containing FAK Y397F point mutation abrogated ET-1-induced motility. In spite that ET-1 also increased Y925 phosphorylation, transfection of Y925F point mutant had no impact on ET-1-induced migration (figure 3F). These results indicate the crucial participation of FAK Y397 in ET-1-mediated migration in primary TA-derived VSMC.

It has been previously reported that integrin engagement and FAK signalling trigger rapid secretion of gelatinases MMP9 and MMP2 by lymphoid cells.[20 33] Based on the important role of ET-1 in inducing FAK activation, we explored whether ET-1 modulated secretion of gelatinases by VSMC. ET-1 slightly increased secretion of pro-MMP2 (figure 3G) and this effect was reduced by BQ788 ($ET_BR$ antagonist) (figure 3H).

ET-1 induced FAK phosphorylation and recruitment of phosphorylated FAK at the focal adhesions in the leading and rear edges and colocalisation with αSMA (figure 4A,B).

To confirm the relevance of the above results in GCA, cultured TA sections from patients with GCA were assessed for FAK phosphorylation. As shown in figure 4C,D, Y397-phosphorylated FAK was detected in GCA lesions, particularly at the intima and intima/media junction and FAK phosphorylation decreased upon exposure to $ET_AR$ and $ET_BR$ antagonists.

### Src and PI3kinases mediate ET-1-induced VSMC migration

Considering the relevance of FAK Y397 in ET-1-induced VSMC migration, we next explored FAK downstream pathways involved in cell migration including ERK, Src and PI3K.[26–30] ET-1 promoted Src activation revealed by increased phosphorylation of the Y416 Src residue and this was inhibited by both $ET_AR$ and $ET_BR$ antagonists (figure 5A).

ERK1/2 activation has a crucial role in cell motility, by phosphorylating myosin light chains and as scaffolding molecule.[26 27 34] Although transfection with Y925F point mutant did not substantially reduce ET-1-induced migration, ET-1 increased ERK1/2 phosphorylation and this was reduced by ET-1 receptor antagonists (figure 5A), consistent with the existence of alternative ERK activating mechanisms dependent and independent of FAK.[26 30] Although the effect of ET-1 on baseline-activated Src and ERK phosphorylation was modest, it was consistently observed.

In accordance with the crucial role of Src in cell migration, Src inhibitor PP2 reduced baseline and ET-1-induced VSMC migration. Interestingly, PI3kinase inhibition with LY294002 selectively reduced ET-1-increased migration (figure 5B). ERK inhibition of ET-1-induced migration could not be assessed with PD98059 due to the decreased viability observed after the 6-hour exposure required for migration experiments (see online supplementary figure S4). Short-term exposure to Src and ERK inhibitors, not reducing cell viability, virtually impeded cell spreading (figure 5C) whereas PI3kinase inhibition only reduced the increase in cell protrusions induced by ET-1 (figure 5C,D).

FAK Y397 interaction with p85, the regulatory subunit of PI3kinase, is crucial to cell migration in other experimental contexts.[35 36] ET-1 promoted colocalisation of PI3kinase p85 with FAK at VSMC focal adhesions (figure 5D). This interaction was abrogated by both $ET_AR$ and $ET_BR$ antagonists and by inhibition of FAK kinase activity. Inhibition of PI3kinase by LY294002 prevented formation of fully developed cell protrusions induced by ET-1, but did not prevent ET-1-induced recruitment of p85 and FAK at the focal contacts in nascent, immature buds (figure 5D).

### ET-1 induces neointima formation in ex vivo-cultured normal TA

In control arteries, αSMA-expressing quiescent VSMCs were concentrically organised (figure 6A). In contrast, in GCA-involved arteries the muscular layer was disrupted and αSMA-expressing VSMCs were mostly located at the neointima (figure 6A). Treatment of cultured TA explants with ET-1 at concentrations similar to those found in patient plasma[18] or in the coculture supernatants increased αSMA expression (figure 6B,C). Exposure of cultured normal TA to ET-1 also resulted in a striking disruption of the muscular layer and migration of VSMC towards the intima (figure 6B).

### $ET_AR$ and $ET_BR$ antagonists reduce αSMA expression and VSMC outgrowth from ex vivo-cultured arteries from patients with GCA

ET-receptor antagonists BQ123 and BQ788 dramatically reduced αSMA expression in cultured artery sections for a patient with

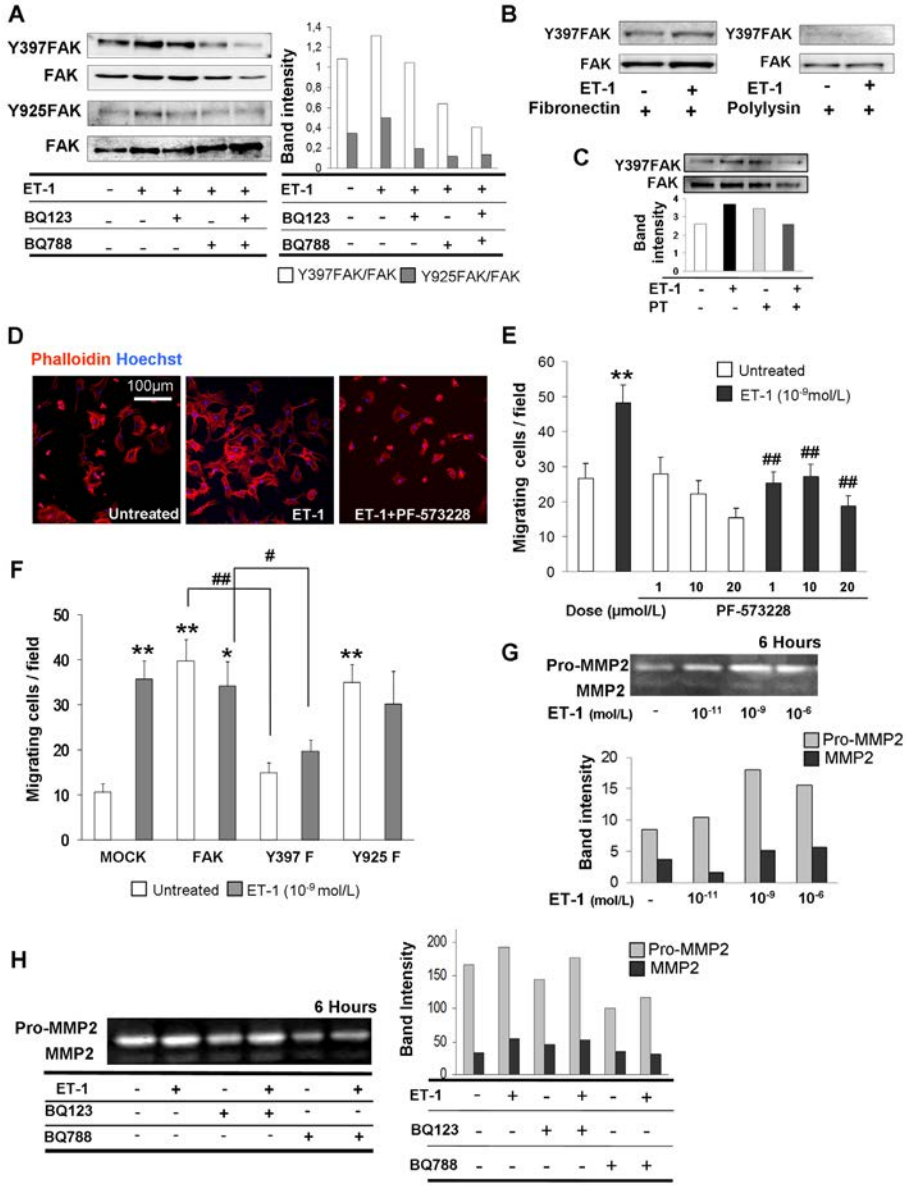


**Figure 3** Y397 FAK phosphorylation is essential for ET-1-induced TA-derived VSMC migration. (A) Immunoblot and corresponding quantification of total FAK or FAK phosphorylated at the indicated tyrosine residues in lysates of VSMC cultured for 90 min in the presence or the absence of ET-1 with or without pretreatment with ET-1 receptor antagonist BQ123 or BQ788 at the same concentrations as in previous experiments. (B) Immunoblot and corresponding quantification of p-FAK and total FAK in cell lysates of VSMC seeded on fibronectin (5 µg/cm$^2$) or polylysine (10 µg/mL) and cultured for 90 min in the presence of absence of ET-1 ($10^{-9}$ mol/L). (C) Immunoblot and corresponding quantification of tyrosine 397 and total FAK from lysates of VSMC cultured for 90 min with or without ET-1 and with or without previous incubation with PT (1 µg/mL). (D) Immunofluorescence of VSMC f-actin cytoskeleton with phalloidin-rhodamine (red) and nuclei (blue). ET-1 ($10^{-9}$ mol/L) was added at the time of VSMC seeding. When indicated, VSMCs were preincubated with a FAK inhibitor (PF-573228) at 20 µmol/L for 30 min before ET-1 exposure. Representative pictures are displayed. (E) Boyden chamber migration assay of VSMC preincubated with increasing concentrations of FAK inhibitor (PF-573228) with or without subsequent addition of ET-1. **p<0.005 untreated cells versus ET-1-treated cells. ##p<0.005 comparing ET-1-treated cells versus ET-1-treated cells preincubated with PF-573228. Cells were counted at 10× magnification. (F) Boyden chamber migration assay of VSMC, 3 days after transfection with empty pcDNA3 vector (MOCK), wild-type FAK (FAK) or FAK mutated at the phosphorylation site Y397F or Y925F. Bars represent the number of migrating cells (mean and SEM) of quadruplicates at 10× magnification. *p<0.05/**p<0.005 untreated cells versus ET-1-treated cells or FAK-transfected cells. #p<0.05/##p<0.005 for the indicated comparisons. Notice that baseline migration in transfected cells is globally inferior than in non-manipulated cells displayed in figure 2. (G) Gelatin zymography of serum-free supernatants of VSMC cultured in the absence or in the presence of ET-1 for 6 hours. A representative experiment out of three is displayed. (H) Gelatin zymography of serum-free supernatants of VSMC cultured in the presence or in the absence of ET-1 ($10^{-9}$ mol/L) and ET-1 receptor antagonists BQ123 and BQ788 (20 µmol/L) for 6 hours. A representative experiment out of two is displayed. ET-1, endothelin-1; FAK, focal adhesion kinase; MMP2, matrix metalloproteinase 2; PT, pertussis toxin; TA, temporal arteries; VSMC, vascular smooth muscle cells.

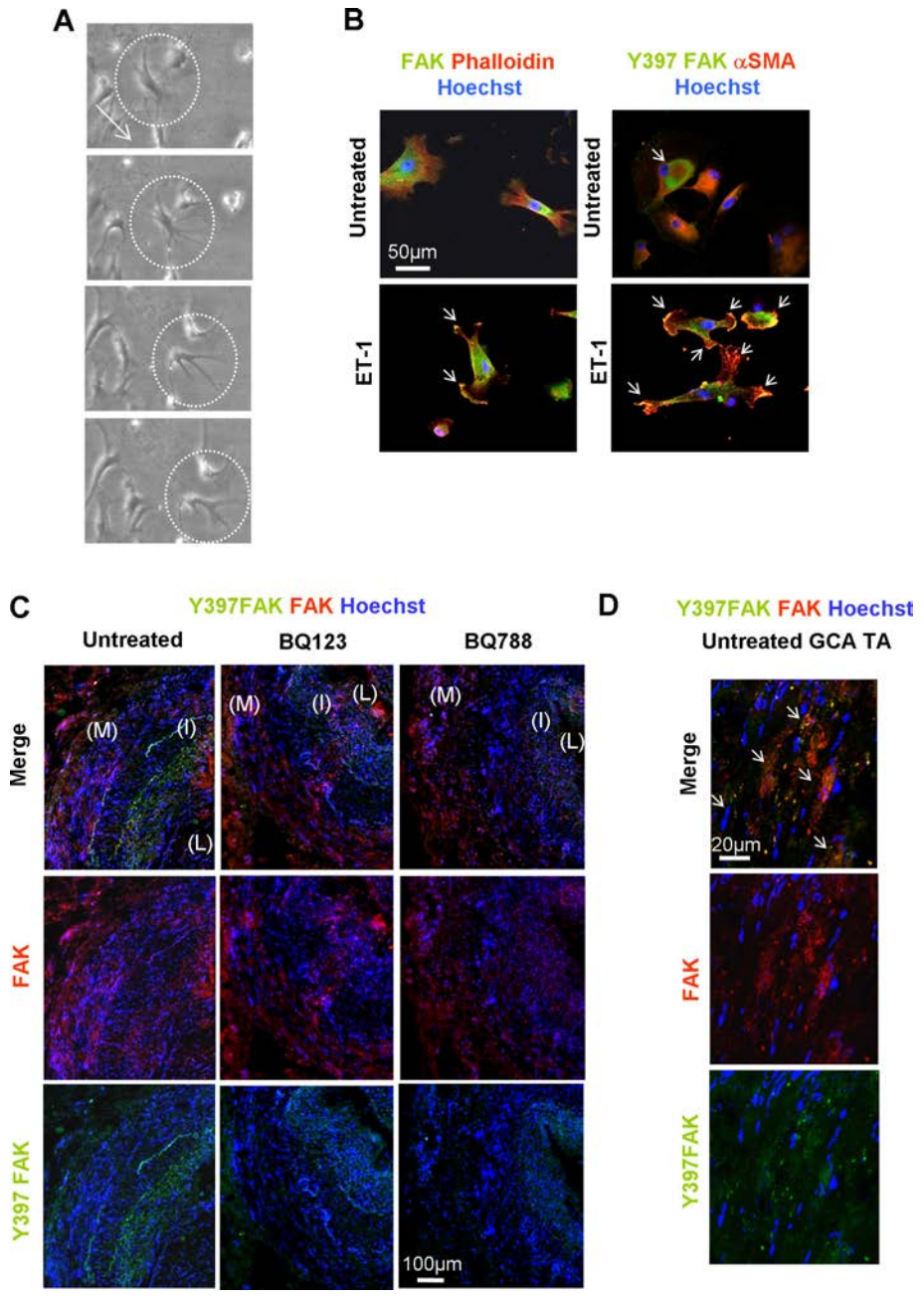


**Figure 4** FAK recruitment and phosphorylation at the cell protrusions of ET-1-stimulated VSMC and in ex vivo-cultured TA from patients with GCA. (A) Tracked migratory VSMC exposed to ET-1 (see online supplementary movie). Direction of the migration is indicated by the arrow. (B) Immunofluorescence of total FAK (red) or phospho-Y397 FAK (green), nuclei (blue) and actin cytoskeleton (red) of VSMC cultured in the presence or in the absence of ET-1 ($10^{-9}$ mol/L) as labelled. Arrows indicate FAK colocalisation with αSMA at the focal adhesions in cell protrusions of migrating cells. (C) Immunofluorescence of pY397 FAK (green), total FAK (red) and nuclei (blue) of a TA from a patient with GCA cultured on Matrigel for 5 days untreated or treated with $ET_AR$ or $ET_BR$ antagonist (BQ123 and BQ788, respectively) at 20 μmol/L. L, lumen; I, intima layer; M, media layer; Adv, adventitia. (D) Magnified VSMC from the media-intima junction of an untreated TA shown in C. Arrow indicates coexpression of phospho-Y397 FAK and total FAK at the media-intima junction of the untreated GCA artery. Separated channels and merge are displayed. Representative picture of multiple cells coexpressing Y397 FAK with FAK. αSMA, α-smooth muscle actin; ET-1, endothelin-1; $ET_AR$, ET-1 receptor A; $ET_BR$, ET-1 receptor B; FAK, focal adhesion kinase; GCA, giant-cell arteritis; TA, temporal arteries; VSMC, vascular smooth muscle cells.

GCA (figure 6D). Blocking $ET_BR$ with BQ788 remarkably inhibited VSMC outgrowth from GCA-involved arteries (figure 6E). The effect of blocking $ET_AR$ with BQ123 was less intense but also delayed VSMC outgrowth (figure 6E). Taking together, these data support a seminal role of ET-1 in inducing neointima formation in GCA.

## DISCUSSION

Expression of ET-1 was increased in GCA lesions compared with normal arteries. In GCA, infiltrating leukocytes accounted for the majority of ET-1 expression, which was also enhanced in the luminal endothelium. Some ET-1 expression was also observed in remaining VSMC. Coculture experiments supported that, in an

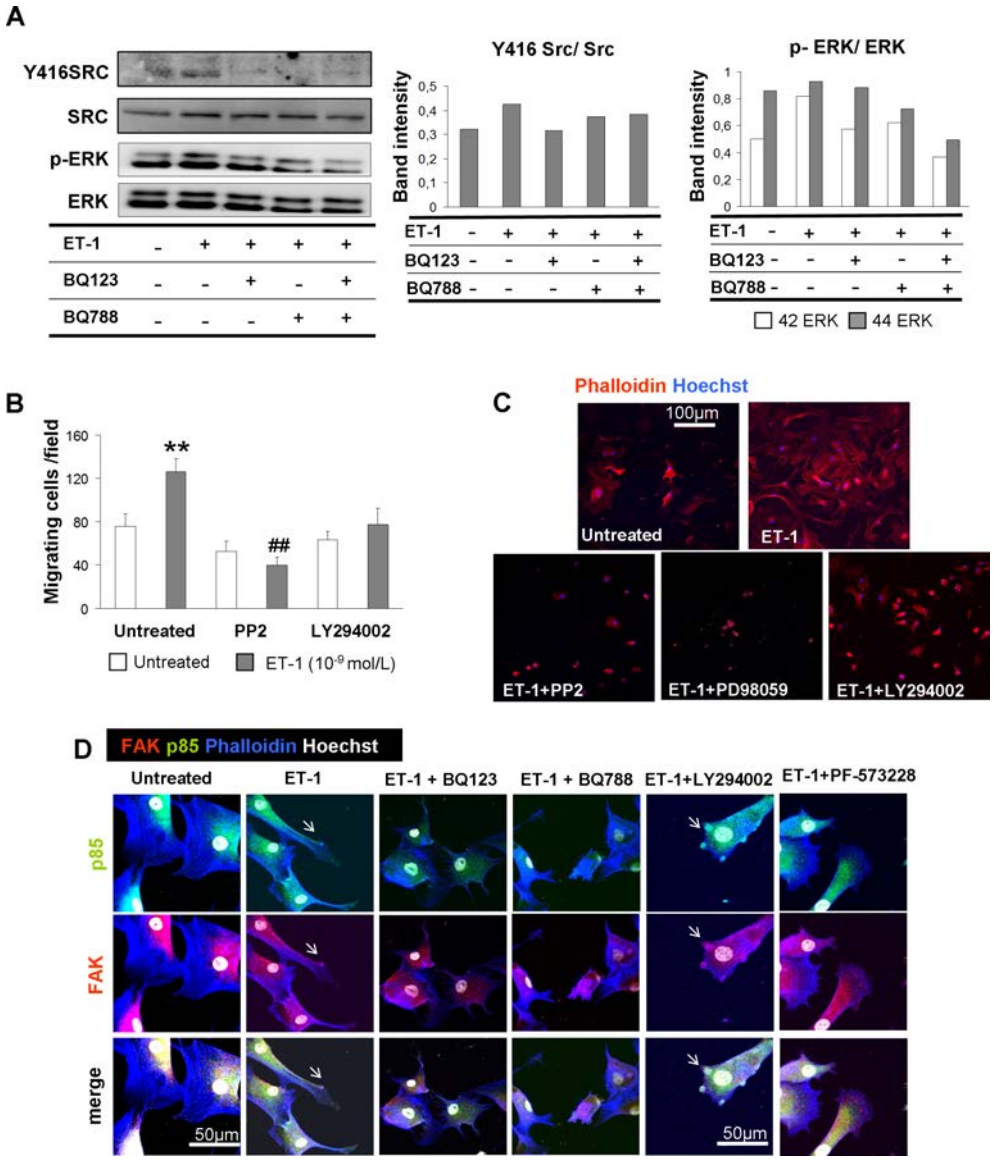


**Figure 5** ERK, Src and PI3K participation in ET-1 induced VSMC migration. (A) Immunoblot of phospho-Src (Y416)/total Src and phospho ERK/total ERK in lysates of VSMC treated with ET-1 ($10^{-9}$ mol/L) for 90 min. Graphs show quantification of a representative experiment out of three independent experiments. (B) VSMC migration in Boyden chambers with or without preincubation with Src inhibitor PP2 (10 µmol/L), or PI3K inhibitor LY294002 (20 µmol/L), with or without subsequent addition of ET-1. Bars represent the number of migrated cells (mean and SEM of quadruplicates). **p<0.005 for untreated cells versus ET-1-incubated cells. ##p<0.005 for ET-1-treated cells versus VSMC preincubated with inhibitors. (C) Immunofluorescence of VSMC f-actin cytoskeleton (red) and nuclei (blue). ET-1 was added at the time of VSMC seeding. VSMCs were preincubated 30 min in suspension with Src inhibitor (PP2), ERK inhibitor (PD98059) or PI3K inhibitor (LY294002) before addition of ET-1 ($10^{-9}$ mol/L). Representative pictures of each situation are shown. (D) Immunofluorescence staining of f-actin cytoskeleton (blue), p85 (green), total FAK (red) and nuclei (white) in VSMC treated with ET-1, or ET-1 plus $ET_AR$ antagonist (BQ123), $ET_BR$ antagonist (BQ788), PI3K inhibitor (LY294002) or FAK inhibitor (PF-573228) as labelled. Arrows highlight p85 and FAK colocalisation in the cell protrusions of ET-1-treated VSMC or p85/FAK clusters in immature cell protrusions triggered by ET-1 in the presence of PI3kinase inhibitor LY294002. ET-1, endothelin-1; $ET_AR$, ET-1 receptor A; $ET_BR$, ET-1 receptor B; FAK, focal adhesion kinase; VSMC, vascular smooth muscle cells.

inflammatory microenvironment, ET-1 production is increased in mononuclear cells and decreases in VSMC. $ET_AR$ was constitutively expressed by VSMC in normal arteries but, in the context of vascular inflammation, both ET receptors were remarkably increased and expressed by endothelial cells, VSMC and infiltrating leukocytes. As previously reported, the increase in $ET_BR$ was much more prominent.[17]

ET-1 stimulated VSMC migration through FAK activation, revealed by ET-1-enhanced FAK autophosphorylation at Y397, creating binding sites for Src kinase and the p85 regulatory subunit of PI3kinase, a crucial process in cell motility.[29 30 35] ET-1 promoted colocalisation of activated FAK and p85-PI3kinase at the focal adhesions. Subsequent signalling cascades participating in cell motility in other cell types, such as Src and ERK, were also slightly activated. Interestingly, while FAK and Src inhibitors strongly reduced both baseline and ET-1-induced migration, PI3kinase inhibitor selectively inhibited the increase in migration induced by ET-1. Class I PI3kinases are activated by tyrosine kinases whereas class II PI3kinases are activated through GPCR.[36 37] It is likely that ET-1 promotes activation of both classes of PI3kinases through FAK activation and through GPCR $ET_AR$ and $ET_BR$. In addition, GPCR-induced heterotrimeric G-protein activation may

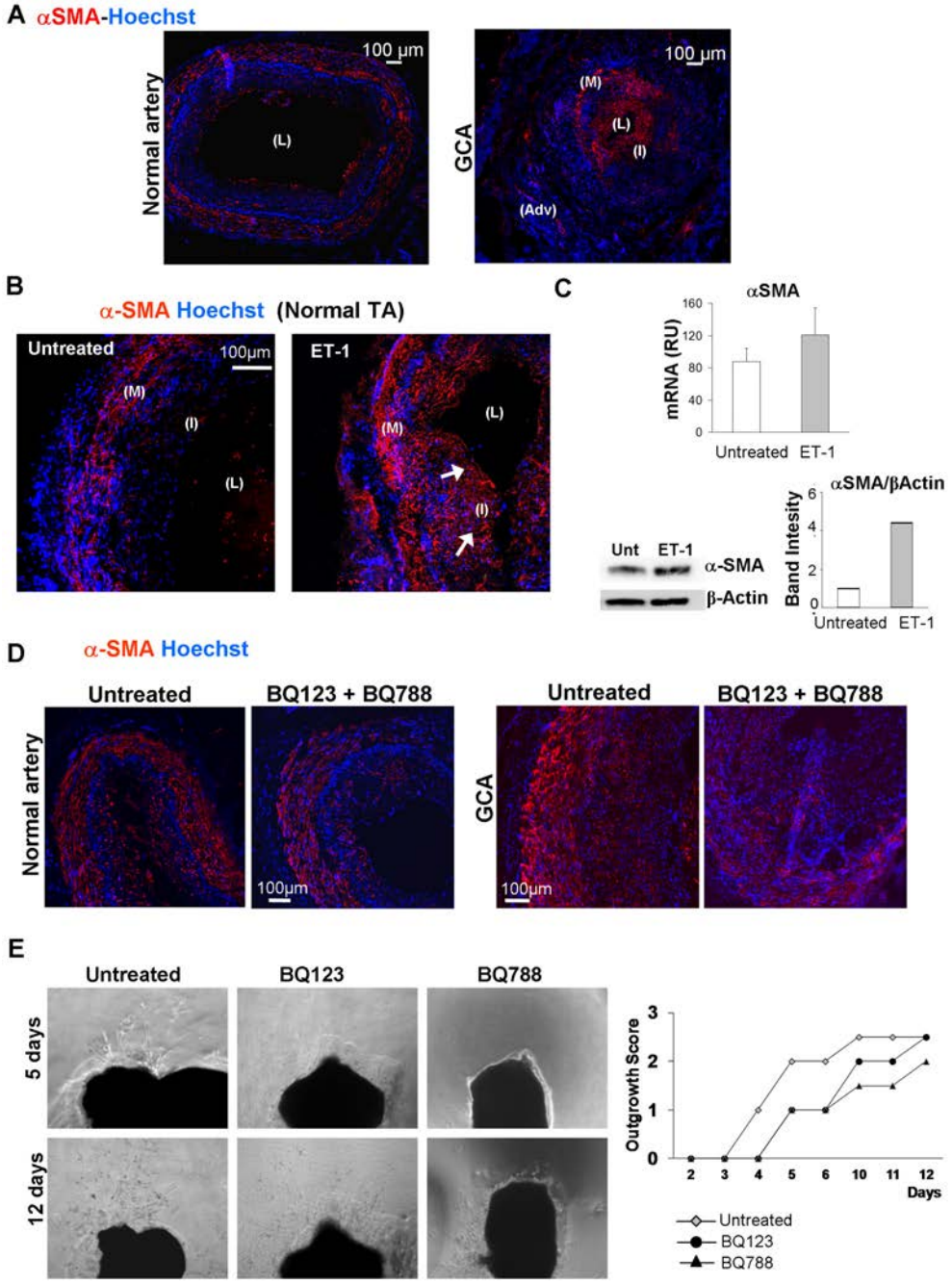


**Figure 6** ET-1 induction of VSMC migration in ex vivo TA cultures. (A) Immunofluorescence staining of αSMA (red) and nuclei (blue) in a control TA (left panel) or in a GCA artery with typical lesions (right panel). (B) Immunofluorescence staining of αSMA (red) and nuclei (blue) in a control TA cultured on Matrigel for 5 days in the presence or in the absence of ET-1 ($10^{-9}$ mol/L). Arrows indicate αSMA-positive cells migrating from the media to the intimal layer. Pictures are representative of three different control arteries. L, lumen; I, intimal layer; M, media layer. (C) αSMA mRNA expression in three cultured normal arteries in the absence or in the presence of ET-1 ($10^{-9}$ mol/L) (upper panel). Immunoblot of αSMA and β-actin, with the corresponding quantifications, in a normal TA cultured for 5 days in the absence or in the presence of ET-1 at the same concentration. The experiment was repeated twice with consistent results. (D) Immunofluorescence staining of αSMA (red) and nuclei (blue) in a control TA cultured in Matrigel for 5 days (left panel) and in a GCA-involved artery (right panel) in the presence or absence of ET-1 receptor antagonists (BQ123 and BQ788) at 20 µmol/L. (E) VSMC outgrowth from three TAs from patients with GCA cultured on Matrigel for the indicated periods of time with or without the presence of ET-1 receptor antagonist BQ123 (20 µmol/L) or BQ788 (20 µmol/L). αSMA, α-smooth muscle actin; ET-1, endothelin-1; GCA, giant-cell arteritis; RU, relative units; TA, temporal arteries; VSMC, vascular smooth muscle cells.

also contribute to FAK activation since pertussis toxin abrogated ET-1-induced FAK phosphorylation.

It has been previously shown that FAK coordinates migration with matrix metalloproteinase (MMP) release, which is necessary for cell progression through the extracellular matrix.[20] ET-1 moderately stimulated release of MMP2 by VSMC, a process mostly mediated by $ET_BR$ in our experimental conditions. Since MMP2 has elastinolytic activity, ET-1-induced MMP-2 release may be relevant to the disruption of the internal elastic lamina, characteristically observed

in GCA lesions, allowing VSMC migration from the muscular to the intimal layer.[6 21 38]

FAK has received substantial attention in pathological processes where cell migration is seminal including cancer and fibrosis.[14 28] Our data suggest that FAK is involved in vascular remodelling. Supporting our findings, a recent study has evidenced Y397 phosphorylated FAK in the resistance arteries undergoing vascular remodelling in hypertension.[39] Selective myeloid deletion of FAK does not influence vascular remodelling in a mouse model, suggesting that expression and activation of FAK in VSMC rather than inflammatory cells may be relevant to vascular occlusion.[40] Moreover, a naturally occurring truncated form of FAK, FRNK, which acts as competitive inhibitor of FAK, inhibits VSMC invasion in a carotid rat injury model.[41] Consequently, our results indicate that ET-1-mediated activation of FAK in VSMC may have a seminal role in vascular remodelling in the context of vascular inflammation where ET-1 is mainly produced by inflammatory cells and their production is amplified through interactions with VSMC. These newly recognised functions of ET-1 on VSMC may have a broader impact and may operate in vascular diseases with prominent vascular remodelling beyond GCA. To date, in the field of vascular biology, attention has mainly focused on the vasoconstriction role of ET-1 and only responses related to vascular reactivity or hypertension have been explored after conditional deletion of $ET_AR$ in VSMC.[9 10 41] In a pioneer study performed in mice more than one decade ago, induced overexpression of ET-1 in endothelial cells resulted in increased vascular remodelling.[42] However, this interesting observation has not been further explored.

Based on its presumed major function, ET-1 has been considered a therapeutic target for vascular diseases where vasoconstriction is thought to play a major role such as systemic or pulmonary hypertension or, more recently, fibrotic diseases, such as scleroderma or lung fibrosis, according to the newly recognised functions of ET-1 on fibroblasts.[9 10 13 14] However, to date, clinical trials with ET-1 receptor antagonists have shown the best efficacy for diseases with prominent vascular remodelling such as ischaemic ulcers in systemic sclerosis or pulmonary hypertension rather than vasoconstriction or fibrotic diseases.[9 10 43]

There is an unmet need of treatments reducing inflammation-induced vascular remodelling in GCA since patients with systemic vasculitis may develop complications derived from vascular occlusion in spite of glucocorticoid or immunosuppressive therapy.[44] Our data underline an unprecedented and crucial role for ET-1 in inducing vascular remodelling and vascular occlusion in the context of vascular inflammation and point towards endothelin receptor antagonists as potential therapeutic targets to avoid maladaptive vascular remodelling in inflammatory diseases of blood vessels.

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**Contributors** MCC and EPR designed the experiments and wrote the manuscript. EPR, NTG and MCB performed the experimental work. EL and MS contributed preliminary results essential for the development of the study. MAA, GEF, SPG and JHR contributed to clinical selection and TA biopsy collection. SP and RL produced and provided BQ123. All authors read, made improvements and approved the final version.

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**Competing interests** None declared.

**Patient consent** Obtained.

**Ethics approval** Ethics Committee Hospital Clinic of Barcelona.

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# Erratum: *Baricitinib in patients with inadequate response or intolerance to conventional synthetic DMARDs: results from the RA-BUILD study*

Dougados M, van der Heijde D, Chen Y-C, *et al*. Baricitinib in patients with inadequate response or intolerance to conventional synthetic DMARDs: results from the RA-BUILD study. *Ann Rheum Dis* 2017;76:88–95.

Page 91 states: Herpes zoster infections (n=7) were seen in the baricitinib 2 and 4 mg groups with similar frequency; none were visceral or disseminated. Herpes zoster distribution beyond the primary or adjacent dermatomes was seen in one patient (baricitinib 4 mg).

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# Giant cell arteritis, infections and biologics

To the editor,

Numerous infectious agents have been implicated in the aetiology and/or pathogenesis of systemic vasculitides through direct damage of the vessel wall or autoimmune disorders. Mechanisms by which pathogens cause autoimmunity may include molecular mimicry (cross-reactivity between pathogen-derived and self-derived epitopes), epitope spreading (the immune response to a persisting pathogen), bystander activation (non-specific activation of autoimmune cells by the inflammatory environment during infection) or immune response to cryptic antigens (subdominant epitopes which are normally hidden from T-cell recognition).[1] The causative role of infectious agents is clearly established in polyarteritis nodosa and cryoglobulinemic vasculitis that are commonly associated with hepatitis B virus and hepatitis C virus (HCV), respectively. Microbial pathogens may probably contribute to the development of other systemic vasculitides as well. However, the evidence for a definitive link between infection and induced autoimmunity in many vasculitides is less strong or lacking. Epidemiological studies showing an increased incidence of systemic vasculitis in people infected with a particular agent, while not wholly definitive, may strengthen the infection-induced autoimmunity concept.[1]

In the impressive nested case–control study recently published in the *Annals of the Rheumatic Diseases*,[2] Rhee *et al* examined the relationship between any infection or herpes zoster infection and the development of giant cell arteritis (GCA) in a large cohort of patients (n=4559) and controls (n=22 759). Herpes zoster was associated with a higher risk of developing GCA ($p<0.01$). However, the incidence rate ratio was relatively low (1.17) and suggests that clinically overt herpes zoster is unlikely to play a major causal role in the pathogenesis of GCA. These data contradict an earlier study that showed the presence of Varicella-zoster virus (VZV) antigen in the majority of GCA-positive temporal artery biopsies, supporting the hypothesis that VZV may trigger GCA.[3] Rhee *et al* study does not refute a link between VZV and GCA, since the authors did not evaluate latent VZV infection or reactivation of virus that can explain histological findings in the temporal arteries in the absence of clinical signs and symptoms of infection. It is tempting to speculate that persistence of VZV during glucocorticoid therapy may account for the recurrent course of GCA in a proportion of patients. Therefore, proof-of-concept trial may be justified to study whether antiviral treatment with acyclovir or valacyclovir confers additional benefit to patients with GCA receiving corticosteroids. The excellent results of treatment with newer direct antiviral agents for HCV-associated cryoglobulinemic vasculitis indirectly support feasibility of such approach. Notably, VZV is an established aetiological agent of central nervous system vasculitis (or vasculopathy) that can involve large and small intracranial vessels and is typically treated with intravenous acyclovir.[4 5]

In Rhee *et al* study, the risk of incident GCA was higher among patients who had any prior infections though this association was also quite modest (the incidence rate ratio of 1.26). However, it was 'dose dependent', and the incidence rate ratio reached 2.18 in patients with a history of ≥5 infections. The authors suggested that infections may be directly involved in the pathogenesis of GCA or may be just a marker of immune dysfunction. In a previous matched historical cohort study, Durand and Thomas showed that treated patients with GCA were also at increased risk of infections, particularly in the first few months following diagnosis.[6] In the latter study, an increase in the risk of lower respiratory tract and urinary infections during immunosuppressive treatment was comparable to that in Rhee *et al* study. In a smaller population-based retrospective cohort study, there was no overall increased risk of infections requiring or acquired during hospitalisation in patients with GCA who were taking glucocorticoid therapy.[7] Therefore, patients with GCA may be more prone to infections than controls even prior to onset of systemic vasculitis, while glucocorticoid therapy does not seem to increase significantly the occurrence of infections in these patients.

In our opinion, these findings have important implications for a choice of treatment. Currently, we face an increase in the number of clinical trials evaluating different biological agents as a treatment for recurrent or newly diagnosed GCA.[8] A list of promising biological agents that may be beneficial in patients with GCA already includes tocilizumab, abatacept, sirukumab and ustekinumab. It is expected that a wider use of biological agents will avoid multiple adverse events related to glucocorticoids. The steroid-sparing effect of biological therapy will be particularly valuable for the elderly patients with GCA who frequently develop metabolic complications during treatment with glucocorticoids. However, we should keep an eye on the risk of infections that can be associated with biological therapy. In the recent systematic review, Singh *et al*[9] identified 106 clinical trials that reported serious infections and included patients with rheumatoid arthritis who received biological therapy. Compared with traditional disease-modifying antirheumatic drugs (DMARDs), standard-dose and high-dose biological agents were associated with an increased risk of serious infections, although low-dose biological agents were not. The absolute increase in the number of serious infections per 1000 patients treated each year ranged from 6 for standard-dose biological drugs to 55 for combination biological therapy, compared with traditional DMARDs. Biological agents have different mechanisms of action and safety profiles. Therefore, it would be a mistake to assume that all of them increase the risk of infections compared with standard treatment. However, the balance between possible benefit and harm should be carefully evaluated.

In summary, Rhee *et al* study suggests that clinically overt herpes zoster infections play a minor role, if any, in the pathogenesis of GCA. However, this case-control study does not rule out the hypothesis that latent or subclinical VZV infection may contribute to the development of GCA. Meanwhile, the clinical significance of VZV antigen identification in the temporal arteries should not be overstated. Numerous studies demonstrated associations between a large variety of pathogens and atherosclerosis, partly by the presence of the infectious agent (eg, *Chlamydophila pneumoniae*) in the human atherosclerotic tissue.[10] Many molecular mechanisms have been suggested by which microbes may affect atherogenesis. Nevertheless, in the large-scale randomised clinical trials, evaluating the efficacy of antibiotic treatment for the secondary prevention of coronary events, there was no reduction in the rate of cardiovascular events, thereby challenging the validity of the infection hypothesis.[11] The most compelling proof of infectious theory of GCA would be disappearance of symptoms or prevention of recurrences with the clearance of the infection. The story of infections and systemic vasculitides will be continued.

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# Response to eLetter: 'Infections in giant cell arteritis and therapeutic implications' by Moiseev *et al*

We thank Professor Moiseev and colleagues for showing their interest in our work.[1 2] While we agree that our study does not completely refute the link between varicella zoster virus (VZV) and giant cell arteritis (GCA), we feel that stronger evidence of a link between VZV and GCA are needed before clinical trials are conducted in which patients with GCA are given antiviral therapy. If VZV, whether clinically overt, subclinical or latent, triggers the onset of GCA, we would have expected a stronger association between herpes zoster and GCA compared with the association of other infections with GCA. Although outside the scope of our study, we agree that the risk of infection following the institution of immunosuppressive therapy, including biological agents, for GCA is extremely important and needs to be further defined.

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# Clinical benefit of vedolizumab on articular manifestations in patients with active spondyloarthritis associated with inflammatory bowel disease

Vedolizumab (VDZ) is a new biological agent which was recently approved for the treatment of inflammatory bowel disease (IBD)[1] following the good clinical responses reported by clinical trials for both Crohn's disease[2] and ulcerative colitis.[3] However, the effects of VDZ on extraintestinal manifestations were not reported in these trials, and the 'real life' experience is still limited. On these premises, we read with interest the recent work by Varkas *et al*[4] reporting a series of five patients with IBD who were treated with VDZ and promptly developed new onset or exacerbation of spondyloarthritis (SpA), irrespective of the response to treatment on intestinal symptoms. Although the hypotheses proposed by the authors to explain such events sound reasonable, we would like to report our different preliminary results on the effect of VDZ on IBD-associated SpA. From June to December 2016, a treatment with VDZ was started in 53 patients. Data were collected prospectively. Patient characteristics and main results are shown in table 1. Notably, 81.1% of patients had been previously treated with at least one TNF-α inhibitor, and almost all (96.2%) were steroid dependent. Overall, 36 out of 53 patients (67.9%) completed the induction phase at last observation, and the mean follow-up of the entire cohort was 2.6±1.6 months. Eight (15.1%) patients had a history of IBD-associated SpA but were inactive at the time of initiation of VDZ, whereas 14 (26.4%) had active SpA when VDZ was started. First, no case of induction or flare of arthritis and/or sacroiliitis was reported among the entire cohort, including the patients without a prior SpA diagnosis. Second, 6 out of the 14 patients with active SpA (46.2%)—all complaining of peripheral arthropathy—experienced a sharp clinical benefit after the initiation of VDZ. About gut inflammation of these six patients, three of them were in clinical remission after 6 and 12 weeks of therapy, two were in remission after 6 weeks (they have not reached week 12 yet) and one patient did not experience any response on intestinal symptoms after 14 weeks of treatment. As a consequence, our preliminary prospective data indicate a potential benefit of VDZ on IBD-associated SpA. Even if we do not reject the possibility that VDZ may induce new onset or exacerbation of arthritis and/or sacroiliitis, the previous demonstration of α4β7 in the joint[5 6] and the recent evidence of the upregulation of mucosal vascular address in cell adhesion molecule (MadCAM-1) in the high endothelial venules of bone marrow in patients with active axial SpA[7] seem to strengthen the hypothesis of a beneficial rather than a paradoxical effect of α4β7 blockade on articular manifestations of IBD. Obviously, more details about the molecular mechanisms underlying the α4β7 blockade in the joints are required, and large cohort studies are needed to provide more evidence on these preliminary findings.

**Table 1** Characteristics of patients and main results

| Variable | n=53 |
|---|---|
| Age (years), mean±SD | 51.5±15.7 |
| Male gender, n (%) | 28 (52.8) |
| Smokers, n (%) | |
| Never | 50 (94.3) |
| Current | 2 (3.8) |
| Ex | 1 (1.9) |
| Type of disease, n (%) | |
| Crohn's disease | 34 (64.2) |
| Ulcerative colitis | 19 (35.8) |
| Duration of disease (years), mean±SD | 13.6±9.4 |
| Localisation of the disease, n (%) | |
| Crohn's disease | |
| Ileal | 3 (8.8) |
| Ileocolic | 26 (76.5) |
| Colic | 4 (11.8) |
| Upper gastrointestinal tract* | 1 (2.9) |
| Perianal disease | 7 (20.6) |
| Ulcerative colitis | |
| Proctitis | 0 (0.0) |
| Left-sided | 6 (31.6) |
| Extensive | 13 (68.4) |
| Behaviour (Crohn's disease), n (%) | |
| Inflammatory | 16 (47.1) |
| Stricturing | 17 (50.0) |
| Fistulising | 1 (2.9) |
| Previous resections (Crohn's disease), n (%) | 21 (61.8) |
| Previous biological treatments | |
| Yes | 43 (81.1) |
| No (naïve to biologics) | 10 (18.9) |
| Steroid-dependent, n (%) | 51 (96.2) |
| IBD-associated SpA | |
| No history | 31 (58.5) |
| History (inactive at initiation of VDZ) | 8 (15.1) |
| Active at initiation of VDZ | 14 (26.4) |
| Peripheral arthropathy | 12 (85.7) |
| Axial and peripheral arthropathy | 2 (14.3) |
| Clinical benefit on SpA following initiation of VDZ (n=14) | |
| No clinical benefit | 8/14 (57.1) |
| Improvement | 6/14 (42.9) |
| New onset/exacerbation of SpA induced by VDZ | 0 |

*In addition to an ileocolic localisation.
IBD, inflammatory bowel disease; SpA, spondyloarthritis; VDZ, vedolizumab.

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# Response to: 'Clinical benefit of vedolizumab on articular manifestations in patients with active spondyloarthritis associated with inflammatory bowel disease' by Orlando *et al*

We thank Orlando *et al*[1] for the critical appraisal of our paper.[2] However, we believe that the limited sample size and the lack of sufficient follow-up do not support unmistakable evidence of clinical benefit of vedolizumab in spondyloarthritis (SpA). First of all, merely 36 out of 53 patients with inflammatory bowel disease (IBD) completed the 6-week induction phase at the last recorded observation, which makes the interpretation of data premature. Hence, as stated in our paper, mean time to flare in our patients was calculated at 64 days after the initiation of vedolizumab, ranging up to 114 days in these selected cases. The patients presented in the series from Orlando *et al* are also much older (mean 51.5 years vs 36.0 years) and display a higher rate of surgical intervention (>60%) compared with our cases, which may respectively reflect a population less likely to develop SpA features and a disturbed gastrointestinal architecture in therapy-resistant patients. Similarly, no data on concomitant medication, which might be synergistic, are provided. Nevertheless, no induction or flare of SpA was seen in this small prospective cohort. This observation does not necessarily contradict with our case series, as the prevalence of these findings in clinical practice is currently unknown and should be further investigated.

Orlando *et al* do not report any induction or flare of SpA in their small prospective cohort, but go as far as suggesting a clinical benefit. However, less than half of patients of 14 active patients with SpA experienced a response, which was vaguely described as a 'sharp clinical benefit' at the level of the joint. Unfortunately, objective signs of disease activity and/or outcome measures such as MRI inflammation or imaging are lacking in the report, making the data difficult to interpret. Surprisingly, one of these six patients even responded well at the level of the joint, in absence of a gut response. Although we cannot exclude some efficacy, taking the high placebo response in SpA in up to 20%,[3 4] the follow-up time and the small sample size of this cohort into account, precaution is needed to make firm conclusions based on these results. In any case, the efficacy of vedolizumab in IBD-associated joint disease, if any, does not seem to measure up to the efficacy of anti-tumour necrosis factor reported previously in over 60% in IBD-related arthritis.[5 6] The lack of mucosal vascular addressin cell adhesion molecule 1 (MADCAM-1) expression in synovial tissue despite the presence of α4β7 on synovial T cells in SpA, which contrasts with the gut-specific interaction with MADCAM-1, provides scientific rationale for a differential response on joint versus gut symptoms.[7 8]

In conclusion, the overall efficacy of vedolizumab in IBD-associated SpA remains unclear. The report of Orlando *et al* suggests some level of response in selected cases but the series is not sufficiently powered and the follow-up is too short in duration to permit firm conclusions on efficacy in SpA. It is clear that only placebo-controlled trials—and not cohort studies—will be able to address the remaining questions regarding the impact of vedolizumab in IBD-associated SpA.

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# Anticitrullinated protein antibodies: taking into account antibody levels improves interpretation

Hensvold *et al*[1] reported on the discriminatory capacity of anticitrullinated protein antibodies (ACPA) for diagnosing rheumatoid arthritis (RA).[1] A major strength of the study is that it was performed in a population setting and that a large number of controls (n=12 434) were included, thereby allowing a reliable estimate of the specificity of ACPA. The authors give detailed information on the diagnostic performance of anti-CCP2 antibodies (Euro-Diagnostica) for two cut-off points, namely the cut-off point recommended by the manufacturer and a cut-off point that is three times higher than the manufacturer's cut-off point. The latter high cut-off was defined in accordance to the European League against Rheumatism/American College of Rheumatology 2010 RA classification criteria.[2] The authors show that the positive likelihood ratio (LR) was higher for the high cut-off (LR=74) than for the cut-off point recommended by the company (LR=33).

It is increasingly recognised that the likelihood for disease increases with increasing antibody levels. Nevertheless, most laboratories report results using a single cut-off value. Reporting test-result interval specific LRs can give additional diagnostic depth to a lab result.[3] The LR (probability of a specific result in patients divided by the probability of the same result in controls) is independent of prevalence or pretest probability and can be applied for test result intervals. An LR >10 or <0.1 indicates a clinically significant difference in pretest to post-test probability.

The unique and large dataset presented by Hensvold *et al*[1] allows to deduce test-result interval specific LRs. The LRs are 0.35 (95% CI 0.28 to 0.43), 3.4 (95% CI 1.5 to 7.5) and 73.6 (95% CI 58.7 to 92.3) for an anti-CCP2 test result <25 AU/mL, between 25 and 75 AU/mL and ≥75 AU/mL, respectively. Only a small fraction (4%) of the patients (in total 156 patients with RA were included) had a low positive anti-cyclic citrullinated peptide antibody (CCP). The data are for prevalent RA at inclusion (based on table 2 in Hesvold *et al*[1]).

LRs can be used to estimate post-test probabilities for any given pretest probability.[3] Figure 1 illustrates the post-test probability as a function of pretest probability for different anti-CCP test result intervals (<25 AU/mL, between 25 and 75 AU/mL and ≥75 AU/mL). For example, for a pretest probability of 1.2% (which corresponds to the prevalence of RA in the general population), the post-test probability for RA is estimated to be 0.4%, 3.9% and 47.2% for an anti-CCP2 test result of <25 AU/mL, between 25 and 75 AU/mL and ≥75 AU/mL, respectively. For a pretest probability of 10% (which corresponds to a clinical presentation of a 50-year-old women presenting with a slightly elevated C-reactive protein (CRP) (10 mg/L) and recent onset undifferentiated arthritis with intermittent asymmetric tender and swollen small joints (n=5) of the hands (deduced from Van der Helm-van Mil *et al*[4])), the post-test probabilities are 3.7%, 27.2% and 89.1% for an anti-CCP2 test result of <25 AU/mL, between 25 and 75 AU/mL and ≥75 AU/mL, respectively.

The 2010 RA classification criteria assign a score of 2 for a low-positive ACPA and of 3 for a high-positive ACPA. Our analysis revealed that the difference in pretest to post-test probability is clearly higher for a high positive ACPA than for a low-positive ACPA (LR 73.6 vs 3.4). Future refinements of the RA classification criteria might give a higher relative weight to a high-positive ACPA compared with a low-positive ACPA. Studies are needed to evaluate whether cut-off points or ACPA assays are aligned between different manufacturers.

In conclusion, interpretation of ACPA must be done in the clinical context and in function of pretest probability and test characteristics. The work presented by Hensvold *et al*[1] allows to deduce reliable estimates of test result interval specific LRs of ACPA for RA. A high ACPA has a higher LR for RA than a low ACPA. Such knowledge helps to better interpret ACPA test results.

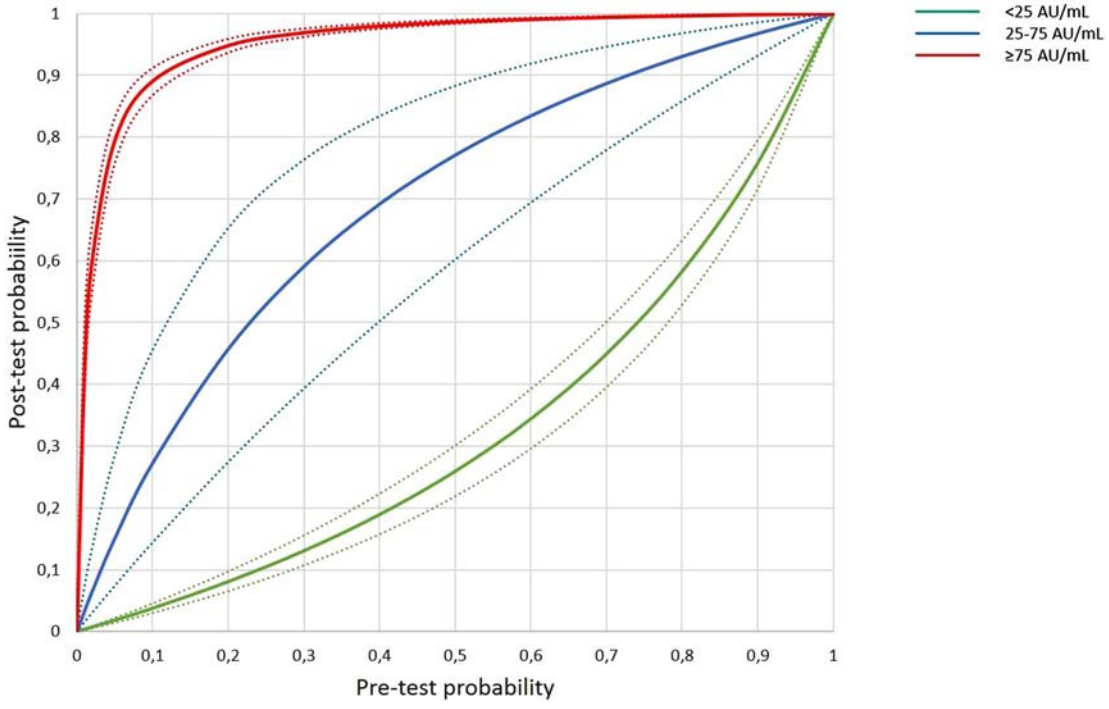


Figure 1 Post-test probabilities (with 95% CIs) as a function of pretest probability for different test result intervals (<25 AU/mL, between 25 and 75 AU/mL and ≥75 AU/mL).

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# Preoperative MRI to plan infrapatellar fat pad resection during total knee arthroplasty

We thank Pan *et al* for their initial paper[1] and subsequent response to our comment.[2–4] We agree with the proposed biphasic role of the infrapatellar fat pad (IPFP). Although there is evidence to support a change of practice towards preservation of the IPFP,[5] we agree that there should not be a 'one shoe fits all approach', there being cases in which benefit may be derived from IPFP resection.

A considered approach offered by Han *et al*[4] involves using screening MRIs to identify IPFP signal intensity alterations and a subsequent indication for resection. However, this technique is not yet validated with high-quality randomised controlled trials (RCTs).[4] An alternative solution proposed by Sekiya *et al*[6] may be the use of postarthroplasty arthroscopic IPFP debridement.

Currently the incidence of knee pain post total knee arthroplasty (TKA) is low, with approximately 10% of patients reporting mild to moderate pain[7 8] and 4.8% reporting severe pain.[6] The study by Sekiya *et al*[6] found that of the 4.8% of patients with severe knee pain, a significant proportion had scar tissue between the IPFP and the tibiofemoral space, impinging the femorotibial joint. Following arthroscopic resection of this scar tissue, 63% of the patients reporting severe pain were now pain free and a further 23% had their pain at least halved.[6]

This suggests that less than 2% of patients will report severe knee pain post TKA if the IPFP is preserved and arthroscopic debridement is performed as required. However, IPFP screening as proposed by Han *et al* has the potential to reduce reoperation and perhaps reduce morbidity beyond this. Consequently, we eagerly await RCTs investigating its use and the possibility of reducing severe pain post TKA to less than 1%.

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## Correction

Based on information provided by the World Health Organization (WHO), the authors wish to correct statements implying that WHO is associated with or endorses the FRAX® model or treatment recommendations of at-risk populations. The FRAX® tool was not developed, endorsed, evaluated or validated by WHO.

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