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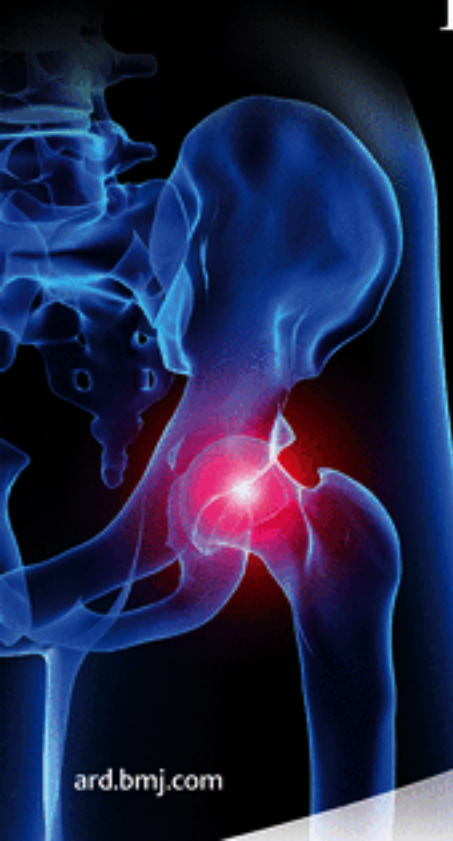

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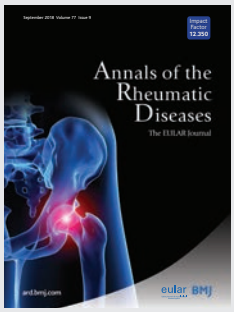

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Bisphosphonates: a therapeutic option for knee osteoarthritis?

Willem F Lems[1,2]

Osteoarthritis (OA) is one of the rheumatic diseases with the highest incidence and prevalence, which are expected to rise in current ageing populations.[1] Unfortunately, in contrast to inflammatory rheumatic diseases such as rheumatoid arthritis, ankylosing spondylitis and psoriatic arthritis, there are hardly any treatment options that modify the course of OA,[2] except for some data on glucosamine and chondrotoine on radiological progression.[3 4] In fact, the only instruments we have are symptomatics: analgesics, exercise therapy, weight loss for those with a high body mass index (BMI), intra-articular injections[2] and orthopaedic surgery for those with severe or end-stage OA.

Nowadays, the disease is regarded as a whole joint disease that affects different structures in and around the joint capsule: the cartilage and also the synovial tissue, subchondral bone, muscles, ligaments and entheses are involved.[5 6] In the absence of effective therapeutic options that modify the course of the disease, it is attractive to split up the options for interventions in different targets: cartilage, subchondral bone, synovitis and central pain regulation.[5–7] Against that background, the study by Neogi *et al*[8] is very welcome: they showed that the risk of knee replacement (KR) therapy in patients with knee-OA was 25% lower in bisphosphonate (BP) users than in non-BP users, a remarkable difference, with large potential consequences, at least for elderly individuals with osteoporosis and OA.

DO ANTIRESORPTIVE DRUGS HAVE FAVOURABLE EFFECTS IN OA?

Recent experimental and clinical studies have demonstrated that changes in subchondral bone and a crosstalk between subchondral bone and cartilage play an important role in the development of OA,[9] although the pathophysiology and changes in subchondral bone have not been fully elucidated. In the early phase, increased osteoclast activity, elevated bone remodelling and hypomineralisation can be found,[7 9] while in later stages low bone turnover and densification of the bone ('bone sclerosis') can be observed. Additionally, bone marrow lesions (BML) can be detected on MRI.[5 9]

Thus, theoretically, the use of antiosteoporotic drugs that can be regarded as antiresorptive drugs (BPs such as alendronate, risedronate, zoledronic acid, but also denosumab, a monoclonal antibody against receptor activator of nuclear factor kappa-B ligand (RANKL)) might have effects on subchondral bone, particularly in the phase of elevated bone remodelling and hypomineralisation. Indeed, for risedronate a reduction in the urinary excretion of crboxy-terminal telepeptides of type II collagen (CTX II), usually regarded as a marker of cartilage breakdown, was found, but not on symptoms and/or radiological progression.[10] For alendronate and strontium ranelate, a reduction in osteophytes and disc-space narrowing of the spine was found in a post-hoc analysis of studies with patients with osteoporosis.[11 12] Additionally, in a small study with zoledronic acid, a reduction in BMLs was found.[13] With another antiosteoporotic drug, strontiumranelate, which is not a pure antiresorptive drug, a reduction in joint space width was demonstrated in a 3-year trial versus placebo.[14]

There are remaining questions how a primary effect on subchondral bone might have subsequent effects on cartilage metabolism, and on anatomical structural abnormalities such as disc-space narrowing, osteophytes and BMLs. Nevertheless, all these data point in the direction that BPs might have an effect on OA (progression).

Since denosumab is also an antiresorptive drug, it is plausible that it also might have favourable effects in OA.

HOW STRONG ARE THE DATA, IS BIAS BY INDICATION FULLY EXCLUDED?

Neogi *et al* used the The Health Improvement Network (THIN) database, in which data are collected by general practitioners in UK. Only patients with incident knee-OA were enrolled, and 2006 patients who initiated BPs at least 1 year after diagnosis of knee-OA were compared with 2006 propensity-matched 'non-BP initiators'. After a mean follow-up of 3 years, the crude incidence of KR was 22.0 per 1000 person-years among the initiators, vs 29.1 among the non-initiators, with an HR of 0.76 (95% CI 0.59 to 0.93). Obviously, the key question is whether this is a direct effect of BPs on the osteoarthritic joint, or might confounding by indication play a role? For example, do BP users have a more healthy lifestyle, or higher vitamin D levels, or a higher socioeconomic status, or less frailty, and so on?

Without doubt, the authors tried to minimise the effect of confounding by indication, by performing a propensity score-matched cohort study, and by sensitivity analysis for those without data on BMI, smoking and/or alcohol, which all support the protective effect of BPs. Even the potentially lower mortality risk for patients with BP use was calculated. Nevertheless, although these all seem to be optimal and robust, it is not possible to completely rule out the risk of bias, because unknown bias cannot be detected and thus not calculated.

ARE THESE DATA IN LINE WITH OTHER STUDIES?

Remarkably, this year Fu *et al*[15] published a more or less comparable, nationwide, study from Taiwan. They observed more than 12 000 BP users and 123 000 non-users, all with knee-OA, and they found a 24% reduction in the percentage of patients for KR (HR 0.75, 95% CI 0.69 to 0.83), which is remarkably in line with the THIN data. The study from Taiwan is larger, which is reflected in the smaller CI. But the authors found also a dose–effect relation: in those individuals who used BPs for more than 2 years and with a medication possession ratio >80%, the risk reduction for a KR was even larger: HR 0.66 (95% CI 0.43 to 0.95). Moreover, the authors observed over a 5-year follow-up period a significantly greater reduction in the use of non-steroidal anti-inflammatory drugs, acetaminophen and glucosamine in the BP users versus the non-users (P<0.001).

WHAT ARE THE CONSEQUENCES?

Obviously, the data from Neogi *et al* with the UK database and the data from Fu *et al* with the larger Taiwan database are in line

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with each other, which is very important because these data validate each other. Since both studies suggest that BPs reduce the percentage of patients with knee-OA who need a KR with around 25%, this might indicate that patients with knee-OA who also have osteoporosis and an elevated fracture risk should preferably be treated with a BP. It is too early to conclude that BPs should also be prescribed in patients with knee-OA without osteoporosis, since prospective data on randomised studies in patients with knee-OA in which reduction in patients with KRs who are BP users versus placebo are lacking.

It is important to realise that both studies have the same case–control design, so it cannot completely be ruled out that they may have both the same structural limitation of confounding by indication in its design. Nevertheless, the larger reduction in KRs in patients with greater exposure to BPs is a strong argument in favour of a drug-induced effect.

What would be the next step? Given the paucity of therapeutic options in OA, these data strongly suggest that BPs have a favourable effect on the natural course of OA, which might have important consequences for our patients and for societies, since reduction or postponing of KRs might have large financial consequences. In my opinion, the data should preferably be confirmed in a randomised controlled trial in which elderly patients with incident knee-OA should be randomised to a BP (or another antiresorptive drug, such as denosumab) versus placebo. Because of the placebo arm, patients with high fracture risk resulting from generalised osteoporosis should be excluded, for ethical reasons. As a consequence, it could be that patients with osteoporotic subchondral bone will be excluded. However, a large sample size and long study duration will be necessary, unless we are able to select patients with OA with osteochondral bone that is vulnerable to the positive effects of BPs, thus with a high bone turnover and some hypomineralisation. Unfortunately, reliable, quantitative and validated measurements are even with new techniques as dual x ray absorptiometry (DXA), CT scan and/or MRI not yet possible.

To summarise, two comparable and high-quality studies have documented and suggested that BP use is associated with a 25% reduction in KRs. Since KR is a clinically relevant and hard endpoint, these studies hopefully might stimulate further research in the role of subchondral bone in OA, which is still urgently needed given the paucity of therapeutic options in OA.

Competing interests None declared.

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Role of Epstein-Barr virus infection in SLE: gene-environment interactions at the molecular level

A paper by Harley *et al* in *Nature Genetics* provides an important new perspective on the mechanisms by which infection with Epstein-Barr virus (EBV) can promote autoimmunity and, in particular, systemic lupus erythematosus (SLE).[1] An interest in the role of EBV in lupus pathogenesis has a long history beginning in the 1970s with observations that patients with SLE have increased titres of anti-EBV antibodies.[2] Subsequent studies have strengthened the link between EBV and SLE as well as other diseases such as rheumatoid arthritis (RA) and multiple sclerosis (MS).[3] To many investigators, the question has not been whether EBV infection can lead to autoimmunity but rather how since the evidence for causality appears high.

Like other autoimmune diseases, SLE is likely the outcome of an encounter of a genetically susceptible individual with an environmental trigger such as an infection. With EBV, classic epidemiology faces a major challenge since infection with this virus, a member of the herpes family, is almost invariable in the adult population.[3] While the study of disease associations in children can be informative because of their lower infection rate,[4] it is difficult if not impossible to sort adults into infected and uninfected bins to investigate disease associations. Furthermore, it is always possible that abnormalities that predispose to autoimmunity also predispose to infection with EBV, complicating the situation further.

Given the limitations of epidemiology, investigators have explored other mechanisms by which EBV can trigger disease. One of the most informative has involved the analysis of the fine specificity of autoantibodies.[5] These studies have provided compelling evidence of molecular mimicry involving sequences of the EBNA1 protein and the SmB and Ro60 proteins. These shared sequences suggest that an aberrant response to an EBV protein can lead to the expression of cross-reactive autoantibodies that target nuclear proteins. With cross-reactive anti-Sm and anti-Ro antibodies in the system, epitope spreading can occur, leading to antibodies binding other epitopes on the Sm and Ro60 proteins as well as other nuclear antigens. Immunisation experiments in mice support this model.[5]

Another mechanism by which EBV could promote SLE pathogenesis relates to the impact of genetic polymorphisms on the host response to viral infection testing the possibility that, with almost everyone infected by EBV, genetic differences among infected individuals determines who will go on to autoimmunity and clinical disease events. At this point in time, genome-wide association studies (GWAS) have identified over 50 susceptibility loci for SLE in the population. Since most of these loci occur in regulatory regions of genes, Harley and colleagues asked whether EBV gene products that serve as transcription factors (TFs) have preferential interaction with loci containing risk alleles. This type of analysis is an exemplar of big data approaches and necessitates powerful bioinformatic platforms.

To identify TFs that bind to risk alleles, the investigative team developed a bioinformatics algorithm called RELI which stands for Regulatory Element Locus Intersection. Using data from a number of experimental sources, RELI can assess 'the significance of the interactions of the genomic coordinators of plausibly causal genetic variation and DNA sequences bound by a particular TF, as determined through chromatin immunoprecipitation and sequencing (ChIP-seq)'.[1] The authors first validated this approach with prostate and breast cancer cell lines and then went on to study SLE and other autoimmune diseases, focusing on the interaction of EBNA2 with European ancestry risk alleles.

Box 1 EBNA2 disorders

Systemic lupus erythematosus
Multiple sclerosis
Rheumatoid arthritis
Inflammatory bowel disease
Type 1 diabetes
Juvenile idiopathic arthritis
Celiac disease

The results with SLE are both impressive and intriguing. Thus, almost half of SLE risk alleles can be occupied by the EBNA2 protein, pointing to an important mechanism by which EBV can perturb immunity. Given the nature of these genes and their expression in EBV-infected B cells, the B cell is likely to be a key cell in disease pathogenesis, consistent with the many serological disturbances in patients with SLE. Furthermore, ChIP-seq data from cell lines with informative genotypes indicated the variants may affect the binding of transcription complexes containing EBNA2 and host proteins, thus modifying host gene regulatory interactions and allele-dependent expression of nearby genes.

Other studies demonstrated an association of EBNA2 and genes implicated in diseases in addition to SLE and MS. The authors term these diseases the EBNA2 disorders (box 1). Since the relevant loci could differ across diseases despite the common interaction of susceptibility loci with EBNA2, the actual mechanisms by which EBV can impact on autoimmune disease may vary. Such differences would be consistent with the distinct clinical and immunological findings in these conditions as well as the potential roles in pathogenesis of B cells, T cells and NK cells. As the authors note, since much of the data on the effects of EBV on gene expression concerns B cells, a more complete picture of the impact of EBV on immune regulation will require studies of other cell populations.

While this study provides a novel perspective on the potential role of EBV in autoimmune disease, many questions remain. Certainly one issue relates to the lag between infection and the development of autoimmunity, benign or pathogenic. Years may pass between the infection, the expression of autoantibodies and the onset of clinical events.[5] Perhaps the onset of disease in an infected, susceptible host requires another triggering event (eg, infection with another agent). Another salient issue concerns the marked differences in the clinical and immunological features of the various EBNA2 diseases.

While the generation of cross-reactive anti-EBNA1 antibodies is a prominent feature of SLE, the other EBNA2 diseases, in general, lack these specificities or have antibodies to other targets (eg, citrullinated proteins in RA). On the other hand, studies also beginning in the 1970s showed that patients with RA have quantitative differences in the expression of antibodies to EBV proteins as well as reactivity to an antigen called RA-associated nuclear antigen (RANA) found in B cell lines transformed by EBV.[6 7] While investigative interest in the serology of RA tends to focus on the role of citrullinated molecules, patients with RA commonly express ANAs, perhaps also related to B cell events during EBV infection.

The discovery of an association between a disease and infecting agent always prompts speculation about the implications for treatment and prevention. With respect to treatment,

the identification of risk alleles with which EBNA2 interacts leads directly to a search for agents that target these pathways. In the realm of prevention, a vaccine for EBV could have major health benefits since infectious mononucleosis remains a very common and sometimes serious infection.[8 9] In addition, EBV infection can be associated with malignancy. The idea that one vaccine could impede the development of diverse disease types (ie, infection, malignancy and autoimmunity) is exciting but perhaps not surprising in view of the high frequency of encounters with the virus in the population and the nature of the infection itself.

The study of Harley *et al* is a tour de force of computational analysis of large and complicated DNA data sets. Research in the next few years should be very informative and will hopefully show how big data can lead to big advances in the treatment of SLE and other autoimmune diseases.

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2018 EULAR recommendations for physical activity in people with inflammatory arthritis and osteoarthritis

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ABSTRACT

Regular physical activity (PA) is increasingly promoted for people with rheumatic and musculoskeletal diseases as well as the general population. We evaluated if the public health recommendations for PA are applicable for people with inflammatory arthritis (iA; Rheumatoid Arthritis and Spondyloarthritis) and osteoarthritis (hip/knee OA) in order to develop evidence-based recommendations for advice and guidance on PA in clinical practice. The EULAR standardised operating procedures for the development of recommendations were followed. A task force (TF) (including rheumatologists, other medical specialists and physicians, health professionals, patient-representatives, methodologists) from 16 countries met twice. In the first TF meeting, 13 research questions to support a systematic literature review (SLR) were identified and defined. In the second meeting, the SLR evidence was presented and discussed before the recommendations, research agenda and education agenda were formulated. The TF developed and agreed on four overarching principles and 10 recommendations for PA in people with iA and OA. The mean level of agreement between the TF members ranged between 9.8 and 8.8. Given the evidence for its effectiveness, feasibility and safety, PA is advocated as integral part of standard care throughout the course of these diseases. Finally, the TF agreed on related research and education agendas. Evidence and expert opinion inform these recommendations to provide guidance in the development, conduct and evaluation of PA-interventions and promotion in people with iA and OA. It is advised that these recommendations should be implemented considering individual needs and national health systems.

INTRODUCTION

Physical activity (PA) is defined as 'any bodily movement produced by skeletal muscles that results in energy expenditure above resting (basal) levels. PA broadly encompasses exercise, sports and physical activities done as part of daily living, occupation, leisure and active transportation'.[1 2] Exercise is a subcategory of PA 'that is planned, structured and repetitive and [that] has, as a final or intermediate objective, the improvement or maintenance of one or more dimensions of physical fitness'.[1 2] PA-interventions can be provided or performed individually or in groups, supervised or non-supervised, in acute or chronic health states, but should always include behavioural change techniques (BCT) to promote long-term adherence.[3 4]

To promote the health benefits of PA in the general population, the WHO[5] and American College of Sports Medicine (ACSM)[2] have provided internationally accepted recommendations for PA (table 1). In this manuscript, the term PA always includes both physical activity and exercise according to the definitions above.

Inflammatory arthritis (iA, in this manuscript encompassing rheumatoid arthritis (RA) and spondyloarthritis (SpA)) and osteoarthritis (OA) (in this manuscript encompassing hip/knee OA (HOA/KOA)) are major causes of pain and disability worldwide.[6] There is strong evidence for the benefits of PA on improvements on disease activity,[7] activities and participation; however, people with rheumatic and musculoskeletal diseases (RMDs) are in general less active compared with healthy controls.[8–10] Possible underlying reasons could be that healthcare providers (HCP, including rheumatology health professionals (eg, physiotherapist (PT), occupational therapist (OT), nurse, podiatrist, psychologist), physical education professions and medical doctors (rheumatologists and other specialists)) and people with iA and OA may be reluctant towards engaging in PA, fearing flare-up or joint damage by exercising.[11] Furthermore, current clinical management recommendations such as the European League Against Rheumatism (EULAR) recommendations on the management of RA,[12] SpA[13] or HOA/KOA[14] and the ACSM guidelines for exercise testing and prescription[15] recommend exercise and/or PA, but none of these is specific regarding the required type and dosage. Therefore, it is not clear how these recommendations should be used in routine clinical care. In particular, the evidence on the effectiveness and safety of exercise and PA to a level that meets public health (PH) recommendations has not yet been clearly examined and defined in people with RMDs. A EULAR task force (TF) was therefore set up (1) to evaluate if the PH recommendations for PA are applicable for people with iA and OA; (2) to

Table 1 Public Health recommendations for PA

The ACSM-AHA primary physical activity recommendations*	
► All healthy adults aged 18–65 years should participate in moderate intensity aerobic PA for a minimum of 30 min on 5 days/week or vigorous intensity aerobic activity for a minimum of 20 min on 3 days/week.	
► Combinations of moderate and vigorous intensity exercise can be performed to meet this recommendation.	
► Moderate intensity aerobic activity can be accumulated to total the 30 min minimum by performing bouts each lasting ≥10 min.	
► Every adult should perform activities that maintain or increase muscular strength and endurance for a minimum of 2 days/week.	
► Because of the dose-response relationship between PA and health, individuals who wish further improve their fitness, reduce their risk of chronic diseases and disabilities and/or prevent unhealthy weight gain my benefit by exceeding the minimum recommended amounts of PA.	
Cardiorespiratory ('aerobic') exercise†	
Frequency	≥5 days/week of moderate exercise or ≥3 days/week of vigorous exercise or a combination of moderate and vigorous exercise on ≥3–5 days/week is recommended.
Intensity	Moderate and/or vigorous intensity is recommended for most adults. Light to moderate intensity exercise may be beneficial in deconditioned persons.
Time	30–60 min/day (150 min/week) of purposeful moderate exercise or 20–60 min/day (75 min/week) of vigorous exercise or a combination of moderate and vigorous exercise per day is recommended for most adults. ≥20 min/day (150 min/week) of exercise can be beneficial, especially in previously sedentary persons.
Type	Regular, purposeful exercise that involves major muscle groups and is continuous and rhythmic in nature is recommended.
Volume	A target volume of ≥500–1000 MET min/week is recommended. Increasing pedometer step counts by ≥2000 steps per day to reach a daily step count ≥7000 steps per day is beneficial. Exercising below these volumes may still be beneficial for persons unable or unwilling to reach this amount of exercise.
Pattern	Exercise may be performed in one (continuous) session per day or in multiple sessions of ≥10 min to accumulate the desired duration and volume of exercise per day. Exercise bouts of ≥10 min may yield favourable adaptations in very deconditioned individuals. Interval training can be effective in adults.
Progression	A gradual progression of exercise volume by adjusting exercise duration, frequency and/or intensity is reasonable until the desired exercise goal (maintenance) is attained. This approach may enhance adherence and reduce risks of musculoskeletal injury and adverse CHD events.
Resistance exercise†	
Frequency	Each major muscle group should be trained on 2–3 days/week
Intensity	60%–70% of the 1RM (moderate to hard intensity) for novice to intermediate exercisers to improve strength.
	≥80% of the 1RM (hard to very hard intensity) for experienced strength trainers to improve strength.
	40%–50% of the 1RM (very light to light intensity) for older persons beginning exercise to improve strength.
	40%–50% of the 1RM (very light to light intensity) may be beneficial for improving strength in sedentary persons beginning a resistance training programme.
	≤50% of the 1RM (light to moderate intensity) to improve muscular endurance.
	20%–50% of the 1RM in older adults to improve power.
Time	No specific duration of training has been identified for effectiveness.
Type	Resistance exercises involving each major muscle group are recommended. A variety of exercise equipment and/or body weight can be used to perform these exercises.
Repetitions	8–12 repetitions are recommended to improve strength and power in most adults. 10–15 repetitions are effective in improving strength in middle-aged and older persons starting exercise 15–20 repetitions are recommended to improve muscular endurance.
Sets	Two to four sets are the recommended for most adults to improve strength and power. A single set of resistance exercise can be effective especially among older and novice exercisers. ≤2 sets are effective in improving muscular endurance.
Pattern	Rest intervals of 2–3 min between each set of repetitions are effective.
	A rest of ≥48 hours between sessions for any single muscle group is recommended.
Progression	A gradual progression of greater resistance and/or more repetitions per set and/or increasing frequency is recommended.
Flexibility exercise†	
Frequency	≥2–3 day/week is effective in improving joint range of motion, with the greatest gains occurring with daily exercise.
Intensity	Stretch to the point of feeling tightness or slight discomfort.
Time	Holding a static stretch for 10–30 s is recommended for most adults. In older persons, holding a stretch for 30–60 s may confer greater benefit. For PNF stretching, a 3–6 s contraction at 20%–75% maximum voluntary contraction followed by a 10–30 s assisted stretch is desirable.
Type	A series of flexibility exercises for each of the major muscle–tendon units is recommended. Static flexibility (active or passive), dynamic flexibility, ballistic flexibility and PNF are each effective.
Volume	A reasonable target is to perform 60 s of total stretching time for each flexibility exercise.
Pattern	Repetition of each flexibility exercise two to four times is recommended. Flexibility exercise is most effective when the muscle is warmed through light to moderate aerobic activity or passively through external methods such as moist heat packs or hot baths.
Progression	Methods for optimal progression are unknown.
Neuromotor exercise training†	
Frequency	≥2–3 days/week is recommended.
Intensity	An effective intensity of neuromotor exercise has not been determined.
Time	≥20–30 min/day may be needed.
Type	Exercises involving motor skills (eg, balance, agility, coordination and gait), proprioceptive exercise training and multifaceted activities (eg, tai ji and yoga) are recommended for older persons to improve and maintain physical function and reduce falls in those at risk for falling. The effectiveness of neuromuscular exercise training in younger and middle-aged persons has not been established, but there is probable benefit.
Volume	The optimal volume (eg, number of repetitions, intensity) is not known.

Continued

Table 1 Continued

The ACSM-AHA primary physical activity recommendations*	
Pattern	The optimal pattern of performing neuromotor exercise is not known.
Progression	Methods for optimal progression are not known.

*ACSM, American College of Sports Medicine; AHA, American Heart Association; extracted from the ACSM Guidelines for Exercising Testing and Prescription, chapter 1, p. 4.[15]
†Extracted from ACSM position stand,[2] table 2, p. 1336.
1 RM, one-repetition maximum; CHD, coronary heart disease; MET, metabolic equivalent of task; PA, physical activity; PNF, proprioceptive neuromuscular facilitation.

develop evidence-based recommendations on PA-promotion and -delivery in the management of people with iA and OA and (3) formulate an educational and research agenda.

These EULAR recommendations for PA in people with iA and OA are for HCPs, patient organisations and policy makers.

METHODS

The EULAR standardised operating procedures for the development of recommendations were followed.[16] The AGREE II-instrument[17] was used to structure this manuscript.

The multidisciplinary TF consisted of a selection of 22 European PA-experts (six medical doctors, including three rheumatologists, one of them specialised in cardiovascular diseases, one GP), one orthopaedic surgeon; nine PTs, a psychologist, an OT, a nurse and a human movement scientist) and three patient representatives. A steering group managed the process (convenor KN, methodologist TVV, expert JB, fellow AR).

During the first TF meeting, definitions of exercise and PA were clarified and the TF agreed to follow the ACSM position stand.[2] The TF agreed that RA and SpA as predominant iA conditions, and HOA/KOA as most relevant for PA recommendations would represent the field of iA and OA, respectively. Clinically relevant questions on the provision of advice and guidance regarding exercise and PA, from which 13 research questions were defined by consensus to guide the subsequent detailed systematic literature review (SLR) (online supplementary table S1).

Two SLRs were performed by AR with the support of two librarians and under the supervision of the convenor and methodologists. The questions were written according to the Population, Intervention, Comparison, Outcome (PICO) format,[18] resulting in two PICOs: (1) on effectiveness, safety and feasibility of PA and (2) on facilitators and barriers towards PA (online-supplementary table S2). For the first PICO, the fellow searched for key meta-analyses (MAs) or systematic reviews (SRs) including randomised controlled trials (RCTs) that investigated the effectiveness of PA-interventions in adults with RA/SpA/HOA/KOA. The SLR was performed in PubMed/Medline, Cochrane Library, Embase, Web of Science, Emcare and PsycInfo, using both MeSH terms and freetext, covering the time frame until 4/2017. For the second PICO, a SLR, covering the time frame until 7/2017, was performed in PubMed/Medline and Cochrane Library including qualitative studies if they described facilitators and barriers regarding PA (including exercise) in people with RA/SpA/HOA/KOA. Experts in the field of RA (EH), SpA (HD), OA (CJ) and behaviour change (KK), respectively, checked if all relevant titles and abstracts were included.

Based on the PICOs, the same author (AR) screened the titles and abstracts according to inclusion and exclusion criteria. Potentially relevant articles were identified and full text versions evaluated. Studies including adults (>18 years) with RA/SpA/HOA/KOA that included PA interventions that met the PH recommendations according to the ACSM principles[2] regarding frequency, intensity and duration for effective interventions were eligible for inclusion. All data extractions were checked by experts from the TF.

Studies measuring the effectiveness of PA-interventions were meta-analysed. These results and detailed descriptions of the methods are reported elsewhere.[19] Studies were used for answering more than one research question if appropriate. For clinical studies evaluating the effectiveness of PA, the Cochrane Risk of Bias Assessment Tool was used to assess selection bias, performance bias, detection bias, attrition bias and reporting bias[20] by two independent assessors (AR, CH). An additional person (KN) helped to resolve any differences in rating between the assessors. The research evidence was categorised according to the Oxford levels of evidence.[21]

During the second TF meeting, the results from the SLR were presented, and the experts developed 'overarching principles' (background statements to preface recommendations) and drafted 10 recommendations through an iterative process of discussion and consensus. After the meeting, the recommendations were collated and sent to the TF members by email, to rate the level of agreement (LoA) independently and anonymously on a 0–10 point scale (0=totally disagree, 10=totally agree). Mean LoA >8 would be considered a 'high' LoA. Furthermore, the TF formulated a research agenda and education agenda based on identified gaps in the evidence.

RESULTS

The search yielded 3471 references, 96 of which were included in the SLR: Four MA/SR[7 22–24] and 66 RCTs[25–93] investigated the effects of exercise interventions, 11 RCTs[94–106] investigated the effects of a PA-promotion-intervention, 11 qualitative studies and literature reviews[3 11 107–115] described barriers and facilitators regarding PA (figure 1A,B). The included RCTs were published between 1985 and 2017. Most information is from studies with low (48%) or unclear (39%) risk of bias (online-supplementary figure S1).

The TF agreed on four overarching principles and 10 recommendations for PA in people with RA/SpA/HOA/KOA based on SLR and expert opinion. High loA was achieved for 9 out of 10 recommendations and 2 recommendations were graded as strength level A. Table 2 summarises the overarching principles and recommendations with their associated level of evidence, strength of recommendation and LoA.

Recommendation 1: PA as integral part of standard care

Given the evidence for effectiveness, feasibility and safety, the PH recommendations for PA are applicable, and thus, PA should be an integral part of standard care for people with RA/SpA/HOA/KOA. PA according to PH recommendations[2] is effective on PA level, physical fitness as well as disease-specific and general outcomes in people with RA/SpA/HOA/KOA (category 1 evidence[16]). Our MA including 16 RCTs[26 35 36 42 43 50 54 56 57 61 70] showed that cardiovascular exercises have a moderate beneficial effect on cardiovascular fitness

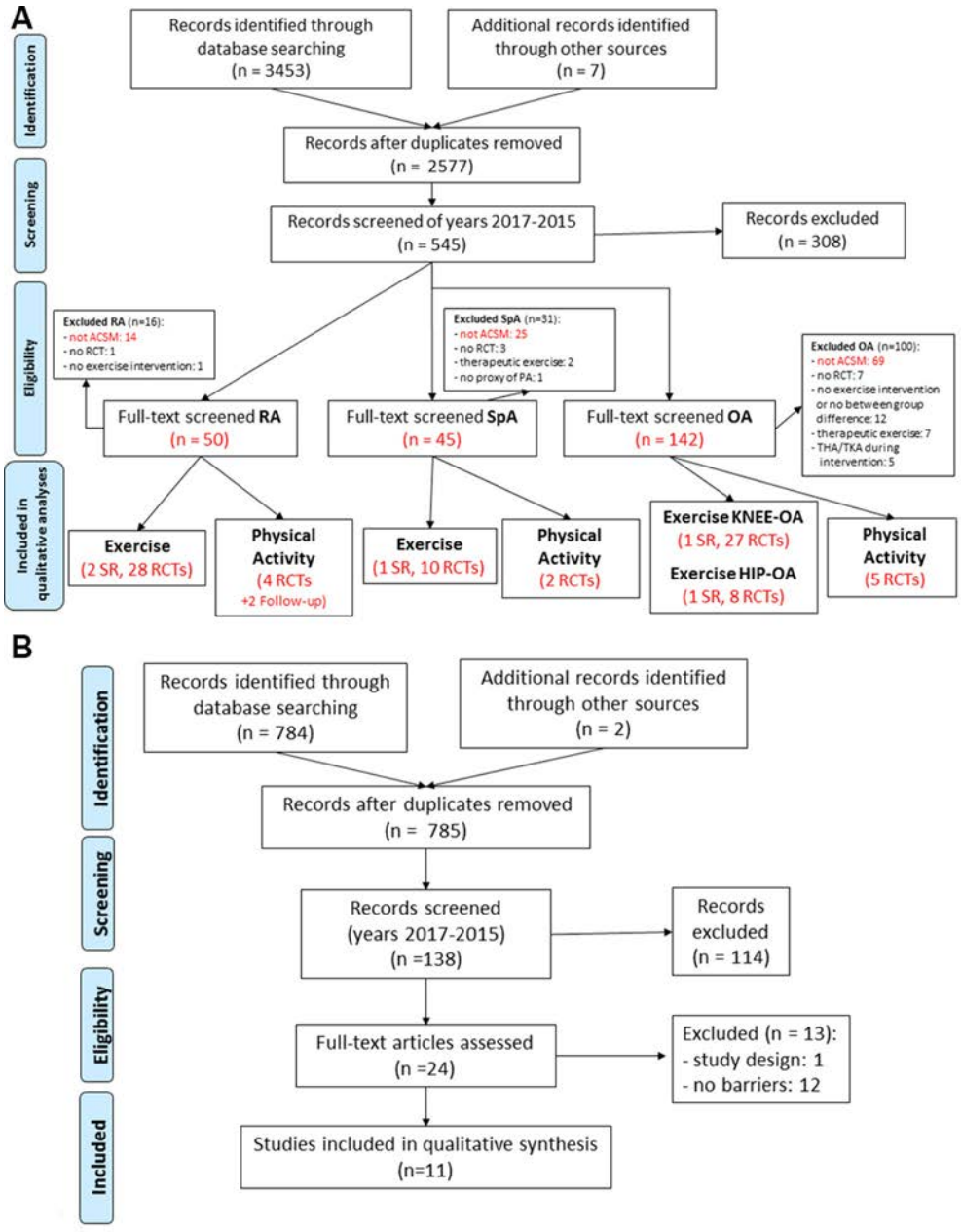

Figure 1 Flowcharts of the literature search related to PICO_1 (A) and PICO_ 2 (B). ACSM, American College of Sports Medicine; OA, osteoarthritis; PA, physical activity; PICO, Population, Intervention, Comparison, Outcome; RA, rheumatoid arthritis; RCT, randomised controlled trial; SpA, spondyloarthritis; SR, systematic review.

(evaluated in VO2 max) in all three conditions. Our MA including 25 RCTs[25 28 31 34 38 39 44 47 49–51 59 62–66 72 75–78 81–83 85 86 88 90 91] showed that muscle strength exercises have a moderate beneficial effect for muscle strength in people with RA and HOA/KOA. Our MA including seven RCTs[52 55 58 78 88 90 116] showed that combined exercises (aerobic or strength exercises plus flexibility exercises) had no effect on flexibility in people with SpA or HOA/KOA. However, exercise conditions, assessments and outcome measures varied greatly. There is no study comparing the effect of flexibility exercises alone versus no exercises. In one RCT,[48] the effect of a neuromotor-exercise programme on neuromotor performance was investigated in people with RA showing a positive effect. Eleven RCTs described the promotion of daily PA. Our MA including six RCTs[95 98 101 102 104 117] applying BCTs for the counselling intervention showed a small beneficial effect.

Feasibility of interventions can be captured by adherence to the intervention or the study protocol.[118] Adherence to interventions (number of sessions attended/total number of sessions) has been reported in 26 RCTs (35%) and the mean adherence was 69% in people with SpA, 71% in people with RA and 79% in people with HOA/KOA. However, the (self-) reported adherence to intervention might be overestimated due to recall bias or social desirability. In 68 RCTs (94%), protocol violations were reported, with approximately 10% of these being disease-related or intervention-related.

PH recommendations for PA can be considered safe. No detrimental effects were reported, rather beneficial effects on disease activity and symptoms in iA.[7] Forty-four per cent of all included RCTs reported on adverse events (AE), of those 62% described no AE and 38% describe minor AE such as transitional exercise related joint or muscle pain.

Table 2 Recommendations for PA and exercise in people with inflammatory arthritis and OA

Overarching principles

1. PA is part of a general concept to optimise health related quality of life.
2. PA has health benefits for people with RA/SpA/HOA/KOA.
3. General PA recommendations, including the four domains (cardiorespiratory fitness, muscle strength, flexibility and neuromotor performance) are applicable (feasible and safe) to people with RA/OA/SpA.
4. The planning of PA requires a shared decision between healthcare providers and people with RA/SpA/HOA/KOA, which takes people's preferences, capabilities and resources into account.

Recommendations	Category of evidence	Strength of recommendation	Level of Agreement mean (SD) Median (Range)
1. Promoting PA consistent with general PA recommendations should be an integral part of standard care throughout the course of disease in people with RA/SpA/HOA/KOA.	1B	A	9.81 (0.39) 10 (9–10)
2. All healthcare providers involved in the management of people with RA/SpA/HOA/KOA should take responsibility for promoting PA and should cooperate, including making necessary referrals, to ensure that people with RA/SpA/HOA/KOA receive appropriate PA-interventions.	4	D	9.14 (0.98) 9 (7–10)
3. PA interventions should be delivered by healthcare providers competent in their delivery to people with RA/SpA/HOA/KOA.	4	D	8.86 (1.48) 10 (5–10)
4. Healthcare providers should evaluate the type, intensity, frequency and duration of the people's actual PA by means of standardised methods to identify which of the four domains of general PA recommendations can be targeted for improvement.	3	C	9.05 (1.04) 9 (6–10)
5. General and disease-specific contraindications for PA should be identified and taken into account in the promotion of PA.	4	D	9.10 (1.41) 10 (5–10)
6. PA interventions should have clear personalised aims, which should be evaluated over time, preferably by use of a combination of subjective and objective measures (including self-monitoring when appropriate).	4	D	9.05 (1.25) 9 (5–10)
7. General and disease-specific barriers and facilitators related to performing PA, including knowledge, social support, symptom control and self-regulation should be identified and addressed.	3	C	9.19 (1.13) 10 (6–10)
8. Where individual adaptations to general PA recommendations are needed, these should be based on a comprehensive assessment of physical, social and psychological factors including fatigue, pain, depression and disease activity.	4	D	9.24 (0.86) 9 (7–10)
9. Healthcare providers should plan and deliver PA interventions that include the behavioural change techniques self-monitoring, goal setting, action planning, feedback and problem solving.	1A	A	9.48 (0.79) 10 (7–10)
10. Healthcare providers should consider different modes of delivery of PA (eg, supervised/not-supervised, individual/group, face-to-face/online, booster strategies) in line with people's preferences.	4	D	9.00 (1.30) 9 (5–10)

HOA, hip osteoarthritis; KOA, knee osteoarthritis; OA, osteoarthritis; PA, physical activity; RA, rheumatoid arthritis; SpA, spondyloarthritis.

Recommendation 2: Responsibility for PA promotion

All HCPs should have a responsibility for PA promotion and collaborative working that facilitate a close cooperation between different professions to support appropriate disease management. This statement was based on the finding that 66% of the included studies reported the profession of the HCP providing the intervention, of which 75% were PTs.[25 31 36 40 44 45 48 50 53 55 58 61 64–66 70 73–79 81 84 87 88 91 94 96 101–105 119 120] However, the functions and responsibilities of HCPs vary across Europe.[121 122] Therefore, the TF agreed that PA advice should be provided by all HCPs.

Recommendation 3: Delivery of PA

The delivery of interventions should be performed by HCPs competent in the field of PA principles and rheumatic conditions. The reporting of training on PA guidelines was rare. One study[59] described a '4 hours education session on cardiovascular training', others described the instructing person as 'trained'[25 50 69 70 84 88 123] or 'experienced'.[31 49 76 77 88] Some studies with focus on the promotion of daily PA described training sessions on behaviour change skills like Motivational Interviewing.[94 96 104]

Recommendation 4: Evaluation of PA

The PA level (active or non-active) and the exercise domains (cardiorespiratory, muscle strength, flexibility and neuromotor) should be routinely assessed. Of 11 trials investigating the effect of PA promotion interventions, three RCTs[94 96 105] described baseline screening to distinguish between active and non-active persons before starting the tailored PA-intervention. Specific tools are needed to assess each domain.[15,p. 68]

Recommendation 5: General and disease-specific contraindications

Tools for specific contraindications (CIs) were found;[15 94 124] however, available general or national guidelines defining absolute or relative CIs should be followed as a priority.

Recommendation 6: Personalised aims and evaluation

The PA-interventions should be based on individual aims, which should be regularly evaluated. This can be done by PA assessments and any other assessments related to the individual aims. As PA assessments, performance–based tests, patient-reported outcome measures (eg, SQUASH,[104] PASE[94]) and self-monitoring

tools (eg, wearables such as Fitbit,[100] pedometer[99] or accelerometer[101]) were identified. However, we did not evaluate the validity and reliability of the assessments applied.

Recommendation 7: General and disease-specific barriers and facilitators

General and disease-specific barriers (that are not CI per se) and facilitators should be addressed as described in 11 studies.[11 107–115 125] Disease-specific barriers included lack of knowledge about the disease, lack of knowledge about safe exercising (both in people with iA/OA and HCPs) and symptoms like pain, fatigue, stiffness, reduced mobility, fear of flare-ups or causing damage. Disease-specific facilitators included positive impact of exercise in symptoms or disease control, information about disease and correct exercising, the use medication for pain prior to exercising, using self-regulation techniques, supportive, but not controlling encouragement from HCPs and a supportive social background.

Recommendation 8: Individual adaptations to PA following individualised assessment

Adaptations to PA should be made on a comprehensive individual assessment. However, no evidence on the necessity of general adaptations in people with RA/SpA/HOA/KOA was found. In some RA studies the '24 hour-rule' was applied, that is, the exercise intensity was reduced when the increased pain persisted for more than 24 hours.[23 40 50] ACSM provides adaptations to exercise testing in people with arthritis (eg, no high-intensity testing if acute inflammation) and training such as exercising when pain is typically least severe or to train carefully in order to reduce risk of associated injuries, although no clear evidence that high-impact activities cannot be engaged during active inflammation.[15, pp. 298–301] Individual disease-related barriers (eg, symptoms) may determine these adaptations.

Recommendation 9: Behaviour change techniques

BCTs should be an integral component of PA-interventions. Several behaviour change theories were used in PA promotion interventions in the field of RA and HOA/KOA,[4 126] but the reporting was poor. Future research based on theories in design, evaluation and interpretation of findings is needed.

A meta-analysis of six RCTs[94 95 98 101 102 104] investigating the effects of a PA promotion intervention according to general PA recommendations[2] and based on counselling interventions that apply BCTs showed a small beneficial effect on PA level.[19] Counselling interventions show a small beneficial effect if BCTs are applied.[19]

Recommendation 10: Modes of delivery

HCPs should consider the whole range of modes to deliver interventions. No evidence on the superiority of specific delivery modes was found. The delivery modes of PA-interventions vary considerably and are mostly described as 'land-based and/or water-based' and 'supervised and individualised', the latter usually applied to group settings. As booster strategies phone calls,[36 96 98 105] devices (eg, pedometer,[98 99] wearable[100 101]), home visits,[63 70] log book,[36 51 76 98] web-based instructions,[127] written material,[51 54 103] visual instructions (eg, video[103]) were reported.

Research and education agendas

Based on the gaps identified in the literature, the TF discussed and proposed a research agenda (box 1) with the prioritised research topics and an education agenda (box 2) with topics for education and training in PA promotion for HCPs. Evidence on impact of (reducing) sedentary behaviour emerged as an important future research topic.

Box 1 Research agenda for physical activity (PA) in people with inflammatory arthritis and osteoarthritis

1. To evaluate the long-term effectiveness of PA at different intensities and types and monitoring of adverse events (AE).
2. To evaluate links between PA behaviour and disease-specific outcomes.
3. To evaluate the long-term effectiveness of sedentary behaviour reduction, including the monitoring of AE.
4. To evaluate links between sedentary behaviour and disease-specific outcomes.
5. To identify which PA-intervention strategies work best to increase PA level and adherence in various subgroups.
6. To identify markers of response and non-response to PA treatment.
7. To identify disease-specific contraindications on different exercise domains (cardiovascular, strength, flexibility, neuromotor).
8. To further develop and evaluate strategies to reduce and monitor a change in sedentary behaviour.
9. To develop PA-interventions targeting all exercise dimensions simultaneously with special focus on feasibility.
10. To evaluate and recommend valid PA assessments feasible for the use in clinical practice.
11. To study how to facilitate PA behaviour change immediately from screening onwards and how to address facilitators and barriers.
12. To identify facilitators and barriers of healthcare providers towards applying the PA recommendations.
13. To perform long-term effectiveness trials on combined interventions including other health behaviours.

DISCUSSION

The TF agreed on 4 overarching principles and 10 recommendations for PA in people with RA/SpA/HOA/KOA, which integrated the perspectives of the TF members from different professional, cultural and personal backgrounds. This led to a broad consensus on the principles and recommendations within the group and ought to foster its feasibility and practicability in the diverging health systems across Europe.

Box 2 Education agenda for physical activity (PA) in people with inflammatory arthritis and osteoarthritis

1. Increase knowledge about PA among health professionals (HPs), physicians and people with inflammatory arthritis and osteoarthritis.
2. Increase HPs' and physicians' skills in communicating the role of PA in managing general health and disease-specific issues.
3. Include knowledge and skills on PA promotion in all HPs' and physicians' undergraduate training curricula.
4. Develop a EULAR training module on PA for HPs and rheumatologists.
5. Propose a session on PA at every EULAR congress.
6. Develop education materials for people with inflammatory arthritis and osteoarthritis.

The LoA on the recommendations among the TF members was very high. The only exception was about the competency of HCP, which may be due to country specific differences in the availability of HCP competent in PA promotion.

Although the PH recommendations for PA are well established, the feasibility and applicability of these for people with iA and OA has not been assessed so far. Accordingly, the development of the recommendations was needed. Expectedly, they emphasise the importance of PA and will guide future PA-interventions in people with chronic rheumatic conditions.

PA promotion is a behavioural intervention and therefore BCT are central components in PA-interventions. Identifying effective and cost-effective BCT within PA promotion intervention in people with chronic conditions is currently a hot topic in research and for example a research priority of the National Institute for Health and Care Excellence, UK.[128]

We decided a priori to include only studies fulfilling the PH recommendations for PA according to ACSM principles.[2] This was a far-reaching decision, which allowed drawing stronger conclusions on the effectiveness and especially the safety of correctly dosed PA-interventions. We followed a pragmatic search strategy with the plan to answer all RQs related to PICO 1 with findings of available SR/MA. However, there were no SR/MA on all exercise dimensions and all conditions available; this led to extracting single RCTs from high-quality SR/MA. This, however, excluded high-quality reviews (eg, Cochrane reviews) and RCTs that did not fulfil the ACSM principles and affected the potential to report 1A evidence according to Oxford levels of evidence.[21] Furthermore, only one reviewer screened the abstracts and decided on unclear abstracts together with a second reviewer, which is not fully in line with standard procedures of a SLR.[129] However, we applied a double-check by experts to ensure that no relevant studies were missed.

A major problem for data extraction and interpretation was that the reporting of interventions in most studies was incomplete. Manuscripts that applied TIDieR[130] (Template for Intervention Description and Replication) guidelines reported more precisely the PA-interventions and substantially improved the objective evaluation of the PA-interventions.

For the research questions related to the effectiveness and safety of PA-interventions and BCT, the PICO scheme was applied, resulting in 1A level of evidence. All other research questions we had to answer in a descriptive way limiting the level of evidence to 3 to 4. However, this limitation is due to the nature of the research questions. Nevertheless, the qualitative studies may provide valuable insight into important PA-related fields, such as assessments, barriers and facilitators, PA promotion strategies.

The recommendations focused on the conditions RA/SpA/HOA/KOA, the most prevalent RMD conditions to increase the generalisability and applicability of the recommendations. However, large heterogeneity between these conditions may limit the precision of the recommendations. Therefore, additional disease-specific recommendations are desirable. In addition, not all subconditions were considered and represented (eg, juvenile arthritis).

The research agenda highlights several areas where scientific evidence is lacking. It is a clear ambition to implement these recommendations into daily clinical routine. Due to the different health systems across Europe, development and evaluation of target group and culture-specific implementation strategies are needed and should involve all stakeholders.

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EXTENDED REPORT

Inadequate response to treat-to-target methotrexate therapy in patients with new-onset rheumatoid arthritis: development and validation of clinical predictors

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ABSTRACT

Objective To identify and validate clinical baseline predictors associated with inadequate response (IR) to methotrexate (MTX) therapy in newly diagnosed patients with rheumatoid arthritis (RA).

Methods In U-Act-Early, 108 disease-modifying antirheumatic drug (DMARD)-naive patients with RA were randomised to initiate MTX therapy and treated to target until sustained remission (disease activity score assessing 28 joints (DAS28) <2.6 with four or less swollen joints for ≥24 weeks) was achieved. If no remission, hydroxychloroquine was added to the treatment regimen (ie, 'MTX+') and replaced by tocilizumab if the target still was not reached thereafter. Regression analyses were performed to identify clinical predictors for IR, defined as needing addition of a biological DMARD, to 'MTX+'. Data from the treatment in the Rotterdam Early Arthritis Cohort were used for external validation of the prediction model.

Results Within 1 year, 56/108 (52%) patients in U-Act-Early showed IR to 'MTX+'. DAS28 (adjusted OR (OR_{adj}) 2.1, 95% CI 1.4 to 3.2), current smoking (OR_{adj} 3.02, 95% CI 1.1 to 8.0) and alcohol consumption (OR_{adj} 0.4, 95% CI 0.1 to 0.9) were identified as baseline predictors. The area under the receiver operator characteristic curve (AUROC) of the prediction model was 0.75 (95% CI 0.66 to 0.84); the positive (PPV) and negative predictive value (NPV) were 65% and 80%, respectively. When applying the model to the validation cohort, the AUROC slightly decreased to 0.67 (95% CI 0.55 to 0.79) and the PPV and NPV to 54% and 80%, respectively.

Conclusion Higher DAS28, current smoking and no alcohol consumption are predictive factors for IR to step-up 'MTX+' in DMARD-naive patients with new-onset RA.

Trial registration NCT01034137; Post-results, ISRCTN26791028; Post-results.

INTRODUCTION

The ultimate treatment goal of rheumatoid arthritis (RA) is remission and with that prevention of structural joint damage and preservation of physical function. Current international guidelines endorse to start treatment with conventional synthetic disease-modifying antirheumatic drugs (csDMARDs) in combination with short-term glucocorticoids (GCs) as bridging therapy early in the disease course to achieve clinical remission.[1 2] Methotrexate (MTX) is considered as initial therapy because of its favourable efficacy/toxicity balance and long-term safety profile.[2–4] However, about 30%–50% of the patients eventually need additional treatment to achieve the treatment goal.[5 6] As early response to treatment strongly correlates with long-term clinical outcomes,[7–9] starting combination therapy with a biological DMARD (bDMARD) in patients less likely to achieve long-term response to MTX treatment (eg, those with autoantibody positivity, high disease activity, early presence of joint damage) could optimise the clinical outcome in these difficult-to-treat patients. Therefore, more reliable predictors are needed to guide clinicians in choosing the initial therapy in patients with early RA. The aim of this study was to identify clinical baseline predictors associated with inadequate response (IR) to MTX therapy and to validate these in another cohort with a different case mix. Furthermore, we also evaluated if those predicted as having IR to MTX therapy would have responded to treatment if combination therapy with a bDMARD or several csDMARDs had initially been started.

METHODS

Development sample: U-Act-Early

The U-Act-Early was a 2-year, multicentre, randomised, double-blind, placebo-controlled strategy trial evaluating sustained remission (disease activity score in 28 joints (DAS28) <2.6 with ≤4 swollen joints for ≥24 weeks) in patients with newly diagnosed RA initiating tocilizumab (TCZ), MTX or their combination (ClinicalTrials.gov no. NCT01034137).[10] Adults with early RA meeting the 1987/2010 classification criteria[11 12] and active disease (DAS28 ≥2.6) were eligible to participate. MTX (oral) was started at 10 mg/week and increased with 5 mg every 4 weeks up to a maximum dose of 30 mg/week until remission or the maximum tolerable dose. If remission was not achieved at week 20, hydroxychloroquine (HCQ) was added to the regimen. If 12 weeks thereafter, remission still was not reached, HCQ was discontinued and patients randomised to initiate TCZ or MTX switched to TCZ+MTX therapy. Oral GCs were not permitted during the first 3 months of the study; later it was allowed, limited to once per year

for a maximum of 2 weeks (≤10 mg prednisone/day). The study design has been described in detail.[10] For identifying clinical predictors at baseline associated with IR to MTX therapy, data were used from the patients (n=108) initiating MTX. Furthermore, to evaluate, by applying the prediction model, if those with a poor prognostic outcome would have responded to initiating TCZ+MTX therapy, we also used data of patients initiating this strategy (n=106).

Validation sample: tREACH

In the treatment in the Rotterdam Early Arthritis Cohort (tREACH), a multicentre, randomised, single-blinded (physician), treat-to-target trial, patients had been stratified into three strategy groups according to their likelihood of progression to persistent arthritis (ISRCTN26791028).[13] Patients were eligible if age ≥18 years, arthritis of ≥1 joints and symptom duration ≤1 year.[14] We used data from those patients (n=83) fulfilling the RA classification criteria and initiating (oral) step-up MTX (week 1, 15 mg/week; week 2, 20 mg/week; week 3, 25 mg/week) with GCs in a tapering scheme (weeks 1–4, 15 mg/day; weeks 5–6, 10 mg/day; weeks 7–8, 5 mg/day; weeks 9–10, 2.5 mg/day prednisone-equivalent). If after 3 months DAS44 was ≥2.4, medication was intensified to MTX plus anti-tumour necrosis factor-alpha (TNF-α) therapy. The need to initiate a bDMARD within the first year was in both the development and validation sample classified as IR to the MTX strategy (designated as 'MTX+'). In addition, we used data from patients in tREACH who initiated MTX with sulfasalazine (SSZ) and HCQ (n=164), to evaluate, by applying the prediction model, if this strategy would have led to better treatment responses in those initiating MTX, but with a poor prognostic outcome.

Study variables

As baseline parameters were evaluated: age, gender, body mass index, current smoking status, alcohol consumption, symptom duration, anticyclic citrullinated peptide (pos/neg), rheumatoid factor (pos/neg), C reactive protein (CRP) and erythrocyte sedimentation rate (ESR). Radiographic scoring of hands and feet was done with the Sharp/van der Heijde score (SHS), consisting of an erosion and joint space narrowing (JSN) score. Disease activity was evaluated by tender joint count (TJC), swollen joint count (SJC), Visual Analogue Scale scores, Clinical Disease Activity Index (CDAI), Simplified Disease Activity Index (SDAI) and DAS28. The Health Assessment Questionnaire (HAQ) was used to assess physical function.

Statistical analyses

Candidate predictors for IR to 'MTX+' were identified in the development sample by using univariate regression preselection (p≤0.15); collinearity between variables was assessed with the Pearson correlation coefficient (PCC). Prior to the preselection, missing data were imputed using multiple imputation (n=20 datasets); complete case analyses (CCA) were additionally performed. Univariately selected variables were included in a multivariable model, which was further reduced by stepwise backward selection (p≤0.10). The association between the outcome and the predictors is expressed in ORs with 95% CI; dose-related effects were examined in logistic regression analyses by categorising the predictor into quartiles. To visualise the predicted risk of IR to 'MTX+', a prediction matrix was developed using the variables of the final multivariable prediction model. The diagnostic performance of the model was assessed using the positive (PPV) and negative predictive value (NPV); discrimination was evaluated by the area under the receiver operator characteristic curve (AUROC), 'excellent' (0.9–1.0), 'good' (0.8–0.9), 'fair' (0.7–0.8), 'poor' (0.6–0.7) and 'fail' (0.5–0.6),[15] and calibration by the Hosmer-Lemeshow test and by plotting calibration curves with observed versus predicted probabilities. To correct our model for overfitting (optimism), we performed an internal validation (bootstrapping procedure, n=250) to calculate a shrinkage factor for adjusting the regression coefficients (ie, slope); the intercept was re-estimated using the offset procedure. Thereafter, the adjusted linear predictor was applied to the validation sample to evaluate discrimination and calibration of the prediction model in a different population (external validation). Statistical analyses were performed using SPSS (V.20, Chicago, Illinois, USA) and R V.3.3.1 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Table 1 shows the baseline characteristics of the patients included in the development and validation samples. Significant differences were found between the cohorts in the proportion consuming alcohol (60% vs 75%, p=0.008), symptom duration (median (IQR) 27 (15–46) vs 140 (93–188), p<0.001), TJC28 (median (IQR) 7 (4–10) vs 5 (2–9), p=0.010), SHS (median (IQR) 0 (0–1) vs 1 (0–3), p<0.001), erosion score (median (IQR) 0 (0–0) vs 1 (0–1), p<0.001) and JSN score (median (IQR) 0 (0–0) vs 0 (0–2), p<0.001). Within 1 year, 56/108 (52%) patients in the U-Act-Early trial showed IR to step-up 'MTX+' for various reasons: TCZ was added to MTX according to treatment protocol (n=42), withdrawal from study because of adverse events (n=4) or inefficacy (n=10). In those

Table 1 Baseline patient characteristics of the two study samples

	U-Act-Early, n=108 (development sample)	tREACH, n=83 (validation sample)	P values
Age, years	52 (14)	53 (13)	0.59
BMI, kg/m^2	26 (4)	27 (5)	0.50
Current smoking	31 (29%)	26 (31%)	0.86
Female gender	69 (64%)	59 (71%)	0.29
Alcohol consumption (≥1 unit per week)	65 (60%)	62 (75%)	0.008
Symptom duration, days	27 (15–46)	154 (93–187)	<0.001
Anti-CCP positive	85 (79%)	68 (82%)	0.58
RF positive	87 (81%)	67 (80%)	0.98
SJC28 (range 0–28; 28=maximum)	6 (3–10)	5 (2–9)	0.21
TJC28 (range 0–28; 28=maximum)	7 (4–10)	5 (2–9)	0.010
ESR, mm/hour	25 (12–46)	23 (14–38)	0.72
CRP, mg/L (normal <10 mg/L)	10 (3–24)	11 (5–26)	0.69
DAS28 (range 0–9.4; 9.4=maximum)	5.1 (1.2)	4.8 (1.3)	0.052
HAQ (range 0–3; 3=maximum)	1.1 (0.6)	1.1 (0.7)	0.81
Sharp/van der Heijde score (range 0–448; 448=maximum)	0 (0–1)	1 (0–3)	<0.001
Erosion score (range 0–280; 280=maximum)	0 (0–0)	1 (0–1)	<0.001
JSN score (range 0–168; 168=maximum)	0 (0–0)	0 (0–2)	<0.001

Data are n (%), mean (SD) or median (IQR).
BMI, body mass index; CCP, cyclic citrullinated peptide; CRP, C reactive protein; DAS28, disease activity score assessing 28 joints; ESR, erythrocyte sedimentation rate; HAQ, Health Assessment Questionnaire; JSN, joint space narrowing; RF, rheumatoid factor; SJC, swollen joint count; TJC, tender joint count.

switching to TCZ+MTX therapy, 32/42 patients (76%) were able to achieve sustained remission. In the tREACH trial, 37/83 (45%) switched within 1 year to a TNF-α inhibitor; of these 37 of whom DAS data were available (n=34), 20 patients (59%) achieved the treatment target (ie, DAS44 <2.4) within 6 months after switching. The mean (SD) MTX dosage used in U-Act-Early and tREACH was respectively 19 (10) and 21 (6) mg/week.

Predictors of inadequate response to 'MTX+'

Univariate preselection of baseline parameters identified age (p=0.10), current smoking (p=0.10), alcohol consumption (p=0.026), ESR (p<0.001), CRP (p=0.011), TJC28 (p=0.07), SJC28 (p=0.049), DAS28 (p<0.001), SDAI (p=0.002), CDAI (p=0.046) and HAQ (p=0.003) as predictors (table 2). CRP and ESR showed collinearity (PCC 0.72, p<0.001) and because DAS28 was calculated using ESR, CRP was kept in the model. Similarly, DAS28, CDAI and SDAI showed collinearity (PCC ≥0.7, p<0.001). Because CDAI does not include an acute phase reactant, it was kept in the model in addition to DAS28: SDAI was excluded as it contained missing data (12%). Logistic regression analysis identified DAS28 (adjusted OR (OR_{adj}) 2.1, 95% CI 1.4 to 3.2; p<0.001), current smoking (OR_{adj} 3.0, 95% CI 1.1 to 8.0; p=0.027) and alcohol consumption (OR_{adj} 0.4, 95% CI 0.1 to 0.9; p=0.021) in the development sample as predictors for IR to 'MTX+'; CCA yielded the same results. For smoking, we found a dose-related effect on the risk of IR: for 1–9 cigarettes/day, the OR was 0.46 (95% CI 0.08 to 2.49); for 10–14 cigarettes/day, it was 1.14 (95% CI 0.2 to 6.0); for 15–19 cigarettes/day, it was 4.0 (95% CI 0.8 to 20); and for ≥20 cigarettes/day, it was 9.11 (95% CI 1.1 to 76); only for this latter category the OR was statistically significant (p=0.042). For alcohol consumption, no dose-related effect was present; only consumption of 1–2 glasses/week was significantly associated with a decreased risk of IR to 'MTX+', when compared with non-consumers (OR 0.10, 95% CI 0.02 to 0.50; p=0.005). The discriminative ability of the final prediction model was 'fair' with an AUROC of 0.75 (95% CI 0.66 to 0.84); we found no statistically significant difference (p=0.96) between the observed and predicted probabilities (figure 1). For predictive accuracy of the model, we used a NPV (ie, predicted risk of IR) >80% as cut-off. Our model predicted that 77/108 (71%) patients would have IR to 'MTX+', of whom 50 (65%, PPV) were correctly classified, and that 31/108 (29%) patients would have no IR, of whom 25 (80%, NPV) were correctly classified; sensitivity: 0.89, specificity: 0.52.

Table 2 Predictors of IR to 'MTX+' therapy

	OR 95% CI, univariate analysis	OR 95% CI*, multivariable analysis
Demographic		
Age	1.03 (0.99 to 1.05)	
BMI	1.05 (0.96 to 1.15)†	
Female gender	1.13 (0.52 to 2.49)†	
Alcohol consumption‡	0.41 (0.18 to 0.90)	0.35 (0.14 to 0.85)§
Current smoking	2.07 (0.88 to 4.90)	3.02 (1.14 to 8.03)§
Anti-CCP positive	0.94 (0.38 to 2.38)†	
RF positive	1.30 (0.50 to 3.37)†	
Disease activity		
DAS28	2.04 (1.38 to 3.02)	2.09 (1.37 to 3.19)§
SDAI*	3.92 (1.77 to 8.64)	
CDAI	1.05 (1.00 to 1.09)	
TJC28*	1.70 (0.95 to 3.02)	
SJC28*	1.74 (1.00 to 3.02)	
HAQ	2.76 (1.34 to 5.71)	
VAS-PGA	1.00 (0.98 to 1.02)†	
VAS-EGA	1.01 (0.99 to 1.04)†	
VAS-GH	1.00 (0.99 to 1.02)†	
Laboratory		
ESR*	3.09 (1.79 to 5.32)	
CRP*	1.49 (1.10 to 2.02)	
Imaging		
Sharp/van der Heijde score*	1.41 (0.80 to 2.49)†	
Erosion score*	1.47 (0.66 to 3.27)†	
JSN score*	1.57 (0.77 to 3.20)†	

Effect estimate expressed in OR with 95% CI, representing the increased risk per unit. 'MTX+' including second-line disease-modifying antirheumatic drug therapy.
*Natural log transformed.
†Not significant (p>0.15) in univariate analyses.
‡≥1 unit per week.
§Adjusted ORs.
BMI, body mass index; CCP, cyclic citrullinated peptide; CDAI, Clinical Disease Activity Index; CRP, C reactive protein; DAS28, disease activity score assessing 28 joints; ESR, erythrocyte sedimentation rate; EGA, evaluator global assessment; GH, global health; HAQ, Health Assessment Questionnaire; IR, inadequate response; JSN, joint space narrowing; PGA, patient global assessment; RF, rheumatoid factor; SDAI, Simplified Disease Activity Index; SJC28, swollen joint count assessing 28 joints; TJC28, tender joint count assessing 28 joints; VAS, Visual Analogue Scale.

Prediction matrix

A risk matrix, categorised by DAS28, alcohol consumption and smoking status, was created to visualise the predictive probability of IR to 'MTX+' (figure 2). To calculate the predicted risk in the development sample, DAS28 was made categorical: 2.6≤3.2 (low), 3.2<5.1 (moderate) and ≥5.1 (high). In general, the risk of IR is visibly lower for patients with a low disease activity compared with those with a higher disease activity. Non-smokers who are alcohol consumers and who have a low disease activity at baseline have the lowest probability (7%) of IR to 'MTX+'; patients currently smoking with high disease activity and who are also alcohol non-consumers have the highest risk (90%) of IR. The prediction matrix, stratified by the main predictors, shows nearly a 12.5-fold risk difference between the subgroups.

External validation

The final, internally validated model was applied to the validation sample, yielding an AUROC of 0.68 (95% CI 0.56 to 0.80), indicative of a slightly lower but similar discriminative ability as in the development sample (figure 1). Similar dose-related effects of smoking and alcohol consumption as in the development sample were observed (data not shown). No significant difference (p=0.28) was found between observed outcomes versus predicted probabilities, indicating good calibration of the model. At a cut-off of NPV >80%, the model predicted in the validation sample that 61/83 (73%) patients would have IR to 'MTX+', of whom 33 (54%, PPV) were correctly classified, and that 22/83 (27%) patients would have no IR, of whom 18/22 (82%, NPV) were correctly classified; sensitivity: 0.88, specificity: 0.38.

Clinical practice

When applying our prediction model to the TCZ+MTX arm of U-Act-Early, 56 patients were predicted to have had IR if they would have initiated 'MTX+' therapy (cut-off NPV >80%), but in fact 53 (95%) of them achieved remission on TCZ+MTX (figure 3). In the MTX+SSZ+HCQ arm of tREACH, 38 patients were predicted to have had IR if they would have initiated

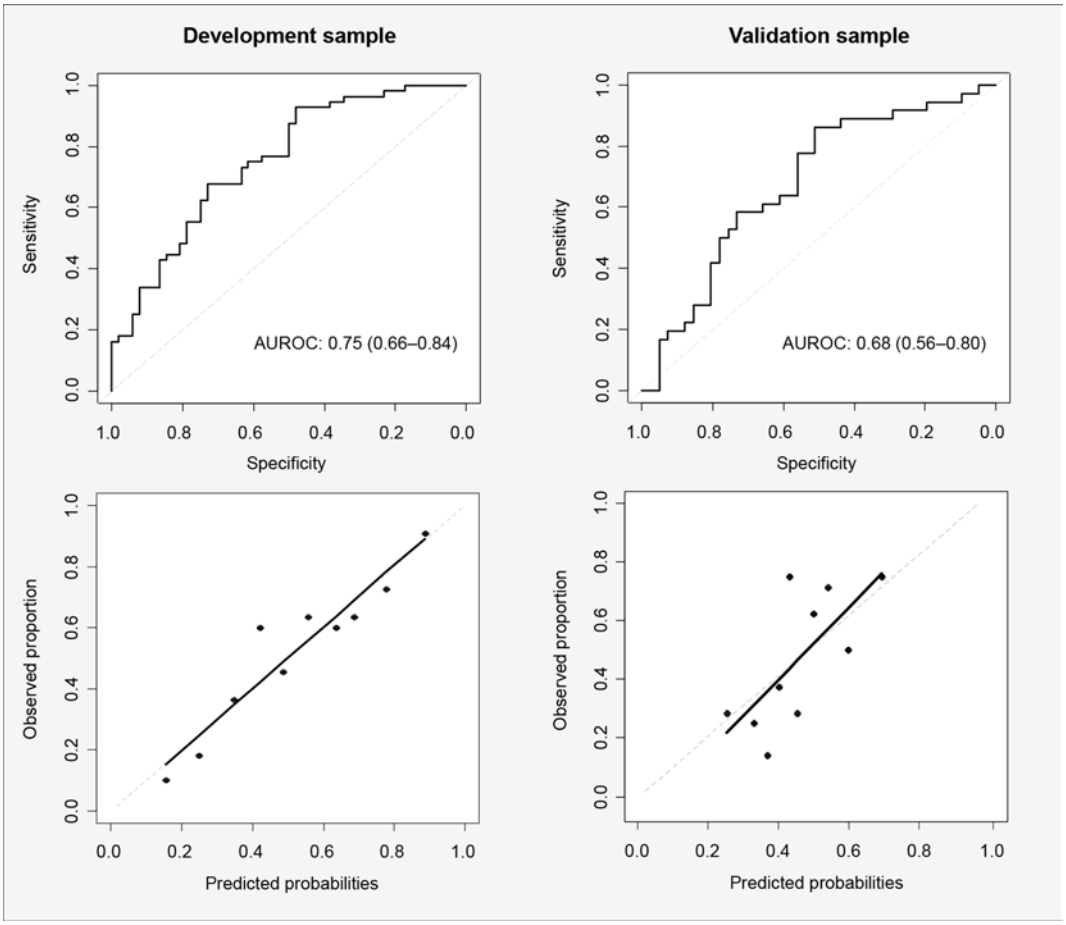

Figure 1 ROC curves and calibration plots of predicting IR to 'MTX+' therapy in the development (left) and validation (right) sample. Dots present deciles of predicted probabilities; dotted diagonal lines in calibration curves indicate perfect calibration between observed and predicted probabilities. AUROC, area under the receiver operator characteristic curve; IR, inadequate response; MTX, methotrexate; ROC, receiver operator characteristics. 'MTX+' including second-line disease-modifying antirheumatic drug therapy.

'MTX+', using the same cut-off, of whom 21 (55%) actually achieved remission. Thus, if our prediction model was to be used in clinical practice, 55%–95% of patients predicted to end up with IR to 'MTX+' would respond to initiation of combination treatment (ie, TCZ+MTX or MTX+SSZ+HCQ) and 5%–45% would not respond. If patients were to receive medication according to this prediction model, 27/108 (25%) initiating TCZ+MTX would have received unnecessary medication as 'MTX+' would have been sufficient to achieve remission; 6/108 (7%) patients initiating 'MTX+' would be undertreated, based on the model, as they would not achieve remission without addition of a bDMARD. When evaluating the prediction model for the entire MTX strategy of U-Act-Early, thus including TCZ therapy, 56% of the patients predicted to have an IR to 'MTX+' was not in remission at 1 year. This demonstrates the reduced chance of adequate disease control in these patients

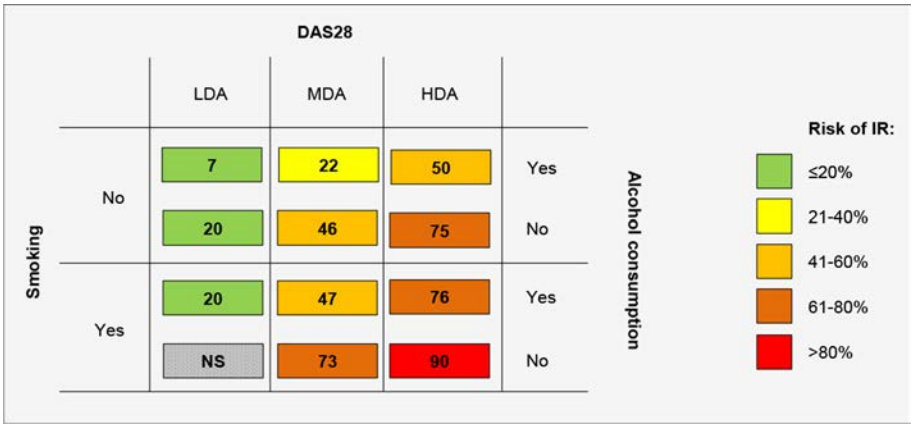

Figure 2 Risk matrix predicted probability (%) of IR to 'MTX+' therapy. Alcohol consumption, yes: ≥1 unit per week. DAS28, disease activity assessing 28 joints; HDA, high disease activity; IR, inadequate response; LDA, low disease activity; MDA, moderate disease activity; NS, not specified, insufficient data to calculate predicted probability.

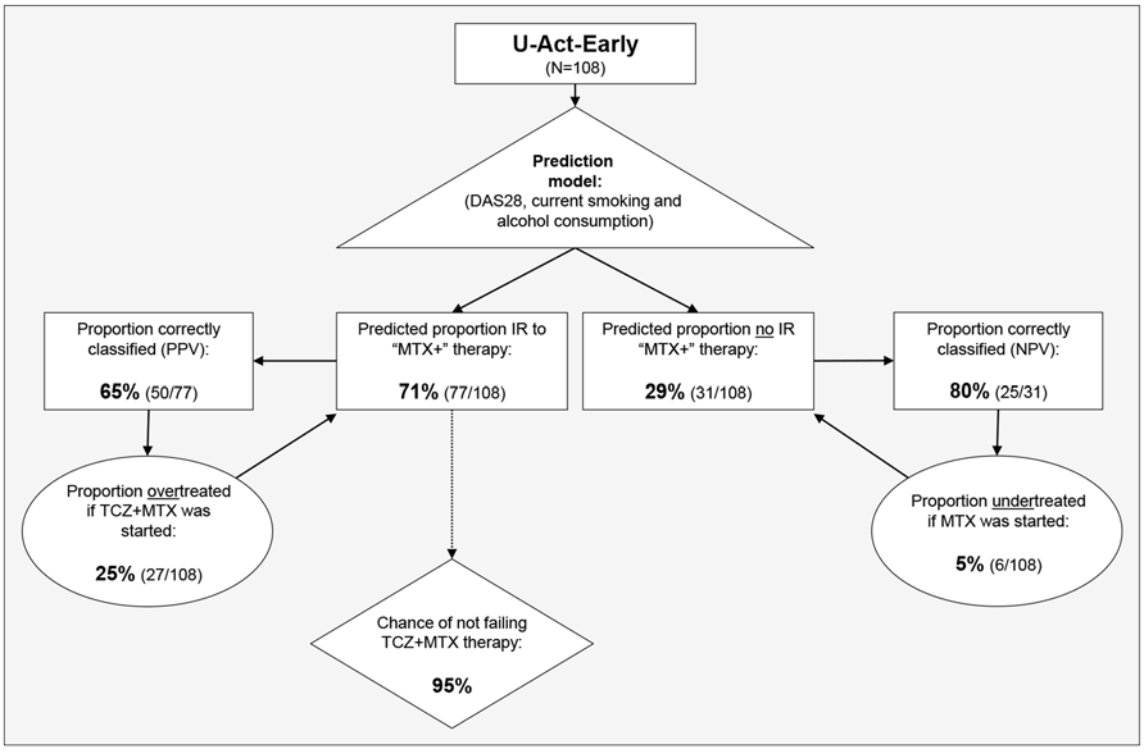

Figure 3 Accuracy of prediction model if used in clinical practice. DAS28, disease activity score assessing 28 joints; IR, inadequate response; MTX, methotrexate; NPV, negative predictive value; PPV, positive predictive value; TCZ, tocilizumab.

with predicted worse outcome, when starting MTX alone, even though in a later phase TCZ could be added.

DISCUSSION

We developed and validated a model for the prediction of IR to treat-to-target 'MTX+' within the first year in DMARD-naive patients with very early RA. Higher DAS28, current smoking and no alcohol consumption were associated with an increased risk of IR to 'MTX+', at a fair discriminative ability with good calibration between observed and predicted probabilities. To our knowledge, this is the first study reporting on clinical baseline predictors for response to 'MTX+' validated in a second cohort treated in another care setting. Additionally, our findings provide further evidence that altering the patients' lifestyle can optimise the treatment outcome. Especially smoking cessation is of importance, particularly as in chronic inflammatory rheumatic diseases the risk of cardiovascular events is already elevated and smoking increases it even more.[16–18]

Although MTX as anchor drug is recommended as initial therapy, a large proportion of patients will not achieve remission with this therapy alone and needs additional treatment. Despite that our clinical model only fairly predicts patients to have IR or no IR to 'MTX+', it still can be of additional value in the decision-making regarding the initial therapy in patients with newly diagnosed RA, considering that 30%–50% will not have adequate disease control by only MTX therapy. Starting combination treatment with MTX and a bDMARD or several csDMARDs in patients with new-onset RA and poor prognostic factors could lead to improved clinical outcomes. It is of course of importance to know if initiating such combination therapy from start would be an adequate alternative, avoiding unnecessary treatment and healthcare cost. To address this issue, we used data from patients in the U-Act-Early and tREACH trial who were randomised to initiate TCZ+MTX or MTX+SSZ+HCQ therapy, respectively, and evaluated if those predicted at baseline as having a high risk of IR based on our prediction model actually responded to the combination therapy. Of those patients, 55%–95% achieved remission within 1 year, supporting the efficacy of initiating intensive strategies in more difficult-to-treat patients with RA. However, when applying the prediction model in the development sample to choose the strategy, 25% (27/108) would have been overtreated as 'MTX+' would also have been sufficient. If 'MTX+' was initiated in those likely to show no IR to 'MTX+', 6/108 (6%) would however have been undertreated. Nevertheless, if applied in clinical practice, the model, especially during the first year of treatment, might reduce the healthcare costs and minimise the risk of adverse events. A possible cost-effective alternative for those likely to show IR to 'MTX+' could be csDMARD combination therapy (eg, MTX+SSZ+HCQ)[19–21]: low-dose (oral) GCs in a tapering scheme or one single intramuscular GC injection might be given in addition as bridging therapy to further improve the response to initial therapy.[14 22]

To appraise the generalisability of the predictors found in our study, we conducted a systematic literature search and found that smoking[23 24] and DAS(28)[23–25] have been associated previously with treatment response to MTX (online supplementary file 1). However, these variables were not validated in a second cohort. To our knowledge, this is the first study reporting a protective effect of (minor) alcohol consumption (median (IQR) 5 (2–14) glasses p/w) on the risk of IR on 'MTX+'. Although alcohol consumption could be a proxy, for example, for general well-being or social status, it is also significantly inversed related to the risk of developing RA[26–30] and with the production of proinflammatory cytokines; via this latter mechanism, it might suppress disease activity.[31–33] In the present analysis, we did not find a dose-related effect of alcohol consumption; however, in patients with RA receiving MTX, weekly alcohol consumption

of <14 units p/w appeared not to be associated with hepatotoxicity, supporting the potential benefit of (low) alcohol consumption on response to this therapy.[34]

Several limitations should be kept in mind when interpreting this data. First, the step-up MTX therapy used in the U-Act-Early trial might not fully be representative of current daily practice. The starting dose of MTX was 10 mg/week and was gradually increased every 4 weeks until remission or maximum tolerable dose was achieved. In patients with poor prognostic factors (eg, high disease activity), MTX may often be started in a higher dose and escalated more rapidly in daily practice. However, in the tREACH trial, in which we found similar results, MTX was dosed at 25 mg/week (dosage reached after 3 weeks). Second, there were differences in initial strategies between the development (ie, MTX+HCQ) and validation (ie, MTX+GCs) sample before a bDMARD was initiated, which might have influenced the findings of the validation. However, the performance of the model in the development cohort was similar to that in the validation cohort. Third, in the present study, IR to 'MTX+' was defined as not achieving the treatment target (ie, remission) while the studies in literature (online supplementary file 1), which we compared our findings with, also used other definitions (eg, drug discontinuation). Fourth, to determine whether and to what degree a predictive model could improve decision-making in daily practice and ultimately the patients' outcome, further impact studies are needed using a comparative design.

In conclusion, our study confirmed the importance of disease activity and lifestyle related factors in the prediction of IR to treat-to-target step-up 'MTX+' in patients with newly diagnosed early RA. By using easily obtainable clinical predictors, the majority inadequately responding to this therapy can be predicted at baseline, in whom starting combination therapy with a bDMARD (eg, TCZ) or csDMARD (eg, SSZ+HCQ) is a feasible alternative to achieve remission. We acknowledge that it is premature to implement this treatment algorithm in clinical practice as further research is needed to quantify the additive effect in improving the management of patients with early RA. Next to applying more individualised care, changing the patients' lifestyle, especially smoking, improves the long-term outcome of our patients.

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Contributors All authors were involved with drafting the article or revising it critically and approved the final draft to be published and agree to be accountable for all aspects. Study conception or design: XMT, JWGJ, PMJW, PHPdJ, FPJGL, JWJB. Acquisition of data: XMT, JWGJ, PMJW, PHPdJ, AP-S, MEAB. Analysis or interpretation of data: XMT, JWGJ, PMJW, PHPdJ, JMWH, AEAMW, AP-S, MEAB, JMvL, FPJGL, JWJB.

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EXTENDED REPORT

Tocilizumab discontinuation after attaining remission in patients with rheumatoid arthritis who were treated with tocilizumab alone or in combination with methotrexate: results from a prospective randomised controlled study (the second year of the SURPRISE study)

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ABSTRACT

Objective To evaluate the sustained remission and low disease activity after discontinuation of tocilizumab in patients with rheumatoid arthritis who were treated with tocilizumab alone or in combination with methotrexate.

Methods The SURPRISE study was a 2-year, open-label randomised controlled study. Among patients who had been randomised to additional tocilizumab (ADD-ON) or switch to tocilizumab (SWITCH) in the first year, those who achieved remission based on the disease activity score for 28 joints (DAS28-ESR<2.6) discontinued tocilizumab at week 52 and were observed for the following 52 weeks. The endpoint of the second year included tocilizumab-free remission and low disease-activity rates, functional outcome, radiological outcomes assessed with the modified total Sharp score (mTSS) and safety. The efficacy of reinstituted tocilizumab/methotrexate was also evaluated.

Results A total of 105 patients who achieved remission at week 52 discontinued tocilizumab; 51 in ADD-ON continued methotrexate and 54 in SWITCH received no disease-modifying antirheumatic drugs. Sustained DAS28 low disease-activity rates were significantly higher in ADD-ON than in SWITCH (55%vs27%, p=0.005). Sustained remission rates at week 104 were 24% for ADD-ON and 14% for SWITCH (p=0.29). Radiological progression was comparable between both groups (mTSS; 0.37vs0.64, p=0.36). The restart of tocilizumab induced remission in all except two patients after 36 weeks, irrespective of concomitant methotrexate.

Conclusion Sustained low disease activity after tocilizumab discontinuation could be maintained with continued methotrexate in more than half of the patients. Retreatment with tocilizumab led to remission in more than 90% of patients.

Trial registration number NCT01120366; Results.

INTRODUCTION

Biologic agents and their effective use have brought about a paradigm shift, which has realised clinical remission in rheumatoid arthritis.[1–3] Recently, there have been rigorous attempts, from the risk–benefit point of view, to identify whether the discontinuation of biologic agents is possible in patients who have achieved remission or low disease activity.[4–21] Accumulated evidence has shown that 0%–59% of patients treated with methotrexate and tumour necrosis factor (TNF) inhibitors can successfully remain in remission or low disease activity after discontinuing TNF inhibitors. The wide range of the success rates is ascribed to the patients' disease stage and the extent and duration of remission. Evidence from several studies suggests that methotrexate-naïve early patients in deep remission can achieve sustained biologic-free remission with no functional or radiographic progression after treatment with TNF inhibitors and methotrexate.[4–15]

Tocilizumab, a humanised antihuman interleukin-6 receptor monoclonal antibody, has been validated to be efficacious in combination with methotrexate and as a monotherapy.[22–25] Compared with TNF inhibitors, little is known regarding tocilizumab-free remission or low disease activity. The DREAM and ACT-RAY studies showed that only a small portion of patients remained in remission after discontinuing tocilizumab without concomitant conventional synthetic disease-modifying antirheumatic drugs (csDMARDs).[16 17] Both studies, however, evaluated drug-free remission. One single-arm study reported that, in less than half of patients, remission was maintained after discontinuation of tocilizumab with sustained methotrexate[18]; however, no study has compared tocilizumab-free remission and low disease activity between with and without methotrexate.

The SURPRISE study was a 2-year, open-label, randomised clinical study in which the efficacy and safety profile of adding tocilizumab to methotrexate (ADD-ON) or switching from methotrexate to tocilizumab (SWITCH) were evaluated in patients with active rheumatoid arthritis despite methotrexate treatment.[26] In this study, tocilizumab

was discontinued in patients in remission at week 52; therefore, patients in the ADD-ON continued methotrexate and those in the SWITCH were observed without DMARDs through week 104. This article reports the results of the second year.

SUBJECTS AND METHODS

Study design and participants

The SURPRISE study was a 2-year, open-label, randomised controlled study conducted at 30 institutes in Japan. In the first year, efficacy and safety were compared between the ADD-ON and SWITCH strategies, and the results have been already published.[26] The current report covers the results of the second year in which tocilizumab-free rates were evaluated after discontinuing tocilizumab with or without methotrexate. The eligibility criteria for this study have been previously described.[26] Briefly, the study included methotrexate and biologic-agents-naïve patients with rheumatoid arthritis according to the 1987 American College of Rheumatology classification criteria whose disease activity score for 28-joints based on erythrocyte sedimentation rate (DAS28-ESR) exceeded 3.2 despite methotrexate treatment.

This report covers the planned analysis of the second year of the SURPRISE study (NCT01120366). This study was approved by the ethics committee at each site and conducted in accordance with the Declaration of Helsinki. All participants gave their written informed consent before inclusion into the study.

Study treatment

Two hundred and twenty-six patients were randomly assigned at baseline to the ADD-ON or the SWITCH treatments. Tocilizumab at a dose of 8 mg/kg every 4 weeks was discontinued in patients in remission (DAS28 <2.6) at week 52; patients in the ADD-ON group continued methotrexate and those in the SWITCH group received no DMARDs. Glucocorticoids and non-steroidal anti-inflammatory drug use were not prohibited. At the investigator's discretion, patients who experienced flare were administered intravenous tocilizumab at the same dose and interval in the ADD-ON group, while methotrexate and/or tocilizumab were administered in the SWITCH group.

Collected patient data and assessments

The treatment status was collected throughout the second year. The following parameters were assessed every 3 months: tender joint count, swollen joint count, Health Assessment Questionnaire Disability Index (HAQ-DI), patient global assessment using a visual analogue scale (VAS), evaluator global assessment using VAS, C reactive protein, erythrocyte sedimentation rate, rheumatoid factor (RF) and matrix metalloproteinase-3 (MMP-3). Radiographs of the hands and feet were obtained at week 104 in addition to those at weeks 0 and 52 from the first year. Each radiograph was assessed with the van der Heijde-modified total Sharp scoring system (mTSS) by two independent readers who were blinded to the patients' clinical status and treatment. At each visit, patients were monitored for physical signs, laboratory parameters and adverse events.

Outcome measures

The primary endpoint of the second year of this study was tocilizumab-free rate and time. The tocilizumab-free rates included patients who had more than low disease activity as long as they did not receive tocilizumab. Secondary endpoints included rates and time of tocilizumab-free remission or low disease activity, change in DAS28-ESR, radiographic progression and rates of remission achievement after the restart of treatment. The restart of tocilizumab and/or methotrexate was deemed as a failure of tocilizumab-free. Four patients in the SWITCH group were started on re-treatment when their DAS28-ESR was still less than 2.6; methotrexate in three patients and tocilizumab in one patient. Their disease activity increased from DAS28 <1.8 when tocilizumab was discontinued to >2.4 when re-treatment was started; therefore, we regarded those patients to have failed to maintain tocilizumab-free remission.

Statistical analysis

Time-to-events was analysed using the Kaplan-Meier method, and comparisons between the two groups were performed using the log-rank test. Patients who were lost to follow-up were regarded as censored. All analyses of proportions for treatment differences were performed with the χ^2 test. The Clopper Pearson method was used to calculate the 95% CIs of proportions. Continuous variables were compared with Student's t-test for two groups and with one-way analysis of variance for three groups. Efficacy analyses were conducted in the full-analysis population with the last-observation-carried-forward method. Safety endpoints including the incidence of adverse events, serious adverse events, infections and specific laboratory abnormalities were analysed in all treated patients. P values of less than 0.05 were regarded as statistically significant.

RESULTS

Patient flow and characteristics at tocilizumab discontinuation

shows the study flowchart through the 104 weeks. Among 83 patients in the ADD-ON group and 78 in the SWITCH group who achieved remission at week 52, 49 in the ADD-ON group and 53 in the SWITCH group agreed to discontinue tocilizumab. The 59 patients who refused to stop tocilizumab were excluded from the analyses. Therefore, a total of 102 patients (49 who continued with a mean dose of 7.7 mg/week of methotrexate and 53 who received no DMARDs) were analysed for efficacy and safety as the full-analysis population in the second year of the SURPRISE study. No difference was found in disease activity at week 52 between patients who stopped tocilizumab per protocol and those who did not (DAS28 was 1.5 in the former and 1.6 in the latter, p=0.33). Five patients in the SWITCH group lacked radiographs of the hands and feet and were excluded from the radiographic analysis.

There were no statistically or clinically significant differences in characteristics between the two groups at tocilizumab discontinuation (table 1). Eight patients (15.1%) in the SWITCH group and 5 (10.2%) in the ADD-ON group received glucocorticoids with a mean dose of 3.4 mg/day and 2.4 mg/day, respectively.

Tocilizumab-free remission or low disease activity

The tocilizumab-free rates are shown in figure 2A. Four patients in the SWITCH group were lost to follow-up and were regarded as censored. The rates of patients who remained tocilizumab-free were significantly higher in the ADD-ON group than in the SWITCH group; 67.3% (95% CI 54.0% to 80.6%) vs 28.5% (95% CI 16.4% to 40.6%) at week 104, p<0.001, respectively (p<0.001). Whereas tocilizumab-free remission rates were not significantly different between the ADD-ON and SWITCH groups (24.4% (95% CI 12.4% to 36.5%) vs 14.3% (95% CI 4.6% to 24.0%) at week 104, p=0.29, figure 2B), tocilizumab-free low disease-activity rates

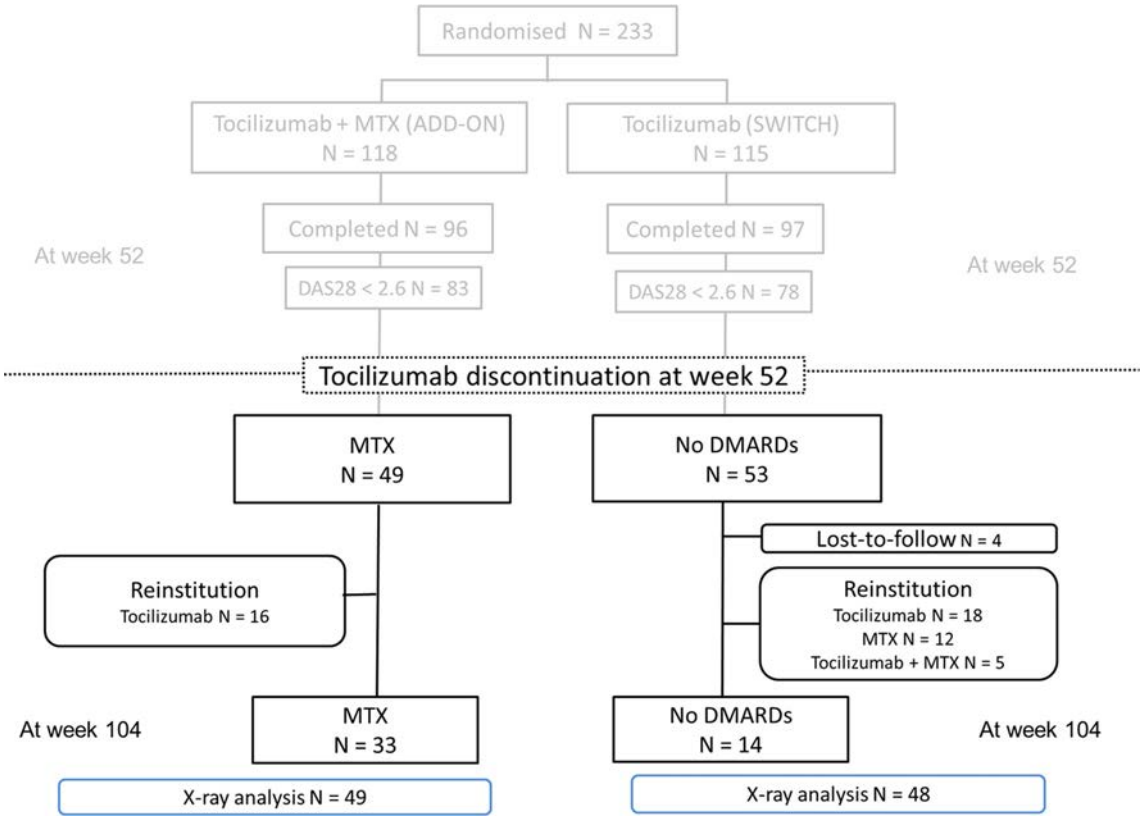


Figure 1 Study flow chart. DAS28, disease activity score for 28 joints; DMARD, disease-modifying antirheumatic drug; MTX, methotrexate.

were higher in the ADD-ON group (55.1% (95% CI 41.1% to 69.1%) vs 26.6% (95% CI 14.5% to 38.7%) at week 104, p=0.005, figure 2C).

If we regarded the restart of methotrexate alone in the SWITCH group as being tocilizumab-free, the difference between the ADD-ON and SWITCH groups became non-significant (67.3% vs 53.1%, p=0.22; online supplementary figure 1). However, the favour of continuing methotrexate at tocilizumab discontinuation still could be seen.

Structural and functional outcome

Probability plots of changes in mTSS from week 52 to week 104 showed slightly more radiological progression in the SWITCH

Table 1 Baseline patient characteristics

	At baseline			At tocilizumab discontinuation		
	Add-on (n=49)	Switch (n=53)	P values	Add-on (n=49)	Switch (n=53)	P values
Age, years	57.5 (11.5)	54.4 (13.7)	0.15	–	–	
Female, N (%)	44 (90)	47 (89)	0.97	–	–	
Disease duration, years	3.6 (3.4)	3.5 (2.9)	0.09	–	–	
RF positivity, N (%)	34 (71)†	40 (82)‡	0.24	–	–	
TJC28	6.1 (4.2)	6.9 (5.5)	0.46	0.3 (0.6)	0.5 (1.0)	0.28
SJC28	5.4 (3.4)	7.0 (4.4)	0.04	0.3 (2.6)	0.2 (0.5)	0.62
TJC68	9.5 (7.3)	8.9 (8.1)	0.69	0.4 (0.7)	0.5 (1.4)	0.53
SJC66	6.3 (4.1)	10.1 (8.1)	0.004	0.4 (0.9)	0.3 (0.7)	0.31
CRP, mg/dL	1.0 (1.2)	1.2 (2.2)	0.56	0.09 (0.49)	0.02 (0.45)	0.06
ESR, mm/h	46 (23)	38 (27)	0.54	6 (5)	6 (4)	0.60
PGA, mm	48 (21)	47 (24)	0.98	12 (14)	9 (11)	0.24
EGA, mm	48 (21)	47 (18)	0.87	5 (5)	6 (12)	0.64
DAS28-ESR	4.9 (1.0)	5.0 (1.1)	0.66	1.4 (0.6)	1.4 (0.6)	0.78
HAQ-DI	0.84 (0.55)	0.89 (0.64)	0.74	0.32 (0.36)	0.31 (0.73)	0.98
MMP-3, mg/dL	190 (180)	159 (179)	0.74	55 (29)	55 (32)	0.73

Values are presented as mean (SD) unless otherwise stated.
*P<0.05.
†n=48.
‡n=49.
CRP, C reactive protein; DAS28, disease activity score for 28 joints; EGA, evaluator global assessment; ESR, erythrocyte sedimentation rate; HAQ-DI, Health Assessment Questionnaire Disability Index; MMP, matrix metalloproteinase; PGA, patient global assessment; RF, rheumatoid factor; SJC, swollen joint count; TJC, tender joint count.

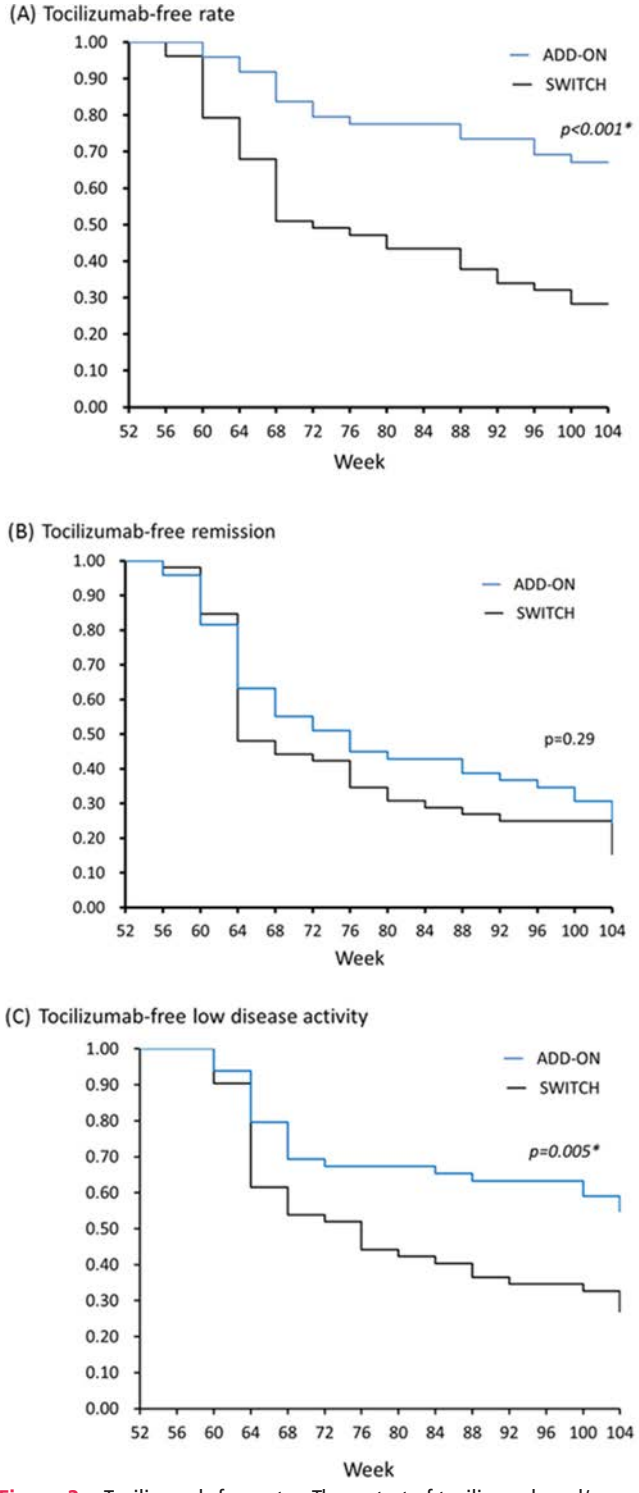
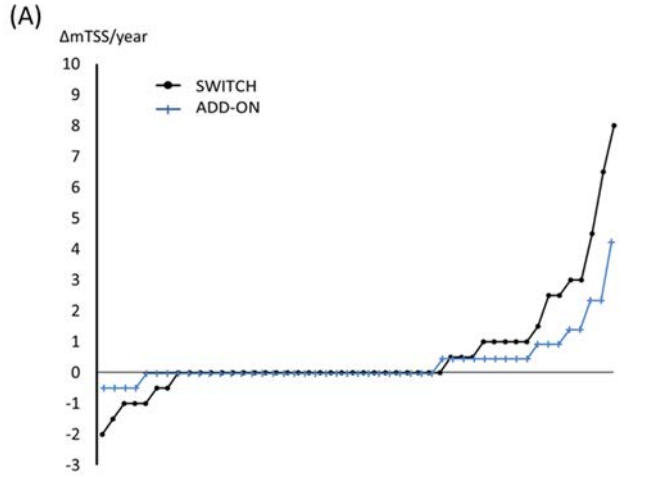


Figure 2 Tocilizumab-free rates. The restart of tocilizumab and/or methotrexate was deemed as a failure of tocilizumab-free remission if the drugs were restarted when the patients were still in remission or with low disease activity. (A) Overall tocilizumab-free rates, (B) tocilizumab-free remission rates, (C) tocilizumab-free low disease-activity rates. *P<0.05.



(B)

ΔmTSS/year (52-104w)	ADD-ON (N = 49)	SWITCH (N = 48)	P
mean	0.37 (0.9)	0.64 (1.8)	0.36
≤ 0, N (%)	32 (65.3%)	32 (66.7%)	0.89
≤ 0.5, N (%)	41 (83.7%)	35 (72.9%)	0.23
≥ 3, N (%)	1 (2.0%)	5 (10.4%)	0.11
≥ 5, N (%)	0 (0%)	2 (4.2%)	0.24

Figure 3 Structural outcome. (A) Cumulative probability plot of change from week 52 to week 104 in mTSS. (B) Mean change in mTSS and the percentage of patients with change in mTSS ≤0, ≤0.5, ≥3 or ≥3. mTSS, van der Heijde-modified total Sharp score.

group than in the ADD-ON group (figure 3A); however, the mean change in mTSS was comparable (0.37 in the ADD-ON group and 0.64 in the SWITCH group, p=0.36, figure 3B). The rates of non-progression or rapid progression (changes in mTSS ≤0, ≤0.5, ≥3 or ≥5) were also not different between the two groups (figure 3B). When we looked at changes in mTSS from week 0 to week 52, the mean change was 0.57 in the ADD-ON group and 0.77 in the SWITCH group (p=0.36; online supplementary figure 2A and 2B). As a whole, the non-progression rates were modestly better in the second year than in the first year.

HAQ-DI was 0.25 in the ADD-ON group and 0.29 in the SWITCH group at tocilizumab discontinuation (p=0.59), and were 0.30 and 0.17, respectively, at week 104 (p=0.29).

Characteristics favourable to sustained tocilizumab-free remission and low disease activity

The comparison of characteristics at tocilizumab discontinuation between patients who achieved tocilizumab-free remission or low disease activity and those who did not revealed that patients who achieved tocilizumab-free remission showed less RF positivity, lower patient global assessment and lower MMP-3. Patients who achieved tocilizumab-free low disease activity used methotrexate more frequently, showed less RF positivity and had a lower patient global assessment (online supplementary table 1). Taken together, RF negativity and lower patient global assessment may be predictors for successful tocilizumab-free achievement.

Efficacy of retreatment with methotrexate, tocilizumab or combination

Tocilizumab was added to methotrexate in 16 patients of 49 (32.7%) in the ADD-ON group because of flare between weeks 52 and 104. In the SWITCH group, 35 of 53 (66.0%) restarted medication: methotrexate (n=12), tocilizumab (n=18) or a combination of both (n=5). The median duration from tocilizumab discontinuation to treatment restart was 16 weeks in the

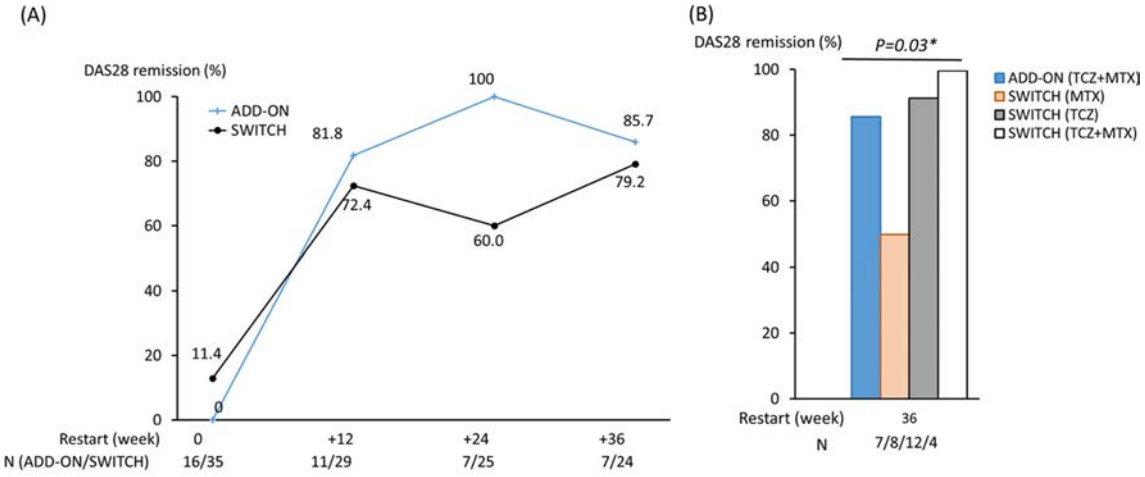


Figure 4 Percentage of patients who achieved DAS28 remission after treatment was restarted (A) in the ADD-ON and SWITCH from the restart through 36 weeks, and (B) in the ADD-ON and the three SWITCH groups (re-treatment with methotrexate (MTX), tocilizumab (TCZ) or tocilizumab plus methotrexate) at week 36. DAS28, disease activity score for 28 joints. *P<0.05.

ADD-ON group and 14, 16 and 8 weeks in the SWITCH group with methotrexate, tocilizumab and methotrexate plus tocilizumab, respectively. Online supplementary table 2 shows the characteristics of the ADD-ON and SWITCH groups at retreatment. Figure 4A shows the DAS28 remission rates at treatment restart and after 12, 24 and 36 weeks in both groups. All but one patient in the ADD-ON group (85.7%) and 79.2% of patients in the SWITCH group achieved remission again at week 36 (p=0.14). In the SWITCH group, 11 of 12 patients (92%) who were treated with tocilizumab alone and all patients treated with tocilizumab concomitant with methotrexate achieved remission at week 36, while remission was achieved in 50% of patients who received methotrexate alone (p=0.03, figure 4B). Overall, the restart of tocilizumab induced remission in 91.3% of patients and its efficacy was independent of concomitant methotrexate.

Safety

The safety results at week 52 through week 104 are presented in table 2. The number of patients with adverse events tended to be higher in the ADD-ON group than in the SWITCH group. The most frequent adverse event in the ADD-ON group was gastrointestinal disorder including oral ulcer and abdominal pain. Two serious adverse events were observed in the ADD-ON group (one gastric cancer without the restart of tocilizumab and one pneumocystis pneumonitis after the restart of tocilizumab), while none were noted in the SWITCH group.

DISCUSSION

The findings of the second-year results of the SURPRISE study showed that maintained remission after tocilizumab discontinuation was similarly low with or without methotrexate in patients with rheumatoid arthritis who had been in remission, while continued methotrexate could double the chance of sustained low disease activity compared with no DMARDs. The reinstitution of tocilizumab achieved remission in more than 90% of patients, with or without methotrexate.

The possibility of discontinuing biological agents in patients with remission or low disease activity has been investigated by weighing the advantage of obviating safety issues and economic burdens and the disadvantage of a potential risk of flare. Whereas the discontinuation of monoclonal antibodies against TNF has been considered feasible, especially in patients in the early stage,

Table 2 Adverse events by group

	Add-on (n=49)		Switch (n=53)	
	AE	SAE	AE	SAE
Infections and infestations	4, 8.2%	1, 2.0%	8, 14.8%	0
Gastrointestinal disorders	9, 18.4%	0	0	0
Hepatobiliary disorders	3, 6.1%	0	2, 3.7%	0
Respiratory, thoracic and mediastinal disorders	4, 8.2%	0	0	0
Laboratory test abnormalities	2, 4.1%	0	0	0
Metabolism and nutrition disorders	3, 6.1%	0	0	0
Skin and subcutaneous tissue disorders	2, 4.1%	0	2, 3.7%	0
Injury, poisoning and procedural complications	3, 6.1%	0	1, 1.9%	0
General disorders and administration site conditions	1, 2.0%	0	0	0
Neoplasms benign, malignant, unspecified	1, 2.0%	1, 2.0%	3, 5.6%	0
Eye disorders	1, 2.0%	0	0	0
Musculoskeletal and connective tissue disorders	1, 2.0%	0	0	0
Blood and lymphatic system disorders	1, 2.0%	0	0	0

Values are presented as N, %.
AE, adverse event; SAE, serious adverse event.

the usefulness of tocilizumab discontinuation is unclear owing to the findings of the DREAM study where tocilizumab-free remission was achieved in only 13% at 52 weeks,[16] and the ACT-RAY study in which 83% of patients experienced flares after stopping tocilizumab and subsequently csDMARDs and methotrexate.[17] Unlike both studies that targeted drug-free remission, the SURPRISE study compared patients' status after tocilizumab discontinuation between the use and non-use of methotrexate. In the study, a tocilizumab-free low disease activity with methotrexate was noted in 55% of patients, which was twice as much as 27% of a tocilizumab-free low disease activity without methotrexate. This rate is comparable with the 55% infliximab-free low disease activity with methotrexate in the RRR study[5] and the 54% adalimumab-free low disease activity with methotrexate in the OPTIMA study,[6] although caution is necessary when comparing results from different studies. While our study showed that tocilizumab-free remission was successful in a minority of patients with or without methotrexate as was shown in the DREAM and the ACT-RAY studies,[16 17] one single-arm study reported a sustained remission rate of 44% after tocilizumab discontinuation with continued methotrexate.[18] Taken together, tocilizumab can be suspended in a part of patients with rheumatoid arthritis in remission for at least several months, which could endorse the concept of biologic DMARD discontinuation, as reported with other biological agents including TNF inhibitors and abatacept.[4–15 20 21]

Radiological progression was modestly lower in the ADD-ON group than in the SWITCH group, although the mean changes in mTSS per year were very low, with no significant difference between both groups. Functional impairment also did not change in the second year in either group. Although retreatment with tocilizumab and/or methotrexate might have affected those results, our study suggests that stopping tocilizumab after remission achievement is worth attempting, in that retaining methotrexate is better for maintaining low disease activity. In addition, those results could re-emphasise the results of the first year of the SURPRISE study; tocilizumab induction would be recommended in combination with methotrexate in patients inadequately responsive but tolerant to methotrexate. However, it should be noted that continued methotrexate caused more adverse events, especially gastrointestinal symptoms, which was a definite drawback of the non-DMARDs-free strategy. The optimal dose of continued methotrexate is another issue that requires clarification.

The reinstitution of tocilizumab was as efficacious as the initial administration. This is in line with the results of the RESTORE study in which 89% of patients achieved DAS28 remission within 12 weeks of retreatment with tocilizumab,[27] and those of the RONIN study in which all of four patients responded to tocilizumab retreatment after long-term discontinuation of tocilizumab.[19] While the results of the first year of the SURPRISE study showed that tocilizumab with concomitant methotrexate induced remission earlier than tocilizumab without methotrexate, the reinstitution of tocilizumab was comparably effective, with and without methotrexate. This could be attributed to disease activity at tocilizumab initiation. A study reported that the concomitant use of methotrexate and tocilizumab was more important in patients whose DAS28 exceeded 5.1 than in those with less activity.[28] Indeed, the mean DAS28-ESR at baseline in the SURPRISE study was approximately 5.1 to 5.3 but was 4.1 to 4.4 at tocilizumab reinstitution. Methotrexate led to remission in half of the patients despite the inclusion criterion in this study of an inadequate response to methotrexate. This may also be due to different disease activities at the start of methotrexate administration. The good results shown by reinstituted tocilizumab in patients with flare-up after discontinuation of tocilizumab can also support the strategy of tocilizumab suspension in clinical, economic and safety aspects.

Some predictors associated with sustained remission or low disease activity after cessation of biologic agents have been reported. Strong opinion is that anticitrullinated autoantibody negativity and absence of synovitis are associated with higher chances of achieving drug-free remission, and long-term remission and non-smoking may be relevant with successful TNF inhibitor withdrawal.[5 9 12 29] Our study shows that RF negativity and lower patient global assessment were predictors for sustained tocilizumab-free remission or low disease activity. These results are consistent with the previous hypotheses because RF negativity could represent immunological remission,[29] and patient global assessment may reflect residual disease activity.[30]

Our study has some limitations. First, this was an open-label study with a limited number of subjects. Both patients and rheumatologists were aware whether patients were receiving methotrexate or not, which could have affected the assessment. Second, the lack of anticitrullinated antibody and the small sample size hampered the solid identification of predictive factors for successful tocilizumab-free remission or low disease activity. Third, we did not have information regarding patients who did not stop tocilizumab after 52 weeks; therefore, a comparison of patients who stopped tocilizumab and those who continued was not performed. Fourth, the inclusion criterion for the second year of the SURPRISE study was a DAS28 <2.6 at a single time point of week 52. However, a sustained remission status has recently been considered to raise the feasibility of DMARDs tapering.[29] Fifth, we did not examine methotrexate-free remission or low disease-activity rates with continued tocilizumab, which is another strategy of treatment reduction that should be investigated in the future. Nevertheless, the results of the second year of the SURPRISE study provided valuable insights into the possibility of stopping tocilizumab after achieving remission.

In conclusion, the results of the second year of the SURPRISE study show that the discontinuation of tocilizumab after remission achievement is feasible for maintaining low disease activity with continued methotrexate. The restart of tocilizumab is efficacious for suppressing flares, with or without methotrexate.

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Contributors YK, NM, HY, KY and TT designed the study and analysed and interpreted the data. YK, MK, YT, MI, HK-H, KA, MM and YM were involved in collecting data and managing their clinical research sites. YK, HY, SH and ET scored mTSS blinded to patients' clinical information. All authors were involved in writing the manuscript and approved the final version.

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Competing interests YK has received grants or speaking fees from from AbbVie, Astellas, Ayumi, Bristol-Myers Squibb, Chugai, Eisai, Eli Lilly, Hisamitsu, Jansen, Kissei, Pfizer, Sanofi, Takeda, Tanabe-Mitsubishi and UCB. MK has received research grants or lecture fees from GlaxoSmithKline K.K. and Actelion Pharmaceuticals. YT has received grants or speaking fees from Mitsubishi-Tanabe, Takeda, Bristol-Myers, Chugai, Astellas, AbbVie, MSD, Eli Lilly, YL Biologics, Daiichi-Sankyo, Sanofi, Janssen, Pfizer, Kyowa-Kirin, Eisai and Ono. HK-H has received speaking fees from Chugai, Astellas and Bristol-Myers Squibb. KA has received research grants from Chugai Pharmaceutical Co. and speaking fees from Daiichi-Sankyo, Eli Lilly, Pfizer Japan and Tanabe-Mitsubishi. MM is an employee of the Japanese Red Cross Society. YM has received grants, instructor fees or speaking fees from Chugai, Ono, Daiichi-Sankyo, Teijin, Eisai, Nippon Kayaku, Tanabe-Mitsubishi, Kissei, Janssen, Eisai, Astellas, Ayumi, Takeda, UCB, Sanofi and Eli- Lilly. HY has received grants or speaking fees from Bristol-Myers Squibb, Takeda, Japan Blood Products Organization, Eizai, Daiichi-Sankyo, Novartis, Chugai and Actelion. SH has received grants, lecture fees or speaking fees from AbbVie, Astellas, Ayumi, Bristol-Myers Squibb, Chugai, Eisai, Eli Lilly, Jansen, Kissei, Pfizer, Sanofi, Takeda, Tanabe-Mitsubishi and UCB. ET received speaking fees from AbbVie, Ayumi Pharmaceutical, Bristol-Myers Squibb, Chugai Pharmaceutical, Eisai Pharmaceutical, Nippon Kayaku, Pfizer, Takeda Pharmaceutical and UCB Pharma. HY has received research grants, consultant fees or speaking fees from MSD, Astellas, AbbVie, Bristol-Myers Squibb, Kaken, UCB, Ono, Ayumi, Eisai, Daiichi-Sankyo, Takeda, Tanabe-Mitsubishi, Chugai, Teijin, Torii, Nipponshinyaku, Pfizer, YL Biologics and Nipponkayaku. KY has received grants or speaking fees from Astellas Pharmaceutical, Chugai Pharmaceutical, Eizai Pharmaceutical, Immunofuture Inc, Mitsubishi Tanabe Pharma Corporation, Santen Pharmaceutical, Pfizer Japan Inc, AbbVie GK, Bristol–Myers KK, Diaichi Sankyo, Eli Lilly, Sanofi, Janssen and UCB. TT has received research grants or speaking fees from Astellas Pharma Inc, Bristol–Myers K.K., Chugai Pharmaceutical Co, Ltd, Daiichi Sankyo Co, Ltd, Takeda Pharmaceutical Co, Ltd, Teijin Pharma Ltd, AbbVie GK, Asahikasei Pharma Corp, Mitsubishi Tanabe Pharma, Astra Zeneca KK, Eli Lilly Japan KK, Novartis Pharma KK, AbbVie GK, Nipponkayaku Co, Ltd, Janssen, Pharmaceutical KK, Taiho Pharmaceutical Co, Ltd and Pfizer Japan Inc. MI and NM have nothing to declare.

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EXTENDED REPORT

Comparative effectiveness of tocilizumab versus TNF inhibitors as monotherapy or in combination with conventional synthetic disease-modifying antirheumatic drugs in patients with rheumatoid arthritis after the use of at least one biologic disease-modifying antirheumatic drug: analyses from the pan-European TOCERRA register collaboration

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ABSTRACT

Objective To compare the effectiveness of tocilizumab (TCZ) and tumour necrosis factor (TNF) inhibitors (TNFi) as monotherapy or in combination with conventional synthetic disease-modifying antirheumatic drugs (DMARDs) in patients with rheumatoid arthritis (RA) after the use of at least one biologic DMARD (bDMARD).

Methods We included patients with RA having used at least one bDMARD from 10 European registries. We compared drug retention using Kaplan-Meier and Cox models and Clinical Disease Activity Index (CDAI) change over time with mixed-effects models for longitudinal data. The proportions of CDAI remission and low disease activity (LDA) at 1 year were compared using LUNDEX correction.

Results 771 patients on TCZ as monotherapy (TCZ mono), 1773 in combination therapy (TCZ combo), 1404 on TNFi as monotherapy (TNFi mono) and 4660 in combination therapy (TNFi combo) were retrieved. Crude median retention was higher for TCZ mono (2.31 years, 95% CI 2.07 to 2.61) and TCZ combo (1.98 years, 95% CI 1.83 to 2.11) than TNFi combo (1.37 years, 95% CI 1.30 to 1.45) and TNFi mono (1.31 years, 95% CI 1.18 to 1.47). In a country and year of treatment initiation-stratified, covariate-adjusted analysis, hazards of discontinuation were significantly lower among patients on TCZ mono or combo compared with patients on TNFi mono or combo, and TNFi combo compared with TNFi mono, but similar between TCZ mono and combo. Average adjusted CDAI change was similar between groups. CDAI remission and LDA rates were comparable between groups.

Conclusion With significantly longer drug retention and similar efficacy to TNFi combo, TCZ mono or combo are reasonable therapeutic options in patients with inadequate response to at least one bDMARD.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease characterised by joint inflammation and structural damage. The management of RA has dramatically changed with the use of biologic disease-modifying antirheumatic drugs (bDMARDs). Tocilizumab (TCZ) is a humanised anti-interleukin (IL)-6 receptor antibody that has shown efficacy in reducing signs and symptoms of RA and in preventing the progression of structural damage and loss of function.[1–6] TCZ is licensed for the treatment of patients with RA with inadequate response to conventional synthetic DMARDs (csDMARDs) and/or bDMARDs.[7] Most international recommendations advocate the use of bDMARDs in combination with methotrexate (MTX) or other csDMARDs in case MTX is not tolerated or contraindicated.[8] However, data derived from various patient registries show that bDMARDs are prescribed as monotherapy in up to 30% of patients, due to patient's preference or occurrence of intolerance to csDMARDs.[9–16] The ACT-RAY study examined the efficacy and safety of switching to TCZ monotherapy or adding TCZ to MTX in patients with active disease despite MTX therapy. The results at 24 weeks showed that efficacy was largely similar in both treatment arms,[17] but this first analysis and later follow-up during 2 years overall suggested that TCZ performed better in combination with MTX than as monotherapy.[18 19] In a 52-week prospective, randomised controlled study, adding TCZ to MTX more rapidly achieved remission than switching to TCZ monotherapy, in patients with RA refractory to MTX.[20] In the FUNCTION randomised placebo-controlled trial in early arthritis, the combination of MTX and TCZ seemed to be more efficacious than TCZ in monotherapy, but the study was not powered to detect difference between these groups.[21] On the other hand, a study combining several European registries found that TCZ as monotherapy (TCZ mono) had similar effectiveness as compared with TCZ in combination with MTX and/or csDMARDs when assessed as changes in Clinical Disease Activity Index (CDAI) and Disease Activity Score

28 (DAS28) from baseline values.[9] The ADACTA study demonstrated in a head-to-head randomised controlled trial setting that TCZ monotherapy was superior to adalimumab monotherapy for reduction of signs and symptoms of RA in patients for whom MTX was deemed inappropriate.[22] However, one of the criticisms of this study is that adalimumab was used as monotherapy, which does not represent the best comparator since TNF inhibitors (TNFi) are notoriously more efficacious when used in combination with MTX.[23–25] Since TCZ is largely used as a second-line bDMARD in numerous countries, we decided to compare the effectiveness of TCZ and TNFi as monotherapy or in combination with csDMARDs in patients with inadequate responses to at least one bDMARD followed longitudinally in 10 European registries, with a special interest in the comparison between TCZ mono and TNFi in combination with csDMARDs (TNFi combo).

METHODS

The TOCERRA collaboration of registries (TOcilizumab Collaboration of European Registries in RA) is an investigator-led, industry-supported project aiming at evaluating clinical aspects of TCZ use in patients with RA. Each registry obtained ethical approval for the use of anonymised data for research in their local ethics committee. TOCERRA includes data from 10 countries (see online supplementary table S1). All patients included in the different registries and starting treatment with TCZ or any TNFi between 16 January 2009 and 1 January 2017 were considered eligible for the present study. Inclusion criteria were diagnosis of RA established by a rheumatologist, being aged 18 years or more, having used at least one bDMARD, baseline information on prior use of bDMARDs or csDMARDs and information on concomitant use of csDMARDs. When patients had several treatment courses with either TCZ or TNFi, all treatment courses were used and statistical models included a stochastic term to account for the non-independence of the data.

Exposure of interest

bDMARDs were classified either as monotherapy or in combination therapy with any csDMARDs, depending on the presence of concomitant csDMARDs at baseline. The main exposures of interest were TCZ or TNFi as monotherapy or in combination with one or several csDMARDs. We also performed three secondary analyses. In the first, we carried out additional detailed analysis between TCZ mono and TNFi combo. In another secondary analysis, patients treated with TNFi and MTX as the only csDMARD were further categorised as having low-dose MTX (<10 mg/week), medium dose (10–15 mg/week) versus high dose (>15 mg/week), yielding four groups (TCZ mono, TNFi combo MTX low dose, TNFi combo MTX medium dose, TNFi combo MTX high dose). Finally, patients treated with TNFi were categorised by their type of concomitant csDMARD (only MTX, MTX +another csDMARD, other csDMARD without MTX).

Study outcomes

Our main focus was drug retention and the change of disease activity in terms of CDAI following initiation of bDMARDs.

Drug retention reflects both effectiveness and tolerance of a drug and is reliably assessed in all registries.[26 27] It was defined as the time from the start date of TCZ or TNFi treatment until the treatment discontinuation date plus one dispensation interval. If treatment had not been discontinued, retention was censored at the date of the last reported follow-up visit.

CDAI was considered both as a continuous outcome over time and as a measure of remission or low disease activity (LDA) at 1 year, using the validated thresholds.[28 29] The frequency of assessments in most available registries did not allow for shorter evaluations of remission or LDA. We used the CDAI as a measure of disease activity instead of the DAS28 to avoid an assessment bias in favour of TCZ that has a strong impact of acute phase reactants.[30] We also used the DAS28-eythrocyte sedimentation rate (ESR) as a secondary outcome measure.

Covariates

The baseline covariates considered were sex, age, disease duration, number of previously used bDMARDs, seropositivity (presence of rheumatoid factor (RF) or anticyclic citrullinated peptide antibodies), glucocorticoid (GC) use and daily dosage, functional disability (Health Assessment Questionnaire (HAQ)), DAS28-ESR, year of treatment initiation and country of registry. Seropositivity was operationally defined as positive if RF and/or anticitrullinated protein antibody were positive according to each national registry, negative if both were negative and missing if one was missing and the other was negative. This algorithm is designed to limit misclassification of exposure and assign seronegative status to patients with missing data.

Statistical methods

Baseline characteristics across treatment were compared using generalised estimating equations, to account for the nested structure of the data, since patients could have several treatment courses and come from separate centres (registers). Drug retention was analysed using Kaplan-Meier and Cox models. In the Cox models, the baseline hazards were allowed to vary by country of registers and year of treatment initiation, and a cluster term was added to account for the fact that the same patients could have both TNFi and TCZ treatment. Missing covariates were imputed using multiple imputations with chained equations. CDAI and DAS28 change over time were analysed with mixed-effects models for longitudinal data. The frequency of disease remission or LDA under treatment was assessed at 1-year post-treatment start. When no observed values within a 3-month window were available, they were interpolated using a quadratic interpolation for each patient. The proportions of patients reaching remission or LDA by treatment group were then estimated using frequency and proportion (raw estimates) and corrected for drug discontinuation using the LUNDEX index (index combining the proportion of patients fulfilling specific response criteria with the proportion of patients still adhering to therapy).[31]

RESULTS

A total of 8308 eligible treatment courses were retrieved before January 2017, including 771 TCZ mono, 1773 TCZ in combination therapy (TCZ combo) (87.5% of all TCZ by intravenous administration), 1404 TNFi mono and 4660 TNFi combo. All registries contributed patients to both the TCZ and TNFi groups (mean proportion of TCZ patients across registries: 38.8%, range: 9.2%–73.7%). Among TNFi patients, 24.8% were on adalimumab, 15.1% on certolizumab, 34.2% on etanercept, 15.8% on golimumab and 10.0% on infliximab. On average, TCZ patients were slightly older, had longer disease duration and more previous bDMARDs. Baseline disease characteristics were slightly more severe in TCZ patients, with higher HAQ values, higher patient global assessment and higher C reactive protein levels (table 1). Patients in monotherapy (TCZ and

Table 1 Patient characteristics at baseline

	TCZ mono	TCZ combo	TNFi mono	TNFi combo
N	771	1773	1404	4660
Age, year (median, IQR)	55.8 (47.5, 64.5)	55.4 (46.8, 62.2)	54.5 (45.3, 63.8)	54.3 (44.0, 61.9)
Female gender, N (%)	639 (82.9%)	1421 (80.2%)	1165 (83.2%)	3718 (79.9%)
Disease duration, year (median, IQR)	10.2 (4.6, 17.2)	9.0 (4.3, 15.3)	8.7 (3.7, 15.6)	7.9 (3.4, 14.6)
Seropositivity (RF and/or ACPA), N (%)	458 (83.7%)	1134 (83.0%)	689 (79.5%)	2772 (80.8%)
Previous bDMARDs, N (%)				
1	301 (39.0%)	737 (41.6%)	822 (58.5%)	3204 (68.8%)
2	256 (33.2%)	572 (32.3%)	276 (19.7%)	641 (13.8%)
≥3	214 (27.8%)	464 (26.2%)	306 (21.8%)	815 (17.5%)
Glucocorticoids, N (%)	193 (25.0%)	893 (50.4%)	308 (21.9%)	2625 (56.3%)
Glucocorticoids dose, mg/day (median, IQR)	5.0 (4.0, 10.0)	5.0 (5.0, 10.0)	5.0 (5.0, 7.5)	5.0 (5.0, 7.5)
Concomitant csDMARD, N (%)				
MTX	–	931 (52.5%)	–	2097 (45.0%)
MTX+other		363 (20.5%)		1421 (30.5%)
Other		479 (27.0%)		1142 (24.5%)
DAS28	4.1 (1.7)	4.6 (1.4)	4.1 (1.4)	4.0 (1.4)
CDAI	23.3 (15.9)	27.7 (14.8)	22.6 (15.3)	21.4 (14.4)
HAQ	1.3 (0.7)	1.4 (0.7)	1.2 (0.8)	1.1 (0.8)
TJC (over 28 joints)	7.7 (7.2)	9.1 (6.8)	7.5 (6.9)	6.8 (6.3)
SJC (over 28 joints)	6.0 (6.0)	7.4 (6.0)	5.9 (5.9)	5.9 (5.6)
ESR (mm/hour)	29.6 (25.5)	34.4 (26.7)	27.6 (23.8)	26.5 (22.6)
CRP (mg/L)	16.7 (26.8)	19.9 (26.6)	16.4 (25.5)	14.9 (21.4)
Patient global assessment	5.5 (2.8)	6.0 (2.5)	5.5 (2.7)	5.1 (2.7)
Physician global assessment	4.1 (2.5)	5.1 (2.4)	4.2 (2.5)	4.0 (2.4)

Values are mean (SD) when not specified.
ACPA, anticitrullinated protein antibody; bDMARD, biologic disease-modifying antirheumatic drugs; CDAI, Clinical Disease Activity Index; combo, combination therapy; CRP, C reactive protein; csDMARD, conventional synthetic DMARD; DAS28, Disease Activity Score 28; ESR, erythrocyte sedimentation rate; HAQ, Health Assessment Questionnaire; mono, monotherapy; MTX, methotrexate; RF, rheumatoid factor; SJC, swollen joint count; TCZ, tocilizumab; TJC, tender joint count; TNFi, tumour necrosis factor inhibitor.

TNFi) had less GCs than patients in combination therapy. Baseline characteristics of patients of the subanalysis by dose of MTX are in online supplementary table S2.

Crude median drug retention (figure 1) was higher for TCZ mono (2.31 years, 95% CI 2.07 to 2.61) or combo (1.98 years, 95% CI 1.83 to 2.11) than TNFi combo (1.37 years, 95% CI 1.30 to 1.45) or mono (1.31 years, 95% CI 1.18 to 1.47). Among TNFi combo patients, crude median retention by concomitant csDMARD was 1.55 years (95% CI 1.43 to 1.64) for MTX, 1.36 years (95% CI 1.24 to 1.57) for MTX +another csDMARD and 1.09 years (95% CI 1.00 to 1.23) for csDMARD other than MTX (see online supplementary figure S1A). Among TNFi combo patients with MTX as the only concomitant csDMARD (n with available MTX dose=1520), median drug retention was 1.27 years (95% CI 1.04 to 1.62) for patients with low dose of MTX (<10 mg/week, n=170), 1.33 years (95% CI 1.10 to 1.57) for patients with medium dose of MTX (10–15 mg/week, n=697) and 1.64 years (95% CI 1.44 to 1.82) for patients with high dose (>15 mg/week, n=653) (see online supplementary figure S1B).

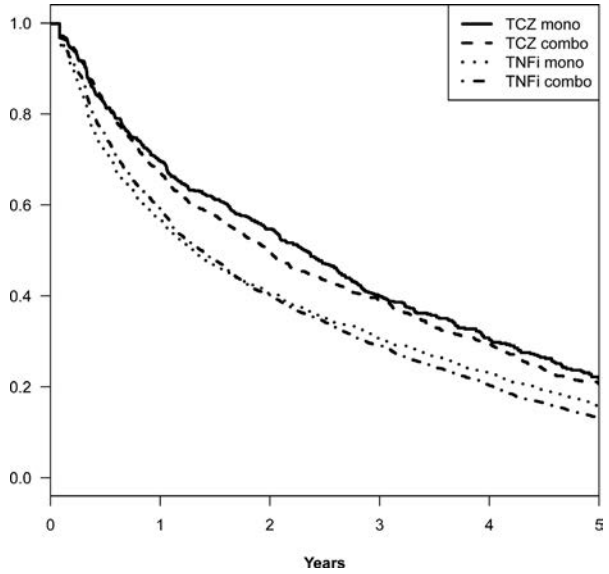

Figure 1 Kaplan-Meier curves of drug discontinuation by biologics and presence or not of concomitant conventional synthetic disease-modifying antirheumatic drugs (csDMARDs). TCZ combo, tociluzimab in combination with csDMARDs; TCZ mono, tocilizumab as monotherapy; TNFi combo, TNF inhibitor in combination with csDMARDs; TNFi mono, TNF inhibitor as monotherapy.

In a country and year of treatment initiation-stratified, covariate-adjusted analysis, we found that hazards of discontinuation of TCZ mono or combo were significantly lower than for TNFi mono or combo, and lower for TNFi combo than mono but similar between TCZ mono and combo (table 2).

When comparing TCZ mono with TNFi combo, TCZ mono was stopped more frequently for ineffectiveness than TNFi combo (24.0% vs 13.9%), whereas discontinuation was equally often recorded for adverse events in TCZ mono-treated and TNFi combo-treated patients (13.0% vs 13.6%). However, most of the causes of treatment discontinuation were recorded as 'other' by the treating physicians, which may include patient preference, remission and pregnancy, as well as a combination of causes (TCZ mono=56.0%, TNFi combo=42.6%). Among the TCZ mono group, 50 treatment courses (6.5%) were changed to include a concomitant csDMARD at some point. Conversely, among the TNFi combo group, 313 treatment courses (6.7%)

Table 2 Multivariable analysis of drug discontinuation.

	HR	95% CI	P values
TCZ mono vs TNFi combo	0.78	0.70 to 0.86	<0.001
TNFi mono vs TNFi combo	1.15	1.06 to 1.23	<0.001
TCZ mono vs TCZ combo	0.96	0.86 to 1.08	0.53
TCZ mono vs TNFi mono	0.65	0.58 to 0.74	<0.001
TCZ combo vs TNFi combo	0.70	0.65 to 0.76	<0.001
TCZ combo vs TNFi mono	0.65	0.59 to 0.72	<0.001

Adjusted by age, gender, disease duration, seropositivity, number of previous biologic disease-modifying antirheumatic drugs, glucocorticoids at baseline, Disease Activity Score 28 at baseline, Clinical Disease Activity Index at baseline, Health Assessment Questionnaire at baseline.
combo, combination therapy; mono, monotherapy; TCZ, tocilizumab; TNFi, tumour necrosis factor inhibitor.

were modified to stop the csDMARD for at least some visits (more than one-fourth of the visits). Shorter disease duration, higher past number of bDMARDs, concomitant GC treatment and higher HAQ at baseline were significantly associated with greater risk of discontinuation (see online supplementary table S3). The hazards of discontinuation were also significantly lower when comparing TCZ mono patients (HR 0.75, p<0.001) with TNFi patients treated with a high dose of MTX (>15 mg/week) (see online supplementary table S3, right columns).

CDAI score significantly decreased over time in the four different groups, and the decrease was not significantly different between them (table 3). The average adjusted CDAI change at 1 year was of −3.54 for TNFi mono patients, −3.34 for TNFi combo patients, −3.68 for TCZ combo and −3.58 for TCZ mono patients. When comparing TCZ mono versus TNFi combo, shorter disease duration, higher number of past bDMARDs, higher HAQ at baseline and concomitant GC treatment were associated with higher CDAI at any time during follow-up (see online supplementary table S4). The pattern of findings was similar when comparing TCZ mono patients with TNFi patients treated with a high dose of MTX (see online supplementary table S4, right columns), though number of past bDMARDs became non-significant.

Two hundred and fifty-one TCZ mono, 737 TCZ combo, 375 TNFi mono and 1995 TNFi combo patients were still under treatment with CDAI information at 1 year and could be included in the LUNDEX calculation. CDAI rates were relatively similar between groups, although CDAI LDA rates seemed lower in TNFi mono patients. However, this trend was not reflected in the CDAI remission rates (figure 2). In contrast, DAS28 remission and LDA at 1 year (LUNDEX corrected) were considerably higher in TCZ patients compared with TNFi patients (see online supplementary figure S2). For TCZ mono vs TNFi in combination with MTX, across doses of MTX, CDAI remission and LDA rates remained similar after LUNDEX adjustment (see online supplementary figure S2). They also remained similar across the type of concomitant csDMARD with TNFi. Conversely, DAS28 remission and LDA rates were higher in TCZ mono than for TNFi patients, across type of concomitant csDMARD or MTX doses after LUNDEX adjustment (see online supplementary figure S4).

Table 3 Multivariable analysis of CDAI over time

	Coeff	95% CI	P values
TCZ mono vs TNFi combo	0.17	−1.33 to 1.66	0.83
TNFi mono vs TNFi combo	−0.23	−1.06 to 0.60	0.59
TCZ mono vs TCZ combo	−0.21	−1.24 to 0.83	0.70
TCZ mono vs TNFi mono	−0.47	−1.60 to 0.66	0.41
TCZ combo vs TNFi combo	0.09	−0.56 to 0.74	0.79
TCZ combo vs TNFi mono	0.21	−0.74 to 1.16	0.67

Adjusted by age, gender, disease duration, seropositivity, number of previous biologic disease-modifying antirheumatic drugs, glucocorticoids at baseline, Health Assessment Questionnaire at baseline.
Coeff, coefficient; combo, combination therapy; mono, monotherapy; TCZ, tocilizumab; TNFi, tumour necrosis factor inhibitor.

DISCUSSION

Our study is one of the largest comparing TCZ and TCZ mono and TCZ combo, and one of the first that compared TCZ mono versus TNFi combo in a large population of patients with RA who used at least one bDMARD. The results showed that drug retention was significantly longer with TCZ than TNFi, even when TCZ was used as monotherapy and TNFi in combination therapy. The clinical effectiveness, as assessed by CDAI changes and CDAI responses, were similar in all treatment groups. In contrast, as expected, changes in DAS28 and DAS28 responses were significantly better in TCZ than in TNFi-treated patients. Altogether, the results indicate that TCZ mono or combination therapy are valuable therapeutic option in patients with an inadequate response to bDMARDs.

The patient populations differed in terms of baseline characteristics with older patients, longer disease duration, higher HAQ and more previous bDMARD failure in the TCZ groups. These results are consistent with other studies showing that bDMARDs as monotherapy are usually prescribed to more difficult-to-treat patients.[9 10 32] However, despite these differences, drug effectiveness as assessed by CDAI was similar in the two groups, whereas drug retention was longer with TCZ compared with TNFi whatever the mode of administration (monotherapy or combination therapy). Patients in monotherapy either with TCZ or TNFi had also less GCs than patients in combination therapy, indicating that patients treated as monotherapy have a different profile in terms of disease characteristics and comorbidities. However, the results regarding drug retention and efficacy were adjusted for the use of GCs.

Although bDMARD retention was higher in TCZ than in TNFi-treated patients, the effectiveness of these treatments, based on the CDAI, was not significantly different. This discrepancy suggests that either CDAI does not allow a comprehensive assessment of drug efficacy, the presence of a difference of tolerance between the two treatment groups, which was apparently not identified in our study, or that retention probably captures something that is not evaluated by CDAI, such as patient or physician preference. For example, it is possible that some minor adverse events, not recorded in the registries but sufficient to discourage patients to continue their treatment, can partly account for the difference. It is also possible that TCZ treatment, being used after several bDMARDs failure, is maintained even if not achieving the ideal target due to the lack of treatment alternatives.

Considering similar effectiveness in terms of CDAI and retention to the TCZ combination therapy, TCZ mono may also be suitable for patients who cannot tolerate csDMARDs or in whom these treatments are contraindicated. Indeed, although MTX is still the mainstay of RA therapy, up to 30% of patients discontinue MTX because of preference[33–35] or toxic effects.[36 37] Thus, there is a need to provide patients with effective alternatives. As consistently reported, contrary to monotherapy with either adalimumab or etanercept, the efficacy of TCZ mono is higher than MTX alone.[6 23 25] Furthermore, TCZ mono was superior to adalimumab as monotherapy in patients with inadequate

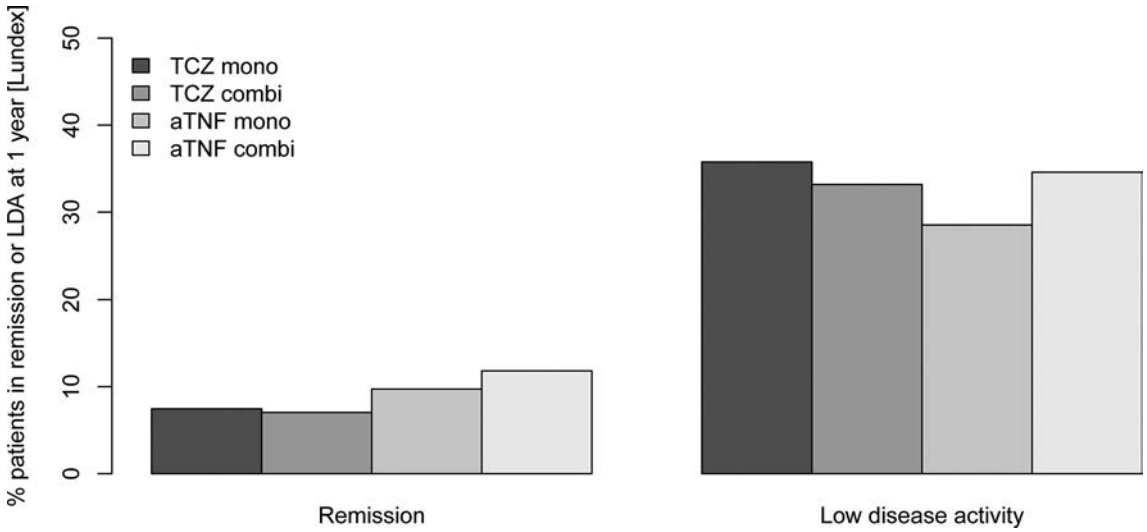

Figure 2 Clinical Disease Activity Index (CDAI) remission or low disease activity (LDA) at 1 year by biologics and presence or not of concomitant conventional synthetic disease-modifying antirheumatic drugs (csDMARDs). Two hundred and fifty-one TCZ mono, 737 TCZ combo, 375 TNFi mono and 1995 TNFi combo patients were still under treatment with CDAI information at 1 year and could be included in the LUNDEX calculation. TCZ combo, tocilizumab in combination with csDMARDs; TCZ mono, tocilizumab as monotherapy; TNFi combo, TNF inhibitors in combination with csDMARDs; TNFi mono, TNF inhibitors as monotherapy.

response to MTX or in whom MTX was not appropriate. Recent data from another head-to-head trial comparing adalimumab with sarilumab, another anti-IL-6R antibody, showed that in monotherapy sarilumab is superior to adalimumab.[38] These results indicate that IL-6 receptor antagonists have an advantage over TNFi when prescribed as monotherapy. Consistent with these findings, the current European League Against Rheumatism recommendations for the management of RA, mention that IL-6 inhibitors and Janus kinase inhibitors may have some advantage over other bDMARDs, if patients cannot use csDMARD.[8] The ACT-iON study has shown that, after an inadequate response to csDMARDs, the efficacy and retention of intravenous TCZ was better than TNFi, both given mostly in combination therapy.[39] However, none of these studies compared TCZ mono with TNFi combo. Furthermore, these studies did not include patients previously exposed to TNFi and other bDMARDs.

The rate of TNFi retention was higher in patients receiving TNFi in combination with MTX alone than with other csDMARD. Similarly, patients treated with the highest MTX doses had longer treatment maintenance than those receiving TNFi with lower MTX doses. The superiority of MTX versus other csDMARDs is consistent with the results of previous clinical trials and observational studies.[10 40 41] Furthermore, MTX has been shown to have a dose-dependent positive influence on adalimumab efficacy.[42] However, in the covariate-adjusted analysis, hazards of discontinuation were still lower with TCZ mono than in TNFi in combination with the highest dose of MTX (>15 mg weekly) (table 2).

We found no difference in the change of CDAI over time between TCZ and TNFi, whereas effects on DAS28 were significantly different between groups, with more patients treated with TCZ achieving remission or LDA. This finding is consistent with the effect of IL-6 blockade on acute-phase reactants.[30]

Our study has several limitations. We took into account only patients who previously used at least one bDMARD, which may reduce the external validity of our findings. However, our results are relevant to clinical practice since TCZ, especially as monotherapy, is commonly prescribed in patients who had previously been exposed to bDMARDs. Because of the observational nature of our data, we cannot exclude potential unmeasured confounders in the baseline characteristics for which we cannot adjust. In particular, only a few registries captured comorbidities and we could not include them in our analysis. The recording of causes of discontinuation in the registries were also not detailed enough to allow further analysis, with the great majority accounted as 'other reason' than lack of effectiveness or adverse events. Another consequence of the observational nature of the data is that patients, initially classified as combo or mono according to baseline data, may have either stopped the csDMARD or started a csDMARD over time. However, this misclassification bias of the exposure seemed relatively small (~7% for TCZ mono and TNFi combo). Finally, several studies reported poor adherence to MTX and under-recognition of this phenomenon by treating physicians.[43 44] Unfortunately, our data do not allow us to evaluate the importance of this phenomenon. The strengths of our study are that we have a large sample of patients with a long duration of follow-up and detailed data on clinical end points. The observational setting also allows the inclusion of a diverse population of patients from different countries without the strict inclusion criteria generally used in randomised controlled trial.

In conclusion, our results support that TCZ mono or in combination with csDMARDs are reasonable therapeutic options in patients with inadequate response to at least one bDMARD, with similar effectiveness in terms of CDAI to the TNFi combination therapy but longer retention.

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Ethics approval Approval of each local ethical committee for the collection of clinical data in each registry.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement All the data belong to the registries. Anonymised data can be shared as long as agreements are made with all participating registries.

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EXTENDED REPORT

The predictive role of ultrasound-detected tenosynovitis and joint synovitis for flare in patients with rheumatoid arthritis in stable remission. Results of an Italian multicentre study of the Italian Society for Rheumatology Group for Ultrasound: the STARTER study

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ABSTRACT

Objective To define the role of ultrasound (US) for the assessment of patients with rheumatoid arthritis (RA) in clinical remission, including joint and tendon evaluation.

Methods A multicentre longitudinal study has been promoted by the US Study Group of the Italian Society for Rheumatology. 25 Italian centres participated, enrolling consecutive patients with RA in clinical remission. All patients underwent complete clinical assessment (demographic data, disease characteristics, laboratory exams, clinical assessment of 28 joints and patient/physician-reported outcomes) and Power Doppler (PD) US evaluation of wrist, metacarpalphalangeal joints, proximal interphalangeal joints and synovial tendons of the hands and wrists at enrolment, 6 and 12 months. The association between clinical and US variables with flare, disability and radiographic progression was evaluated by univariable and adjusted logistic regression models.

Results 361 patients were enrolled, the mean age was 56.20 (±13.31) years and 261 were women, with a mean disease duration of 9.75 (±8.07) years. In the 12 months follow-up, 98/326 (30.1%) patients presented a disease flare. The concurrent presence of PD positive tenosynovitis and joint synovitis predicted disease flare, with an OR (95% CI) of 2.75 (1.45 to 5.20) in crude analyses and 2.09 (1.06 to 4.13) in adjusted analyses. US variables did not predict the worsening of function or radiographic progression. US was able to predict flare at 12 months but not at 6 months.

Conclusions PD positivity in tendons and joints is an independent risk factor of flare in patients with RA in clinical remission. Musculoskeletal ultrasound evaluation is a valuable tool to monitor and help decision making in patients with RA in clinical remission.

INTRODUCTION

The management of rheumatoid arthritis (RA) has changed dramatically over the last 20 years thanks to early intensive treatment and the availability of new drugs. In order to assess disease activity, the European League Against Rheumatism (EULAR) recommends ultrasonography (US) for both assessing inflammatory activity and evaluating patients in remission as it can detect inflammation predicting subsequent joint damage.[1]

On the other hand, the more recent EULAR recommendations for the management of RA[2] state that Boolean and index-based (Simplified Disease Activity Index—SDAI and Clinical Disease Activity Index—CDAI) definitions of clinical remission should be used for defining disease activity and remission. Further, two recent studies that compared targeting US remission with targeting clinical remission or low disease activity demonstrated no advantages of targeted US remission.[3 4] However, such strategic trials in patients in clinical remission are lacking and there is no recommendation on the use of imaging in patients achieving clinical remission.

Musculoskeletal ultrasound (MSUS) can provide diagnostic and prognostic data in terms of risk of flare, disability and damage progression in RA.[5–8] Furthermore, MSUS allows the assessment of periarticular structures such as tendons, which could present inflammatory changes also in clinical remission.[9] In particular, the prognostic value of US tendon inflammation in patients in clinical remission is not known.

On this basis, the MSUS Study Group of the Italian Society for Rheumatology prioritised its research activities on defining the role of US for the assessment of patients with RA in clinical remission, launching the Sonographic Tenosynovitis/arthritis Assessment in Rheumatoid Arthritis Patients in Remission (STARTER) study. The main objective of this study is to determine the prevalence of US tenosynovitis in patients with RA in clinical remission and its association with unstable remission, function and damage. The secondary aim of the study is to assess the prevalence of joint synovitis and its association with flare, function and damage.

METHODS

Patient and study design

This is a longitudinal analysis of the STARTER study, including 25 rheumatology centres. Selection criteria are fully described elsewhere.[10] Consecutive patients with RA (American College of Rheumatology (ACR) criteria 1987[11] or ACR/EULAR 2010 criteria[12]) in clinical remission were recruited between October 2013 and June 2014. Remission was defined as: Disease Activity Score on 28 joints (DAS28) <2.6,[13] SDAI ≤3.3,[14] CDAI ≤2.8,[15] ACR/EULAR Boolean definition,[16] the absence of swollen/tender joints on 28 joints[17] or remission based on clinical evaluation of an expert rheumatologist.[18] For the present analyses, patients with a baseline DAS28 <3.2 were included. A secondary analysis in patients with DAS28 <2.6 was performed for the primary outcome and the functional outcome.

Written informed consent was obtained.

Clinical assessment

A full description of the clinical assessment is reported in the online supplementary file S1. Demographic (age and sex) and clinical variables (disease and remission duration and treatment), rheumatoid factor (RF) and anti-cyclic citrullinated peptide antibody (ACPA) were recorded at baseline. Clinimetric measures (the Italian version of the Health Assessment Questionnaire (HAQ),[19] Visual Analogue Scale for pain, physician global assessment, patient global assessment and global health), erythrocyte sedimentation rate, C reactive protein and 28-joint count were collected at baseline, 6 and 12 months by a rheumatologist blinded to US findings and before the US examination. Treatment (including disease-modifying antirheumatic drugs (DMARDs), biologics, corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs)) could be modified by discretion of the treating physician. Hands, wrists and feet plain radiographs were collected at baseline and 12 months. The Sharp van Der Hejide Score (erosion, joint space narrowing (JSN) and total score) was measured in pairs of radiographs by two external assessors blinded to clinical and US findings.

Outcome measures

Disease flare, defined as change in DAS28 ≥1.2 or ≥0.6 if final DAS28 >3.2,[20] was the primary outcome. An alternative definition of flare, defined as the intention of the treating physician to increase therapy, was also recorded. Secondary outcomes included a change in the HAQ≥0.23[21] and the change in the Sharp van Der Hejide Score (SHS) (total (Δ >4.3), erosion (Δ ≥3) and JSN (Δ ≥2)).[22] For all outcomes, US variables were measured at baseline and outcomes were evaluated at 12 months. A secondary analysis evaluated the impact of baseline US on flare at 6 months and the impact of 6 months US on flare at 12 months, including dichotomous variables in the model for treatment decrease (defined as dose reduction or withdrawals of DMARDs, biologics or corticosteroids, including dose modification related to adverse events) and for treatment increase (defined as dose increase or introduction of a new drug). Further, to test the solidity of our results, the main analysis was repeated introducing the centre as a random effect. Finally, an exploratory analysis of patients fulfilling the SDAI definition of remission (≤3.3) for the primary outcome (flare defined based on the change of DAS28) was performed.

Ultrasonographic assessment

Ultrasonographers were rheumatologists expert in MSUS selected by an inter-observer and intra-observer reliability exercise against a reference standard (AI) on static images using an e-learning platform. A good to excellent reliability (weighted kappa ≥0.7)[23] was required. Centres providing high level US machines (MyLab 70XVG, MyLab Twice, Logiq9, LogiqE9) with high frequency probes (14–18 MHz) were included (online supplementary file S1). MSUS following the EULAR guidelines[24] was performed by a single ultrasonographer blinded to clinical data at the baseline, 6 and 12 months.

A detailed description of the scanning protocol has been published previously.[10] The flexors of the fingers, the flexor carpi radialis and the extensor tendons of the wrist were scanned bilaterally. The dorsal aspects of wrists (radiocarpal and midcarpal joints), metacarpophalangeal joints and the palmar aspects of proximal interphalangeal joints were scanned bilaterally.

Tenosynovitis, joint effusion and synovial hypertrophy were identified according to Outcome Measures in Rheumatology Clinical Trials definitions.[25] Power Doppler (PD) assessment was performed under standardised settings.[10] Representative images were recorded.

Grey scale (GS) and PD tenosynovitis (T) and synovitis (S) were semiquantitatively scored from 0 to 3. Total scores for GS and PD T and S were obtained as the sum of single sites. An image atlas with examples of the scoring was distributed to the sonographers.[10]

T and S were treated as categorical variables, defining their presence in case of GS or PD ≥1. To test the solidity of our results, alternative definitions were tested (GS >1, PD >1 for T and S).

Statistical analysis

The minimal sample size to detect an OR of 1.91 for the primary outcome was defined as 250 subjects. Analyses were performed on complete data. Missing data were handled using available case analysis; in each analysis, all cases with available data on the relevant variables were included. Descriptive statistics were performed for demographic, clinical and US variables, reporting results as percentages, mean with SD or median and IQRs. Patients presenting a flare in the first 6 months were compared with patients with a flare in the second 6 months by Wilcoxon signed-rank test or Pearson's χ^2 test.

The association between US variables and the outcomes was evaluated through logistic regression and the results were presented as OR and 95% CI, both crude and adjusted for prespecified confounders: age, sex, disease duration, remission duration, musculoskeletal comorbidities, RF, ACPA, DMARDs, biologics, NSAIDs, systemic glucocorticoids and local injected glucocorticoids. To fully evaluate the predictive role of US variables, time-to-event analyses using Cox proportional hazard models were also performed, using the same confounders.

To evaluate the additional impact of US and clinimetric variables on top of clinical findings, a model predicting the risk of

flare, including age, gender, disease duration, musculoskeletal comorbidities, RF and ACPA, remission duration, DMARDs, biologics, steroid injections and NSAIDs, was created, presenting the results as area under the curve (AUC) with 95% CI. Each single variable was added to the null model. Since some of the clinimetric variables are included in DAS28 and relate directly to the outcome, flare also defined as intention to change treatment was tested; this definition was correlated with DAS28-defined flare by Pearson's correlation coefficient.

Data were collected and managed using Research Electronic Data Capture (REDCap).[26] Analyses were performed using STATA software package (2009, release 11; StataCorp, Texas, USA) and R statistical software (Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Demographic and clinical characteristics

A total of 361 patients were included, with a mean (SD) age of 56.19 (13.31) years, and 261/361 (72.3%) were women, with a mean (SD) disease duration of 9.75 (8.07) years. Patients who satisfied any remission criteria but had a DAS28>3.2 at the time of enrolment were included for the cross-sectional analysis (66 patients) that has been published previously[10] but excluded from the longitudinal one as they had a partial overlap with the primary outcome. The 283 patients with DAS28 <2.6 had a mean (SD) age of 55.85 (13.55) years and 202/283 (71.3%) were women. Clinical and demographic features of both populations are presented in online supplementary file S2, while treatment patterns are displayed in online supplementary file S3. After 6 months, 344 patients were still followed, while at 12 months 340. The clinical and US features at each time point are shown in table 1.

Primary outcome: disease flare

In the follow-up, 98/326 (30.0%) patients presented a flare. When comparing 56 patients with a flare in the first 6 months with patients with a flare in the second 6 months (40 patients), there were no statistically significant differences in the demographic, clinical and clinimetric variables. For two patients with a flare at 12 months, the 6 months DAS28 were missing. Online supplementary file S4 reports the clinical and US variables in patients with flare at available follow-up times. In the same population, the concurrent presence of PD T and S and GS T and S predicted disease flare, with an OR (CI) of 2.75 (1.45 to 5.20) in crude analyses and 2.09 (1.06 to 4.13) in adjusted analyses for PD and of 2.88 (1.34 to 6.14) in crude models for GS, which was no longer statistically significant (2.25 (1.00 to 4.06)) when adjusted (table 2, figure 1). When applying Cox proportional hazard model, while in crude analysis PD S and the concurrent presence of PD T and S were significant predictors of flare, after adjustment only PD S predicted flare (data not shown). When adding the centre as a random effect, concurrent PD T and S were still significant predictors (OR 2.19 (CI 1.07 to 4.48)) while also concurrent GS T and S achieved statistical significance (OR 2.37 (CI 1.03 to 5.49)). To verify the effect of the inclusion of multiple confounders, we repeated the analysis keeping in the model only significant confounders. This confirmed the previous results (data not shown).

In the 182 patients with DAS28 <2.6, in which 75 flares occurred, the concurrent presence of T and S significantly predicted flare in crude models for both GS and PD (OR 2.59 (CI 1.25 to 5.35) and 2.64 (1.17 to 5.96), respectively), but statistical significance was lost after adjustment (OR 1.87 (CI 0.85 to 4.12) and 1.94 (CI 0.80 to 4.68), respectively). At baseline, 261 patients fulfilled SDAI remission, of whom 61 had a flare in the 12 months of observation. In this population, only the concurrent presence of PD positive synovitis and tenosynovitis significantly predicted flare (OR 2.32 (CI 1.01 to 5.32)) (online supplementary file S5).

Secondary outcome: HAQ

In the 12 months follow-up, 70/326 (21.5%, 14 had missing data) of patients had a significant increase in the HAQ; 33/228 patients (14.5%) in the non-flare group and 35/98 (35.7%) in the flare group (p<0.001), while for 2 the information on flare was missing. In the DAS28 <2.6 cohort, 55/283 (19.4%) had a significant increase in the HAQ. In both cohorts, US variables did not significantly predict the worsening of function (online supplementary file S6).

Secondary outcome: radiographic progression

For 189 of 361 patients of this longitudinal analysis, baseline and 12-month radiographs were available. At baseline, the median total SHS (IQR) was 24.52 (3–31), the median erosion score was 1 (0–5) and the JSN score was 8 (2-25). At 12 months, 39/189 patients (20.6%) had a progression in the total score, 25/189 (13.2%) in the erosion score and 71/189 (37.6%) in the JSN score. The mean (SD) change of the total SHS score was 3.1 (8.28), 1.12 (3.6) for the erosion score and 1.91 (6.84) for JSN score. In the population of patients with DAS28 <2.6 (157/361 patients with complete radiographic data), the median (IQR) baseline total SHS was 9 (2–28), while the scores for erosions and JSN were 1 (0–4) and 7 (2–23), respectively. At 12 months, 34/157 (21.7%) patients had a progression in the total SHS score, 20/157 (12.7%) in the erosion score and 59/157 (37.6%) in the JSN score. The mean (SD) change of the total SHS was 3.06 (6.42); 1.08 (3.7) and 1.98 (4.68) for the erosion and JSN scores, respectively. Patients with radiographic progression were equally distributed in the groups of patients with and without flare (12/55–21.8% and 27/130–20.8%, respectively, p=1; four patients had missing data for flare). None of the investigated US variables significantly predicted radiographic progression also when erosion and JSN scores were examined separately (online supplementary file S7).

Sensitivity analysis—stringent GS and PD definitions

More stringent definitions for GS and PD were applied in patients with DAS28 <3.2, in which 98/326 (30.0%) flares were reported. In crude and adjusted models, concurrent GS T and S predicted flare (OR 2.9 (CI 1.2 to 7.05)). For PD, in both crude and adjusted models, only the presence of isolated S predicted flare (OR 1.98 (CI 1.02 to 3.81)). Three hundred and forty patients were available to assess HAQ progression, and 70 significantly progressed, but no US variable predicted a significant progression (online supplementary file S8). For progression of the SHS, occurring in 39/189 (20.6%) patients, while in crude analysis GS S predicted progression of the erosion score (occurring in 25/189 (13.2%) patients) and JSN score (occurring in 71/189 (37.6%) patients), this was no longer significant when adjusted. In both crude and adjusted analyses, the concurrent presence of GS T and S significantly predicted the progression of the JSN score (OR 5.28 (CI 1.26 to 22.21)) (online supplementary file S9).

Risk of 6-month and 12-month flares

The risk of flare at 6 months based on baseline US and the risk of flare at 12 months based on 6 months US were calculated. In

Table 1 Baseline, 6-month and 12-month demographic, clinical and US features

	Baseline	6 months	12 months
	(361)	(344)	(340)
Age, years (mean, (SD))	56.20 (13.31)		
Female/male (n, (%))	261/100 (72.3/27.7)		
BMI (mean, (SD))	24.42 (4.01)		
Current smokers (n, (%))	65 (18.1)		
Disease duration, years (mean, (SD))	9.75 (8.07)		
Remission duration, months (mean, (SD))	20.30 (21.97)		
Extra-articular manifestation (n, (%))	96 (26.6)		
MSK comorbidities (n, (%))			
Fibromyalgia	8 (2.2)		
Osteoarthritis	74 (20.5)		
Microcrystalline arthritis	3 (0.8)		
Erosions (n, (%))	195 (54.3)		
sDMARDs (n, (%))	276 (76.54)		
bDMARDs (n, (%))	156 (43.2)		
Combination therapy (n, (%))	91 (25.2)		
Corticosteroids (n, (%))	163 (45.2)		
Joint injections in the previous month (n, (%))	7 (1.9)		
NSAIDs (n, (%))			
On demand	198 (54.9)		
Full dosage	6 (1.7)		
Anti-citrullinated peptide antibody positive (n, (%))	207 (57.7)		
Rheumatoid factor positive (n, (%))	201 (55.8)		
DAS28 (mean, (SD))	2.03 (0.68)	2.26 (0.92)	2.33 (0.99)
SDAI (median, (IQR))	1.7 (0.7–3.5)	1.9 (0.5–5.1)	2.26 (0.71–5.33)
VAS PGA (median, (IQR))	4 (0–13)	4.5 (0–20)	7 (0–17.75)
VAS EGA (median, (IQR))	4 (0–10)	5 (0–12)	6 (0–16.5)
Swollen joint count (28 joints, median, (IQR))	0 (0–0)	0 (0–1)	0 (0–1)
Tender joint count (28 joints, median, (IQR))	0 (0–0)	0 (0–1)	0 (0–1)
ESR (median, (IQR))	11 (5–18)	11 (6–19)	12 (6–20)
CRP (median, (IQR))	0.07 (0–0.3)	0.1 (0–0.4)	0.1 (0–0.46)
VAS pain (median, (IQR))	6 (0–16)	6 (0–20)	7 (0–20)
HAQ (median, (IQR))	0 (0–0.38)	0.13 (0–0.38)	0.06 (0–0.38)
van der Heijde modifed Sharp score (median, (IQR))	9 (3–28)		12 (4–40.5)
Erosion score (median, (IQR))	1 (0–4)		2 (0–7)
Joint space narrowing score (median, (IQR))	7 (2–21.75)		9 (2–32.5)
GS_T positive patients (n, (%))	189 (52.3)	157 (46.2)	153 (46.8)
GS_T score in positive patient group (median, (IQR))	2 (1–4)	3 (1–4)	3 (1–4)
GS_T positive tendons per patient (median, (IQR))	1 (0–2)	0 (0–2)	0 (0–1)
GS_T score (median, (IQR))	1 (0–3)	0 (0–2)	0 (0–2)
PD_T positive patients (n, (%))	85 (23.6)	73 (21.5)	68 (20.8)
PD_T score in positive patient group (median, (IQR))	2 (1–4)	2 (1–3)	2 (1–4)
PD_T positive tendons per patient (median, (IQR))	0 (0–0)	0 (0–0)	0 (0–0)
PD_T score (median, (IQR))	0 (0–0)	0 (0–0)	0 (0–0)
GS_S positive patients (n, (%))	260 (72.0)	229 (67.4)	220 (67.3)
GS_S score in positive patient group (median, (IQR))	3 (2–6)	3 (2–6)	4 (2–6.25)
GS_S positive joints per patient (median, (IQR))	2 (0–4)	1 (0–4)	1 (0–4)
GS_S score (median, (IQR))	2 (0–5)	2 (0–4)	2 (0–5)
PD_S positive patients (n, (%))	161 (44.6)	134 (39.4)	132 (40.4)
PD_S score in positive patient group (median, IQR)	3 (2–5)	2 (1–4)	2 (1–5)
PD_S positive joints per patient (median, IQR)	0 (0–2)	0 (0–1)	0 (0–1)
PD_S score (median, IQR)	0 (0–2)	0 (0–2)	0 (0–2)
GS_T+GS_S positive patients (n, (%))	292 (80.9)	256 (75.1)	240 (73.4)
PD_T+PD_S positive patients (n, (%))	184 (51.0)	157 (46.2)	143 (43.7)
Patients with flare positive in US (any item)		53 (15.87)	57 (17.81)
Patients with positive US (any item) without flare		201 (60.18)	182 (56.88)

bDMARDs, biological disease-modifying antirheumatic drugs; BMI, body mass index; CRP, C reactive protein; DAS28, Disease Activity Score on 28 joints; EGA, evaluator's global assessment; ESR, erythrocyte sedimentation rate; GS, grey scale; HAQ, Health Assessment Questionnaire; MSK, musculoskeletal; n, number; NSAIDs, non-steroidal anti-inflammatory drugs; PD, power Doppler; PGA, patients global assessment; S, synovitis; SDAI, Simplified Disease Activity Index; sDMARDs, synthetic disease-modifying antirheumatic drugs; T, tenosynovitis; US, ultrasonography; VAS, Visual Analogue Scale.

Table 2 ORs and CI for flare (increase of DAS28≥1.2 or ≥0.6 if final DAS28>3.2)

	Baseline US → 6 months flare (361)		6 months US→ 12 months flare (291)	
	OR (CI) (338)	Adj OR (CI)(336)	OR (CI)(273/274)	Adj. OR (CI) (271/272)
PD-T	0.7 (0.15 to 3.21)	0.72 (0.15 to 3.44)	2.07 (0.62 to 6.94)	3.03 (0.83 to 11.07)
PD-S	1.84 (0.95 to 3.6)	1.78 (0.87 to 3.64)	*2.75 (1.31to5.77)*	*2.86 (1.3to6.33)*
PD-T+PDs	1.77 (0.81 to 3.86)	1.3 (0.56 to 3.01)	2.27 (0.86 to 6.01)	1.79 (0.63 to 5.12)
GS-T	1.89 (0.53 to 6.76)	2.32 (0.59 to 9.12)	3.15 (0.76 to 13.02)	2.96 (0.67 to 13.07)
GS-S	2.43 (0.91 to 6.45)	2.82 (0.99 to 8.06)	2.76 (0.94 to 8.09)	2.67 (0.89 to 8.08)
GS-T+GSs	2.13 (0.83 to 5.46)	1.88 (0.69 to 5.12)	*4.06 (1.46 to 11.26)*	*4.02 (1.37to11.82)*

Flare occurred in 56 patients in the first semester and in 40 patients in the second one. Baseline US over the risk of flare at 6 months and 6 months US over the risk of flare at 12 months. Analyses adjusted for age, gender, disease duration, musculoskeletal comorbidities, rheumatoid factor positivity +ACPA, remission duration, the use of synthetic disease - modifying antirheumatic drugs, biological disease - modifying antirheumatic drugs, corticosteroids or non-steroidal anti-inflammatory drugs.
DAS28, Disease Activity Score on 28 joints; GS, grey scale; PD, power Doppler; S, synovitis; T, tenosynovitis; US, ultrasonography.

crude and adjusted models, flare at 6 months was not predicted by US variables. Conversely, flare at 12 months was predicted in both crude and adjusted analyses by PD S (OR 2.86 (CI 1.3 to 6.33)) and GS T+S (OR 4.02 (CI 1.37 to 11.82)) (table 2).

Treatment decrease

Analyses of the risk of flare at 12 months, predicted by 6 months US variables, were repeated inserting a dichotomous variable into treatment decrease. With the addition of this variable, in adjusted models, PD S (OR 3.01 (CI 1.36 to 6.63)) and GS T+S (OR 3.86 (CI 1.31 to 11.39)) were still significant predictors of flare (online supplementary file S10).

Application of US information in a clinical context

A weak but significant correlation was found between DAS28-defined flare and the intention to change treatment (r 0.22, p<0.001). The AUC (CI) of the null model was 0.661 (CI 0.598 to 0.725) for DAS28 flare and 0.665 (CI 0.556 to 0.774) for treatment change. When adding US and clinical variables to the model, none of the variables led to a relevant increase with both outcomes. An AUC >0.75 was obtained only with the addition of Visual Analogue Scale pain (table 3).

Table 3 Area under the curve with CI of the prediction models, defining flare as a change in DAS28 (increase of DAS28 ≥1.2 or ≥0.6 if final DAS28 >3.2) or as the intention to change treatment by the clinician

AUC (CI)	DAS28 flare (324)	Change of treatment (330)
Null model	0.661 (0.598 to 0.725)	0.665 (0.556 to 0.774)
Null model+US		
GS_T	0.670 (0.608 to 0.733)	0.663 (0.555 to 0.772)
GS_S	0.674 (0.612 to 0.736)	0.679 (0.576 to 0.781)
PD_T	0.670 (0.606 to 0.733)	0.684 (0.580 to 0.788)
PD_S	0.690 (0.626 to 0.755)	0.716 (0.616 to 0.817)
GS	0.680 (0.618 to 0.742)	0.672 (0.568 to 0.777)
PD	0.690 (0.626 to 0.754)	0.720 (0.621 to 0.819)
Null model+clinimetric variables:		
VAS PGA	0.661 (0.598 to 0.724)	0.734 (0.644 to 0.824)
VAS EGA	0.686 (0.622 to 0.751)	0.668 (0.562 to 0.775)
VAS pain	0.661 (0.597 to 0.724)	0.768 (0.678 to 0.859)
VAS GH	0.661 (0.598 to 0.725)	0.698 (0.596 to 0.800)
Null model+joint count:		
SJC	0.665 (0.602 to 0.728)	0.668 (0.557 to 0.779)
TJC	0.661 (0.598 to 0.725)	0.690 (0.594 to 0.786)

The null model includes age, gender, disease duration, musculoskeletal comorbidities, rheumatoid factor and anti-cyclic citrullinated peptides, remission duration, disease-modifying antirheumatic drugs, biological disease-modifying antirheumatic drugs, steroid injections and non-steroidal anti-inflammatory drugs.
AUC, area under the curve; DAS28, Disease Activity Score on 28 joints; EGA, evaluator's global assessment; GH, general health; GS, grey scale; PD, power Doppler; PGA, patient global assessment; S, synovitis; SJC, swollen joint count; T, tenosynovitis; TJC, tender joint count; US, ultrasonography; VAS, Visual Analogue Scale.

DISCUSSION

According to the latest EULAR recommendations, the treatment of RA should aim at clinical remission,[2] defined by clinical indices, to prevent joint damage and worsening of function. On the other hand, subclinical imaging-detected inflammation in clinical remission leads to flare and radiographic progression.[5 7 18 27 28] Further, clinical indices do not consider tendon involvement, which is frequent[29 30] and has an impact on disability.[31] Finally, the cross-sectional results of the STARTER study show the association between tenosynovitis and FLARE questionnaire in clinical remission.[10]

The longitudinal analysis of the STARTER cohort confirmed that the conjunct presence of PD positive tenosynovitis and synovitis predicts disease flare. While this result emerges consistently in the overall population and in patients in remission according to SDAI, it is not confirmed when limiting the analyses to patients with DAS28 <2.6, possibly because of a smaller sample and a baseline lower risk of flare. With more stringent definitions for synovitis and tenosynovitis, a potential predictivity also emerged for GS. The conjunct effect of tenosynovitis and synovitis was not confirmed in time-to-event analysis, while synovitis was still a significant predictor of flare, suggesting in this case a more prominent role of synovitis. This is the first description of the impact of US-detected tenosynovitis in RA in clinical remission, highlighting a gap in the evaluation of disease activity, which is limited to joints. Taking also tenosynovitis into account could better drive therapeutic decisions, since the impact seems to be more relevant in patients in which treatment is tapered. In addition, patients with positive PD have a higher risk of flare and should be monitored more tightly. This result was achieved defining clinical remission heterogeneously, in a multicentre study, using different US machines with different operators. While this might be regarded as a limitation, it probably implies a larger generalisability of the result, which is more likely to be reproduced in a clinical setting.

Regarding the secondary outcomes, US tenosynovitis or synovitis did not show any correlation with function worsening defined by the HAQ. This could be expected, as the sample size was powered to detect the primary outcome. The detection of a difference in patients who are not likely to progress

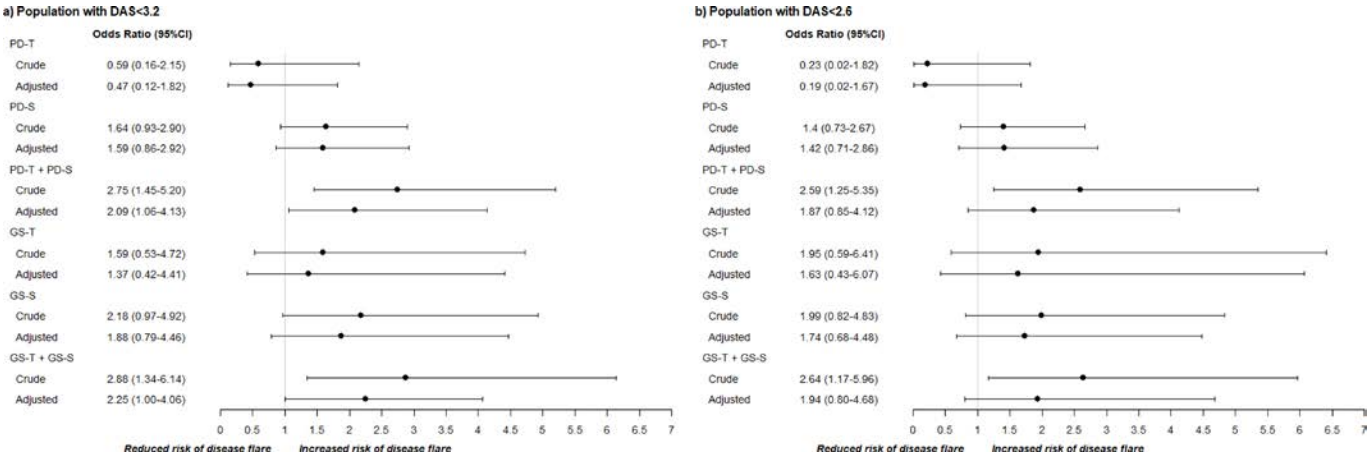

Figure 1 ORs and CI for the occurrence of flare (increase of DAS28≥1.2 or ≥0.6 if final DAS28>3.2). Analyses were performed on 326 patients; 98 flares occurred during observation and were adjusted for age, gender, disease duration, musculoskeletal comorbidities, rheumatoid factor and ACPA positivity, remission duration, the use of synthetic disease - modifying antirheumatic drugs, biological disease -modifying antirheumatic drugs, corticosteroids or non-steroidal anti-inflammatory drugs. DAS28, Disease Activity Score on 28 joints; GS, grey scale; PD , power Doppler; S, synovitis; T, tenosynovitis.

rapidly would have required a larger sample as well as a longer follow-up.

The same considerations can be applied to the radiographic outcome, whose relevance has been questioned very recently,[32 33] based on the reduction of its occurrence[34] and our population is not an exception.

Regarding the timing of US, in our study, US predicts flare at 12 months but not at 6. This suggests that in patients without any US inflammation, it could be useful to repeat US yearly.

The addition of US and clinimetric variables to a model predicting flare did not lead to a relevant improvement of its performance. Neither swollen nor tender joint counts improved the prediction, and both counts, as well as acute phase reactants, remained substantially unchanged at flare, while greater changes were seen in patient's reported outcomes. This aspect raises a further very important question: are the actual clinical indices adequate for defining remission and flare in all patients? In many of our patients, US did not reveal inflammatory exacerbation and flare was mainly PRO driven. It looks like the hot soup paradox of the Italian tradition: 'who was burn with the soup blows also on the water?'

The need for composite disease activity indexes emerged in the 1990s and in a short period different indexes appeared.[15 35] All were meant to assess active disease but later emerged as a milestone in management.[2] Their thresholds for defining remission have been established[16] in randomised clinical trials and even in this context, almost 10% of patients in DAS28 remission had EGA and PGA scores compatible with active disease. In our cohort, comorbidities (with 20% of patients with osteoarthritis and 2% with fibromyalgia) could have interfered with the patient and physician's reported outcomes, shifting patients from stable to unstable remission.

US has demonstrated to be very sensitive in RA and its value has been acknowledged in the EULAR recommendations.[1] However, recent studies questioned the added value of US in guiding therapeutic decision,[3 4] since US did not demonstrate to improve the outcomes, despite some possible methodological limitations.[36] Further, the role of residual US-detectable inflammation in clinical remission is still not clearly defined, considering that inflammatory changes can also be found in healthy subjects.[37]

The STARTER study demonstrated that tendon and joint US can be useful in assessing inflammatory changes in RA in clinical remission to predict disease outcome and the impact of these findings on disease management should be tested in strategic trials.

On the other hand, in this cohort, disease flare is not always accompanied by a clinical and laboratory worsening but mainly by a change in the PROs, which might be influenced by comorbidities. In this scenario, US could confirm active disease and drive the therapeutic decision on top of composite indexes, in accordance with a recent proposal by a group of US experts.[38]

The research agenda on US in patients with RA in clinical remission is rich in unanswered questions regarding both the impact of PROs and the role of US. Decision making in RA should not be based on a single parameter and should be taken after acquisition of as much data as possible regarding the sensations of the patient and objective and reliable data on disease activity. This is a doctor's job, not a machine's or a number's.

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CONCISE REPORT

Health-related quality of life in patients with psoriatic and rheumatoid arthritis: data from the prospective multicentre NOR-DMARD study compared with Norwegian general population controls

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ABSTRACT

Objectives To compare (1) Short Form-36 (SF-36) Physical Component Summary (PCS) and Mental Component Summary (MCS), scale scores and Short Form-6 dimensions (SF-6D) between patients with rheumatoid arthritis (RA) and patients with psoriatic arthritis (PsA) and Norwegian general population controls and (2) improvements in these measures between patients with RA and PsA.

Methods Analyses of covariance were performed to compare SF-36 measures between first-time enrolled patients with RA (n=3898) and PsA (n=1515) from the prospective observational multicentre NORwegian-Disease Modifying Anti-Rheumatic Drug study (6 months follow-up) and general population controls (n=2323).

Results In age and gender-adjusted analyses, patients with PsA compared with patients with RA had similar PCS, MCS and SF-6D ($p \geq 0.14$), worse vitality and general health, but better physical functioning at 0/6 months ($p \leq 0.03$). With additional 28-joint disease activity scores adjustment as a proxy for joint inflammation, PCS, most scale scores and SF-6D were worse in patients with PsA than patients with RA at 0/3/6 months ($p \leq 0.01$). PCS was more impaired than MCS both in RA and PsA compared with general population controls ($p \leq 0.001$). Mean 3-month and 6-month improvements after disease-modifying anti-rheumatic drug treatment were larger in patients with RA than patients with PsA for bodily pain, vitality and mental health ($p \leq 0.02$).

Conclusions Health-related quality of life was overall similar in patients with RA and patients with PsA—with a tendency to worse scores in PsA—and worse compared with Norwegian general population controls.

INTRODUCTION

Health-related quality of life (HRQoL) is part of the core set of data to be collected in patients with psoriatic arthritis (PsA) and recognised to be of major importance also in other rheumatic diseases, including rheumatoid arthritis (RA).[1 2] HRQoL has been found to be impaired in patients with inflammatory arthritides compared with the general population.[3–6] In smaller observational studies performed 1–2 decades ago, similar HRQoL in patients with RA and patients with PsA were described[7 8] and also differences in Short Form-36 (SF-36) scale scores.[5]

To our knowledge no large, prospective observational study has compared HRQoL between patients with RA and patients with PsA and the general population using the widely recognised SF-36.

The aim of this study was to compare SF-36 Physical Component Summary (PCS) and Mental Component Summary (MCS), scale scores as well as Short Form-6 dimensions utility score (SF-6D) between patients with RA and patients with PsA from a large prospective observational study, as well as with general population controls. Furthermore, we aimed to compare improvements in PCS, MCS and scale scores between patients with RA and patients with PsA from baseline to 3-month and 6-month follow-ups after initiation of treatment with disease-modifying anti-rheumatic drugs (DMARDs).

METHODS

Patients

We included first-time enrolled patients with RA and patients with PsA from the prospective longitudinal observational multicentre NORwegian-Disease Modifying Anti-Rheumatic Drug (NOR-DMARD) study,[9 10] starting synthetic and/or biological DMARDs between 1 December 2000 and 6 November 2012 and followed until 1 May 2013 or until stopping DMARD medication. The RA and PsA diagnoses were given by the treating rheumatologist after clinical judgement. Analyses included baseline, 3-month and 6-month follow-ups. Written informed consent was obtained from each patient. For comparison, we included SF-36 Norwegian normative data from 2323 individuals collected in 1996.[11]

SF-36 and SF-6D

The Norwegian translation of SF-36 version 1, used in this study, is validated in Norwegian patients with RA.[12] PCS and MCS were calculated as described by Ware *et al*.[13] Norm-based scale scores were computed by subtracting the general population's respective mean scale score from the 0–100 scale scores, divided by the SD of the data from the general population. Each of these values were multiplied by 10 and 50 was added, as described by Ware *et al*.[14] SF-6D was calculated from SF-36 according to the algorithm developed by Brazier *et al*.[15]

Statistics

Demographic and baseline characteristics are shown as medians (25th and 75th percentiles) for non-normally distributed data and means (SD) for normally distributed data. Continuous variables were compared using independent t-test, Mann-Whitney U test or analysis of variance, as appropriate. Prespecified age and gender-adjusted analyses of covariance were performed to compare PCS, MCS, scale scores and SF-6D between patients with RA, patients with PsA and general population controls at baseline, and between RA and PsA patients with and without additional adjustment for the respective 28-joint disease activity scores (DAS28) at baseline and after 3-month and 6-month follow-up. Prespecified ANCOVA were performed to compare changes in PCS, MCS, scale scores and SF-6D from baseline to 3-month and 6-month follow-up, adjusted for age, gender and the respective baseline values. Radar diagrams were made to visualise differences in scale scores between patients with RA/PsA and the Norwegian general population. Statistical tests were performed using SPSS V.23.0 for Windows. The analyses were performed as completer analyses and without adjustment for multiple comparisons.

RESULTS

Patients with RA (n=3898), patients with PsA (n=1515) and general population controls (n=2323) had mean (SD) age 55.9 (31.6)/48.1 (12.6)/44.9 (16.5) years and 71.4%/50.3%/51.3% were females, respectively. Baseline mean (SD) DAS28 was worse in patients with RA (4.9 (1.4)) than patients with PsA (4.2 (1.3)) (online supplementary table S1).

Analyses adjusted for age and gender

SF-36 PCS, MCS, scale scores and SF-6D were worse in patients with RA and patients with PsA compared with the general population (table 1) but improved during follow-up (online supplementary table S2).

PCS and MCS were not statistically different in patients with RA compared with patients with PsA, while physical functioning was better and general health and vitality worse in patients with PsA at all time-points. Bodily pain was similar between patients with RA and patients with PsA at baseline but slightly worse in patients with PsA at 3 and 6 months (table 1, online supplementary table S2).

Analyses adjusted for age, gender and DAS28

In analyses adjusted for DAS28 in addition to age and gender, patients with PsA had significantly worse PCS and SF-6D at all time-points compared with patients with RA. Baseline MCS and all scale scores, except role emotional, were worse in PsA compared with patients with RA. At 3 and 6 months bodily pain, general health, vitality, social functioning and mental health were worse, and the remaining scale scores were similar in patients with PsA compared with patients with RA (online supplementary table S3).

Longitudinal analyses adjusted for age, gender and baseline values of the respective scores

Patients with RA and patients with PsA had similar improvements in PCS, MCS, all scale scores and SF-6D at 3 and 6 months, except for larger improvements in bodily pain, vitality

Table 1 Unadjusted analyses and analyses adjusted for age and gender of baseline SF-36 component summaries, norm-based scale scores and SF-6D utility scores

Baseline

Component summary/scale score	Unadjusted analyses, mean (SD)				Adjusted analyses, estimated marginal means (95% CI)			
	General population	RA	PsA	P value	General population	RA	PsA	P value
PCS	50.0 (10.1) (n=2012)	29.7 (9.9) (n=3898)	30.5 (9.5) (n=1515)	<0.001*† 0.02‡	49.7 (49.2 to 50.1)	30.5 (30.2 to 30.8)	30.4 (29.9 to 30.9)	<0.001*† 0.69‡
MCS	50.0 (10.2) (n=2012)	46.8 (11.3) (n=3898)	47.1 (11.4) (n=1515)	<0.001*† 0.59‡	50.0 (49.5 to 50.5)	47.1 (46.8 to 47.5)	47.1 (46.5 to 47.6)	<0.001*† 0.90‡
SF-6D	0.80 (0.14) (n=2071)	0.60 (0.12) (n=3787)	0.61 (0.12) (n=1491)	<0.001*† 0.17‡	0.80 (0.80 to 0.81)	0.61 (0.60 to 0.61)	0.60 (0.60 to 0.61)	<0.001*† 0.45‡
Physical functioning	49.4 (10.8) (n=2235)	27.7 (14.5) (n=3898)	30.5 (13.5) (n=1515)	<0.001*†‡	48.9 (48.3 to 49.4)	29.0 (28.5 to 29.4)	30.2 (29.6 to 30.9)	<0.001*† 0.001‡
Role physical	49.6 (10.3) (n=2207)	32.9 (9.1) (n=3898)	34.0 (9.9) (n=1515)	<0.001*† 0.001‡	49.4 (49.0 to 49.8)	33.5 (33.2 to 33.8)	33.9 (33.4 to 34.4)	<0.001*† 0.16‡
Bodily pain	49.6 (10.3) (n=2287)	33.5 (7.5) (n=3898)	33.6 (7.2) (n=1515)	<0.001*† 0.96‡	49.4 (49.1 to 49.8)	34.0 (33.7 to 34.2)	33.5 (33.1 to 33.9)	<0.001*† 0.07‡
General health	49.6 (10.3) (n=2183)	37.5 (9.5) (n=3898)	36.8 (9.7) (n=1515)	<0.001*† 0.02‡	49.5 (49.0 to 49.9)	37.9 (37.6 to 38.3)	36.7 (36.2 to 37.2)	<0.001*†‡
Vitality	49.8 (10.3) (n=2270)	39.6 (10.1) (n=3898)	39.3 (10.3) (n=1515)	<0.001*† 0.69‡	49.9 (49.5 to 50.3)	40.4 (40.1 to 40.8)	39.4 (38.9 to 39.9)	<0.001*†‡
Social functioning	49.5 (10.5) (n=2311)	39.1 (12.6) (n=3898)	39.5 (12.3) (n=1515)	<0.001*† 0.59‡	49.4 (48.9 to 49.9)	39.8 (39.4 to 40.2)	39.5 (38.9 to 40.1)	<0.001*† 0.40‡
Role emotional	49.8 (10.2) (n=2182)	40.2 (13.6) (n=3898)	41.6 (13.6) (n=1515)	<0.001*† 0.002‡	49.5 (49.0 to 50.0)	40.6 (40.2 to 41.1)	41.5 (40.8 to 42.1)	<0.001*† 0.03‡
Mental health	49.8 (10.3) (n=2255)	44.3 (11.5) (n=3898)	44.7 (11.1) (n=1515)	<0.001*† 0.63‡	49.8 (49.4 to 50.3)	44.8 (44.5 to 45.2)	44.7 (44.2 to 45.3)	<0.001*† 0.76‡

*General population compared with patients with RA.
†General population compared with patients with PsA.
‡RA patients compared with patients with PsA.
MCS, Mental Component Summary; PCS, Physical Component Summary; PsA, psoriatic arthritis; RA, rheumatoid arthritis; SF-6D, Short Form-6 dimensions utility score; SF-36, Short Form-36.

Table 2 Mean improvements from baseline until 3-month and 6-month follow-up, adjusted for age, gender and the respective baseline values

Component summary/scale score	Adjusted analyses, mean change (95% CI) from baseline until 3 months*		
	RA	PsA	P values
PCS	4.7 (4.3 to 5.0) n=3081	4.4 (3.9 to 4.9) n=1183	0.42
MCS	1.8 (1.4 to 2.1) n=3081	1.5 (1.0 to 2.0) n=1183	0.41
SF-6D	0.04 (0.03 to 0.04) n=2323	0.03 (0.03 to 0.04) n=872	0.06
Physical functioning	4.6 (4.2 to 5.0) n=3197	4.6 (4.2 to 5.2) n=1217	0.93
Role physical	4.2 (3.8 to 4.6) n=3178	4.3 (3.7 to 4.8) n=1208	0.83
Bodily pain	5.8 (5.5 to 6.1) n=3187	5.1 (4.6 to 5.5) n=1214	0.004
General health	1.4 (1.1 to 1.7) n=3149	1.3 (0.9 to 1.7) n=1203	0.61
Vitality	3.8 (3.5 to 4.2) n=3173	2.8 (2.3 to 3.4) n=1209	0.002
Social functioning	3.7 (3.3 to 4.1) n=3201	3.3 (2.8 to 3.9) n=1216	0.31
Role emotional	2.2 (1.8 to 2.7) n=3161	2.8 (2.1 to 3.4) n=1197	0.20
Mental health	2.5 (2.2 to 2.8) n=3170	1.9 (1.4 to 2.4) n=1210	0.008
Component summary/scale score	**Adjusted analyses, mean change (95% CI) from baseline until 6 months***		
	RA	**PsA**	**P values**
PCS	5.6 (5.2 to 6.0) n=2605	5.4 (4.8 to 5.9) n=1034	0.49
MCS	2.5 (2.2 to 2.9) n=2605	2.1 (1.5 to 2.6) n=1034	0.18
SF-6D	0.04 (0.04 to 0.05) n=1832	0.04 (0.03 to 0.05) n=721	0.42
Physical functioning	5.8 (5.4 to 6.3) n=2676	6.3 (5.6 to 7.0) n=1052	0.26
Role physical	5.6 (5.2 to 6.1) n=2670	5.6 (5.0 to 6.2) n=1048	0.91
Bodily pain	6.7 (6.3 to 7.0) n=2675	5.8 (5.3 to 6.3) n=1051	0.005
General health	1.7 (1.4 to 2.1) n=2659	1.3 (0.9 to 1.8) n=1050	0.17
Vitality	4.7 (4.3 to 5.1) n=2668	3.9 (3.3 to 4.4) n=1051	0.02
Social functioning	4.7 (4.3 to 5.1) n=2687	4.4 (3.8 to 5.0) n=1054	0.39
Role emotional	3.1 (2.6 to 3.6) n=2657	3.4 (2.7 to 4.2) n=1045	0.44
Mental health	3.2 (2.9 to 3.6) n=2665	2.5 (2.0 to 3.0) n=1052	0.02

*Estimated marginal means.
MCS, Mental Component Summary; PCS, Physical Component Summary; PsA, psoriatic arthritis; RA, rheumatoid arthritis; SF-6D, Short Form-6 dimensions utility score.

and mental health in the patients with RA (table 2, adjusted analyses and supplementary table S4, unadjusted analyses).

Graphical comparisons of scale scores

Estimated marginal means of baseline, 3-month and 6-month SF-36 scale scores adjusted for age and gender (figure 1) as well as DAS28 (figure not shown) were impaired in patients with RA and patients with PsA compared with the general population but showed only small differences between patients with RA and patients with PsA.

DISCUSSION

In this large prospective observational multicentre study, SF-36 PCS, MCS as well as the utility measure SF-6D were comparable between patients with RA and patients with PsA at baseline and at 6-month follow-up in age and gender-adjusted analyses. Furthermore, patients with PsA had worse general health and vitality but better physical functioning at all time-points, as well as more bodily pain at 3 and 6 months. With adjustment for DAS28 in addition to age and gender, patients with PsA compared with patients with RA had worse PCS, SF-6D, general health, vitality, bodily pain, social functioning and mental health at all time-points.

Furthermore, the study shows that levels of physical HRQoL were more impaired than mental HRQoL in patients with RA and PsA compared with Norwegian general population controls.

Improvements in scale scores from baseline until 3 months and 6 months were similar between patients with RA and patients with PsA during treatment with DMARDs, except for larger improvements in bodily pain, vitality and mental health in RA.

We have chosen to present norm-based scale scores to facilitate interpretation and comparison across different countries and populations.[14 16] Still, interpretation of the findings is not straight-forward; the statistically significant differences between patients with RA and patients with PsA may not always be clinically significant. As visualised in radar diagrams (figure 1), the differences in scale scores between patients with RA and patients with PsA are small. Still, for example for general health the difference between RA and PsA patients at different time-points is of similar magnitude as the improvement in general health from baseline until 6 months both for patients with RA and patients with PsA and hence of probable clinical significance.

The study underlines the severe impact of both RA and PsA on HRQoL reflected through SF-36, which is in line with a smaller observational study[3] as well as clinical trials.[4 17 18] Furthermore, the study highlights the relatively stronger impairment of HRQoL in patients with PsA compared with patients with RA, when also taking into consideration levels of joint inflammation, as measured by DAS28. Notably, also according to PsA patients' perspective HRQoL is of great importance.[1] The severe impact of PsA on HRQoL may possibly be explained by extra-articular inflammatory affection of, for example, skin or entheses. However, NOR-DMARD does not have data on these disease manifestations. We were therefore unable to identify which disease manifestations that contributed most to the reduced HRQoL in PsA. Of note, general health and vitality were worse in patients with PsA compared with patients with RA in all adjusted analyses at all time-points.

Patients with RA as well as patients with PsA reported better mental than physical HRQoL, which is in line with smaller observational studies on SF-36,[3] as well as with clinical trials.[4 17] Interestingly, the RA and PsA scale score profiles (figure 1) were similar to the RA scale score profile found by Strand and Singh.[17]

Furthermore, the study is partly in line with two considerably smaller NOR-DMARD studies reporting better 6-month improvements in bodily pain and vitality but not mental health

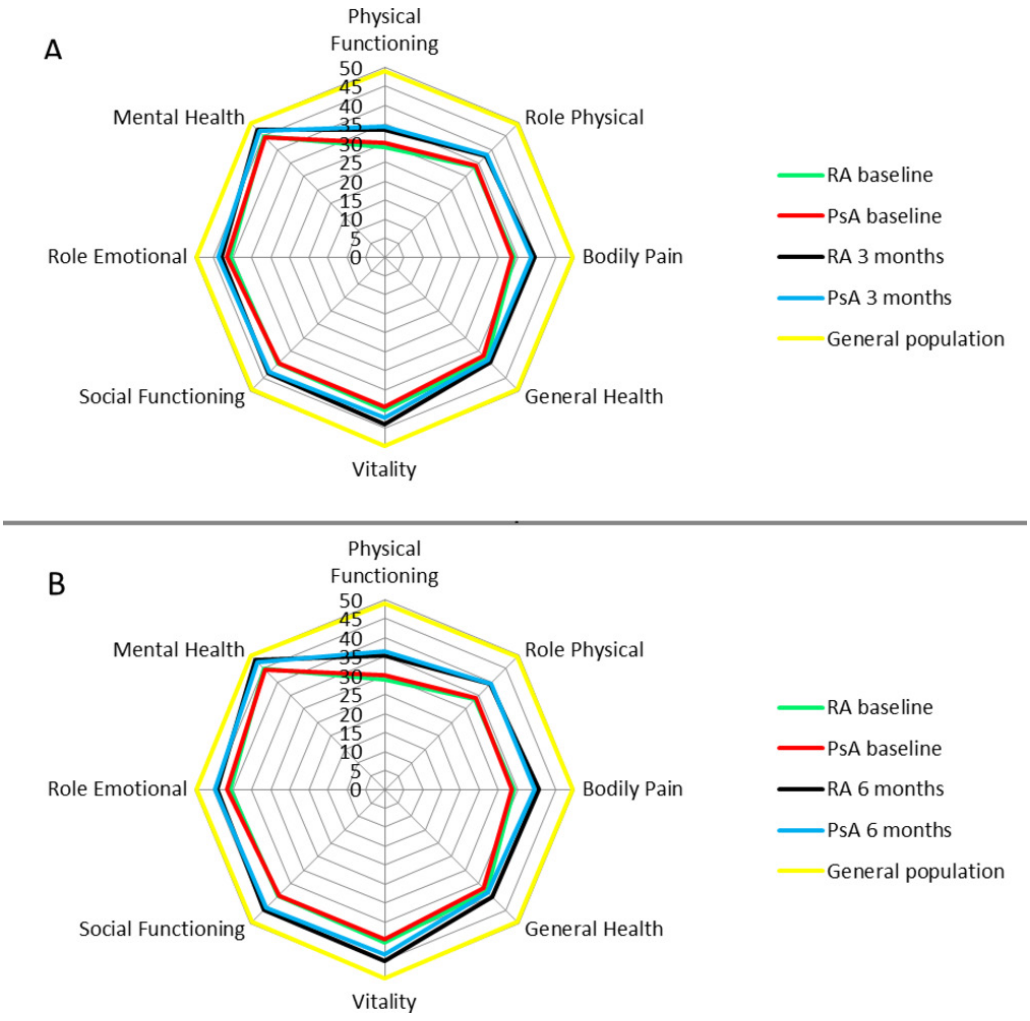

Figure 1 Estimated marginal means adjusted for age and gender of baseline and 3-month (A) and 6-month (B) norm-based scale scores in patients with rheumatoid arthritis (RA) (n=3898) and patients with psoriatic arthritis (PsA) (n=1515) compared with general population controls (n=2323).

in patients with RA compared with patients with PsA.[19 20] These studies did not include general population controls.

The generic SF-36 facilitated comparison of HRQoL across diseases, although it might capture somewhat different aspects of HRQoL than disease-specific measures, for example, the psoriatic arthritis quality of life.[21]

Limitations of the study include lack of 66/68 joint count as well as lack of measures of PsA inflammatory activity other than arthritis (eg, skin involvement, enthesitis, dactylitis and spondylitis), which may have led to underestimation of disease activity in PsA as used in the DAS28-adjusted analyses. Lack of adjustment for comorbidities may have biased the results, although partly corrected for by the age adjustment. Furthermore, completer analyses may have affected the generalisability of the results.

The major strength of the study is the prospective observational multicentre design including large cohorts of patients with RA and patients with PsA over a long time span, as well as Norwegian general population controls. To our knowledge, this is the largest prospective observational study comparing SF-36 between RA and PsA patients, and the first to compare SF-36 component summaries, scale scores as well as SF-6D between patients with RA and patients with PsA and general population controls in the same analyses.

In conclusion, HRQoL was overall similar in patients with RA and PsA—with a tendency to worse scores in PsA—and worse compared with Norwegian general population controls.

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Contributors BM, TU, JS, DvdH, HBH, EKK, JHL, GH and TKK were responsible for study design. TU, AW, GB, ER, FK and TKK were responsible for data acquisition. BM analysed the data and wrote the manuscript. All authors critically revised the manuscript and approved the final version.

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Competing interests None declared.

Patient consent Not required.

Ethics approval The study was approved by the National Data Inspectorate and by the Regional Committee for Medical and Health Research Ethics in Eastern Norway.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement All relevant data are within the text.

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EXTENDED REPORT

Risankizumab, an IL-23 inhibitor, for ankylosing spondylitis: results of a randomised, double-blind, placebo-controlled, proof-of-concept, dose-finding phase 2 study

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ABSTRACT

Objectives To evaluate the efficacy and safety of risankizumab, a humanised monoclonal antibody targeting the p19 subunit of interleukin-23 (IL-23), in patients with active ankylosing spondylitis (AS).

Methods A total of 159 patients with biological-naïve AS, with active disease (Bath Ankylosing Spondylitis Disease Activity Index score of ≥4), were randomised (1:1:1:1) to risankizumab (18 mg single dose, 90 mg or 180 mg at day 1 and weeks 8, 16 and 24) or placebo over a 24-week blinded period. The primary outcome was a 40% improvement in Assessment in Spondylo Arthritis International Society (ASAS40) at week 12. Safety was assessed in patients who received at least one dose of study drug.

Results At week 12, ASAS40 response rates were 25.5%, 20.5% and 15.0% in the 18 mg, 90 mg and 180 mg risankizumab groups, respectively, compared with 17.5% in the placebo group. The estimated difference in proportion between the 180 mg risankizumab and placebo groups (primary endpoint) was −2.5% (95% CI −21.8 to 17.0; p=0.42). Rates of adverse events were similar in all treatment groups.

Conclusions Treatment with risankizumab did not meet the study primary endpoint and showed no evidence of clinically meaningful improvements compared with placebo in patients with active AS, suggesting that IL-23 may not be a relevant driver of disease pathogenesis and symptoms in AS.

Trial registration number NCT02047110; Pre-results.

INTRODUCTION

Ankylosing spondylitis (AS) is a chronic inflammatory rheumatic disease that predominantly affects the axial skeleton, leading to back pain, progressive structural and functional impairment and reduced quality of life.[1] AS is generally unresponsive to conventional disease-modifying antirheumatic drugs (DMARDs), and systemic therapy for AS consists of non-steroidal anti-inflammatory drugs (NSAIDs), tumour necrosis factor inhibitors and, more recently, the interleukin (IL) 17A (IL-17A) inhibitor secukinumab.[2–4]

Several lines of evidence have identified IL-23 as a promising therapeutic target in AS.[5] At the genetic level, case–control genome-wide association studies have demonstrated that IL-23 receptor (IL-23R) polymorphisms are associated with an increased risk of developing AS.[6 7] In addition, a protective effect of the IL-23R^{R381Q} polymorphism is observed in AS.[8] Increased numbers of IL-23-producing cells have been found in facet joints of patients with AS,[9] while the number of IL-23-responsive T helper (Th) 22 (Th22), Th17 and gamma/delta T cells are elevated in blood from patients with AS.[10 11] Stimulation of peripheral blood mononuclear cells isolated from patients with AS leads to enhanced IL-23 production versus controls.[12] Finally, a potential role for the IL-23 pathway in driving entheseal inflammation and bone formation responses in AS has also been highlighted in murine models of spondyloarthritis.[13 14] IL-23 is a key driver in the induction and maintenance of Th17 cells.[15] The recent approval of the IL-17A inhibitor, secukinumab, for the treatment of AS, supported the clinical hypothesis that direct and specific inhibition of IL-23 would be of therapeutic benefit to patients with AS.[2 16 17]

Risankizumab (BI 655066/ABBV-066) is a humanised, immunoglobulin G1 monoclonal antibody that selectively inhibits IL-23 by specifically targeting the p19 subunit[18] and has shown efficacy in psoriasis, psoriatic arthritis (PsA) and Crohn's disease.[19–22] This proof-of-concept, dose-ranging study assessed the efficacy and safety of risankizumab in patients with active AS.

METHODS

Study design

This phase 2, randomised, placebo-controlled, double-blind study was conducted at 47 centres across North America, Europe and East Asia between March and December 2014. Patients were randomly assigned (by interactive response system) in a 1:1:1:1 ratio to one of three regimens of risankizumab (18 mg single dose, 90 mg or 180 mg at day 1 and weeks 8, 16 and 24) or placebo (figure 1A). The doses selected were informed by a phase 1 study in psoriasis and included a 10-fold dose range of risankizumab with a single administration

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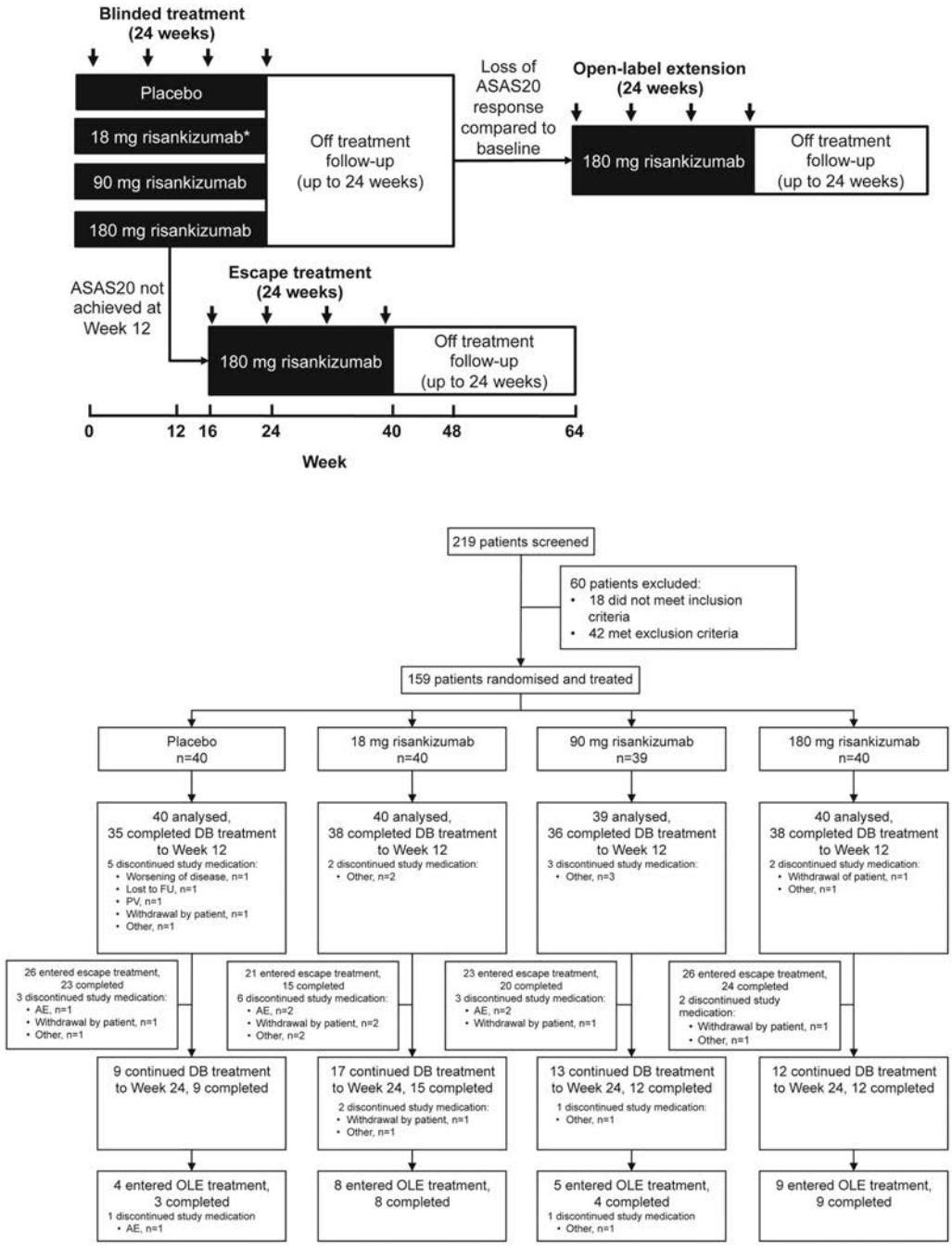

Figure 1 Overview of study design and patient disposition. Overview of treatment and observation periods including escape and open-label extension phases (panel A); patients were randomised 1:1:1:1 to one of three regimens of risankizumab (18 mg single dose, 90 mg or 180 mg at day 1 and weeks 8, 16 and 24) or placebo; patients without ASAS20 response at week 12 received escape treatment; patients with a flare of disease activity within 24 weeks of the last double-blind treatment entered the open-label extension. Arrows represent treatment administration. *Patients received 18 mg single dose at day 1 followed by placebo at weeks 8, 16 and 24. Trial profile (panel B). AE, adverse event; ASAS20, 20% improvement in Assessment in SpondyloArthritis International Society; DB, double blind; FU, follow-up; OLE, open-label extension; PV, protocol violation.

at the low dose (18 mg) that was expected to be subtherapeutic.[19] The study comprised a 24-week blinded treatment period, a potential escape treatment period from week 16 up to week 40 and a 24-week open-label extension period (not reported due to small sample size). Each treatment period had a 24-week follow-up (figure 1B). At week 16, escape treatment with 180 mg risankizumab was available for patients not achieving a 20% improvement in Assessment in SpondyloArthritis International Society (ASAS20) at week 12.

Amendments to the protocol can be found in the online supplementary materials. All authors approved the manuscript for submission and vouch for completeness of the data and the fidelity of the study to the protocol.

Patients

Patients aged 18–70 years were eligible if they had definite AS (1984 modified New York criteria[23] and local X-ray evaluation), active disease defined as a Bath AS Disease Activity Index (BASDAI) score of ≥4,[24] including a value ≥4 for

overall level of AS neck, back or hip pain, and documented inadequate response (30 days of optimal daily doses with ≥2 NSAIDs) or intolerance to NSAIDs. Patients previously treated with any biological for AS were excluded, and other biologicals were not permitted during the study. From 2 weeks prior to randomisation and up to 12 weeks of treatment, conventional DMARDs, low-dose systemic steroids (equivalent to ≤10 mg prednisolone/day), NSAIDs or analgesics at stable doses were permitted under the direction of the investigator. See online supplementary material for full inclusion/exclusion criteria. All patients provided written informed consent.

Assessments

The primary endpoint was the proportion of patients achieving an ASAS40 response at week 12. The key secondary endpoint was the change from baseline in the assessment of disease activity based on the AS Disease Activity Score-C reactive protein (ASDAS-CRP) at week 12. All secondary and further endpoints are listed in the online supplementary material. Of these, MRI assessments of the spine and sacroiliac (SI) joints, using the SpondyloArthritis Research Consortium of Canada (SPARCC) MRI indices for scoring inflammation in the SI joint[25] and spine,[26] were performed pretreatment and post-treatment (at week 24 for patients completing 24-week blinded treatment and at week 12 for patients starting escape treatment) within a subset of patients (see online supplementary material for methodology).

Safety endpoints included adverse events (AEs), serious AEs (SAEs), discontinuation of therapy because of AEs, local tolerability, changes in vital signs and physical examination and laboratory assessments.

Additionally, the IL-23/Th17 pathway biomarker β-defensin 2 and bone remodelling biomarkers associated with AS were evaluated in sera pretreatment and post-treatment at baseline and at week 12. Plasma samples for pharmacokinetic (PK) analysis and immunogenicity assessments were collected at prespecified visits (see online supplementary material for methodologies). A post hoc analysis of change from baseline in C reactive protein (CRP) level was also performed.

Statistical analysis

Sample size was determined based on one-sided comparison between the 180 mg risankizumab dose and placebo using Fisher's exact conditional test, consistent with the one-sided alternate hypothesis of a week 12 ASAS40 response rate of 43% with 180 mg risankizumab and a rate of 13% with placebo; randomisation of 160 patients (40 per study group) was estimated to provide 89% power using a one-sided test of 0.05 significance. The hierarchical inference strategy protects against type 1 error in other comparisons and other endpoints. Analyses were conducted with all randomised patients who received at least one dose of trial medication (full analysis). The Suissa-Shuster unconditional exact test was used to test the difference in the proportion of patients achieving the primary endpoint between treatment groups per intention-to-treat principles. The CI for the difference in the proportion between the treatment groups was obtained by the Clopper-Pearson method. To control the type I error rate, endpoints were tested in a hierarchical fixed sequence, starting with a primary endpoint comparison between the 180 mg risankizumab group versus placebo, with a 5% (one-sided) significance level. Because the primary analysis failed to show superiority of 180 mg risankizumab over placebo, all remaining analyses were exploratory with nominal p values. For any missing week 12 ASAS40 assessment, non-responder imputation was used. Patients receiving prohibited concomitant medication for AS prior to week 12 were considered as treatment failures. Statistical analyses for secondary and further endpoints are described in the online supplementary material.

RESULTS

Study population and patient disposition

Of the 219 patients screened, 159 underwent randomisation: 40 patients were assigned to 18 mg risankizumab, 39 patients to 90 mg risankizumab, 40 patients to 180 mg risankizumab and 40 patients to placebo. Up to week 12, 12 patients discontinued (ie, did not receive an injection at week 8); there was no imbalance in the frequency of premature discontinuation between risankizumab groups. One patient (placebo) discontinued trial medication due to worsening of AS prior to week 12 (figure 1B). A total of 51 patients fulfilled the ASAS20 response criteria at week 12 and continued treatment to week 24, and 96 patients were switched to escape treatment.

Demographic and baseline characteristics were balanced across study groups, with some variation related to the limited sample size (table 1). In particular, a lower number of HLA-B27-positive patients were randomised to the placebo group, most likely reflecting the higher number of Asian patients in the placebo group compared with risankizumab groups. Overall, the baseline disease activity and CRP levels of patients in this study were similar or somewhat less severe to those of patients in recent AS clinical trials.[2 16]

Efficacy

The primary endpoint, ASAS40 response at week 12, was not met. ASAS40 response at week 12 was achieved by 25%, 21% and 15% of patients in the 18 mg, 90 mg and 180 mg risankizumab groups, respectively, compared with 18% in the placebo group. The estimated difference in the proportion of ASAS40 responders between the 180 mg risankizumab group and placebo (primary endpoint) was –2.5% (95% CI –21.8 to 17.0; p=0.42) and 7.5% (95% CI –12.1 to 26.6; p=0.27) and 3.0% (95% CI –15.9 to 20.8; p=0.41) between the 18 mg and 90 mg risankizumab groups versus placebo, respectively (figure 2A). The prolongation of risankizumab treatment for up to 40 weeks by patients receiving escape treatment (180 mg risankizumab) did not substantially improve ASAS40 attainment rates.

ASAS20 response at week 12 was achieved by 45%, 33% and 30% of patients in the 18 mg, 90 mg and 180 mg risankizumab groups, respectively, compared with 20% in the placebo group (figure 2B). ASAS 5/6 response at week 12 was achieved by 20%, 23% and 15% of patients in the 18 mg, 90 mg and 180 mg risankizumab groups, respectively, compared with 5% in the placebo group (figure 2C). Partial remission (ASAS criteria) at week 12 was achieved by 3%, 3% and 10% of patients in the 18 mg, 90 mg and 180 mg risankizumab groups, respectively, compared with 3% in the placebo group (figure 2D).

Median change (IQRs) from baseline in ASDAS-CRP at week 12 were –0.7 (–1.3 to –0.2), –0.6 (–1.2 to 0.0) and –0.7 (–1.1 to –0.3) for the 18 mg, 90 mg and 180 mg risankizumab groups, respectively, compared with –0.3 (–1.0 to 0.2) for the placebo group (figure 3A). A dose-dependent reduction in CRP was observed with risankizumab compared with placebo at week 12 (figure 3B). For the BASDAI score (figure 3C) and other efficacy endpoints (Bath Ankylosing Spondylitis

Table 1 Baseline demographics and clinical characteristics

	Placebo	Risankizumab		
	(n=40)	18 mg (n=40)	90 mg (n=39)	180 mg (n=40)
Age, years (SD)	37.6 (11.0)	38.0 (11.1)	39.5 (10.8)	40.6 (11.9)
Male, n (%)	25 (63)	28 (70)	30 (77)	30 (75)
Race, n (%)				
White	19 (48)	26 (65)	28 (72)	22 (55)
Asian	20 (50)	13 (33)	11 (28)	17 (43)
Other*	1 (3)	1 (3)	0	1 (3)
Geographic region, n (%)				
Europe	18 (45.0)	23 (57.5)	24 (61.5)	20 (50.0)
Asia	20 (50.0)	13 (32.5)	11 (28.2)	17 (42.5)
USA	2 (5.0)	4 (10.0)	4 (10.3)	3 (7.5)
BMI, kg/m^2 (SD)	24.2 (4.3)	26.2 (5.3)	25.9 (4.6)	25.8 (4.5)
HLA-B27 status, n (%)				
Positive	26 (65)	30 (75)	30 (77)	34 (85)
Missing	4 (10)	4 (10)	4 (10)	2 (5)
Duration of disease, years (SD)	8.1 (8.2)	7.4 (8.2)	6.6 (8.8)	10.2 (9.5)
ASAS core components on NRS† (SD)				
Patient global	7.2 (2.0)	7.2 (1.7)	6.5 (1.7)	6.8 (2.2)
Inflammation	6.2 (2.2)	6.4 (2.0)	6.5 (1.8)	6.0 (2.2)
Spinal pain	6.8 (2.0)	6.5 (1.8)	6.3 (1.8)	6.4 (1.9)
Physical function	4.6 (2.3)	4.9 (2.1)	4.9 (1.9)	4.5 (2.6)
ASDAS-CRP	3.5 (3.0, 4.3)	3.6 (2.9, 4.2)	3.5 (2.9, 4.0)	3.6 (2.8, 4.0)
BASDAI	6.3 (5.1, 7.2)	6.4 (5.1, 7.1)	5.8 (4.8, 7.1)	6.1 (4.3, 7.4)
BASMI	3.0 (1.0, 4.5)	2.0 (1.0, 3.0)	3.0 (1.0, 4.0)	3.0 (1.0, 5.0)
CRP level, mg/L				
<2.87 (ULN)	10 (25)	11 (27)	5 (13)	11 (27)
≥2.87	30 (75)	29 (73)	34 (87)	29 (73)
≥2.87 to <8	14 (35)	12 (30)	20 (26)	10 (25)
≥8 to ≤15	9 (23)	6 (15)	5 (13)	11 (28)
>15	7 (18)	11 (28)	9 (23)	8 (20)
SPARCC SI joint, N median (IQR)	14	9	14	16
	0.8 (0.0–4.0)	2.5 (2.0–15.5)	1.5 (0.0–8.5)	3.3 (0.8–7.3)
SPARCC total spine, N median (IQR)	14	9	14	16
	11.3 (3.5–22.0)	9.0 (4.5–24.0)	11.3 (3.8–18.8)	8.3 (0.8–27.5)
Concomitant csDMARD‡	20 (50.0)	8 (20.0)	8 (20.5)	20 (50.0)
Concomitant NSAIDs§ and/or paracetamol	36 (90.0)	35 (87.5)	34 (87.2)	33 (82.5)
Concomitant GCs	3 (7.5)	5 (12.5)	2 (5.1)	3 (7.5)

Data are mean (SD), n (%) or median (IQR).
*Other includes black or African-American and American Indian or Alaska Native.
†Patients assessment of ASAS core components on NRS (0–10): patient global is based on global AS disease activity; inflammation is based on the mean of BASDAI questions 5 and 6 addressing the level of morning stiffness and duration; spinal pain is based on the mean of two questions; physical function is based on BASFI.
‡Concomitant csDMARDs include sulfasalazine, methotrexate, hydroxychloroquine and leflunomide.
§Concomitant NSAIDs include etoricoxib, celecoxib, meloxicam, diclofenac, diclofenac sodium, naproxen, ibuprofen, piroxicam, ketoprofen, indomethacin, aceclofenac, diclofenac resinate, etodolac, vimovo, acemetacin, morniflumate, naproxen sodium, phenylbutazone and sulindac.
AS, ankylosing spondylitis; ASAS, Assessment of SpondyloArthritis International Society; ASDAS-CRP, Ankylosing Spondylitis Disease Activity Score-CRP; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; BASMI, Bath Ankylosing Spondylitis Metrology Index; BMI, body mass index; CRP, C reactive protein; csDMARD, conventional synthetic disease-modifying antirheumatic drug; GC, glucocorticoid; HLA, human leucocyte antigen; NRS, numerical rating scale; NSAID, non-steroidal anti-inflammatory drug; SI, sacroiliac; SPARCC, SpondyloArthritis Research Consortium of Canada; ULN, upper limit of normal.

Functional Index, Bath Ankylosing Spondylitis Metrology Index, tender joint count, swollen joint count and Maastrich Ankylosing Spondylitis Enthesitis Score), there were no meaningful changes over time between the risankizumab groups and placebo (online supplementary table S8).

Only ASAS20 responders at week 12 (32%) continued blinded treatment up to week 24 (94% completing blinded treatment; figure 1), and most ASAS40 responders at week 12 (n=31) remained responders up to week 24 (n=29).

Subgroup analyses of ASAS40 response at week 12 by baseline CRP level, geographic region or morning stiffness did not reveal a higher ASAS40 response for any risankizumab group compared with placebo (online supplementary table S9).

Findings from the MRI analysis were generally consistent with clinical effects (online supplementary table S10 and figure S1). In patients who continued treatment to week 24, risankizumab had no effect on SPARCC SI joint score compared with placebo (online supplementary table S10) but improved

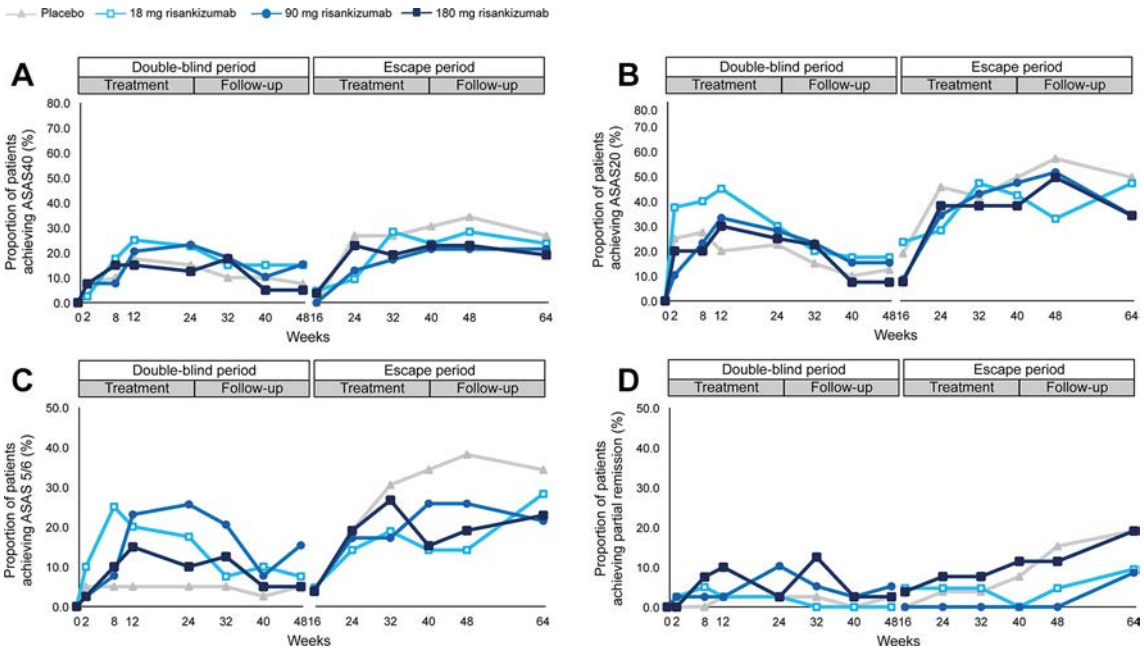

Figure 2 Response rates for ASAS40, ASAS20, ASAS 5/6 and ASAS partial remission during double-blind and escape treatment and follow-up periods. Clinical response rates over time for double-blind and escape treatment periods. ASAS40 (panel A), ASAS20 (panel B), ASAS 5/6 (panel C) and partial remission (panel D). NRI was used for missing data. Number of patients entering the double-blind treatment were: placebo: n=40; 18 mg risankizumab: n=40; 90 mg risankizumab: n=39; and 180 mg risankizumab: n=40. Patients entering escape treatment received 180 mg risankizumab; responses shown for the escape period are by the original randomised treatment (placebo: n=26; 18 mg risankizumab: n=21; 90 mg risankizumab: n=23; 180 mg risankizumab: n=26). Values for all data points are provided in online supplementary tables S1–S4. ASAS, Assessment in SpondyloArthritis International Society; NRI, non-responder imputation.

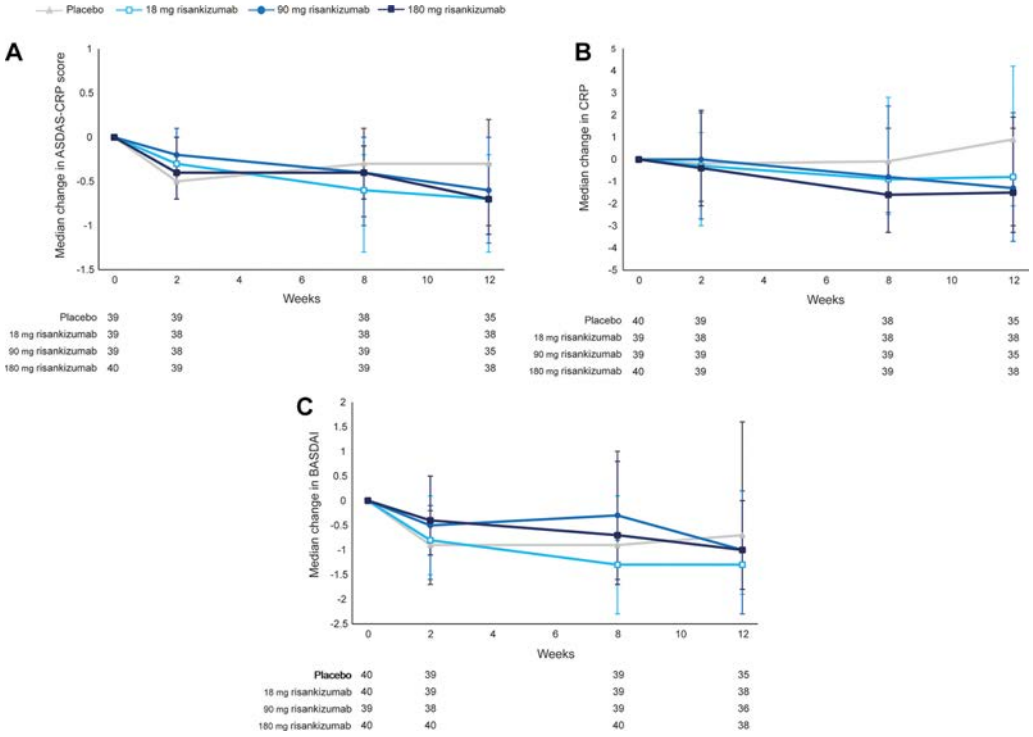

Figure 3 Change from baseline in ASDAS-CRP, CRP and BASDAI over time to week 12. Change from baseline in ASDAS-CRP (panel A), CRP (mg/L) (panel B) and BASDAI (panel C) over time to week 12. Median (IQR) changes are shown (observed). The values under each plot are the number of patients per treatment arm with a valid measurement at the specified time point. *P=0.0229 and p=0.0101 for median change in ASDAS-CRP for 18 mg and 180 mg risankizumab, respectively, versus placebo at week 12. Values for all data points are provided in online supplementary tables S5–S7. ASDAS-CRP, Ankylosing Spondylitis Disease Activity Score-CRP; BASDAI, Bath Ankylosing Spondylitis Disease Activity Score; CRP, C reactive protein.

Table 2 AEs summary up to 16 weeks

	Placebo	Risankizumab		
AEs, n (%)	(n=40)	18 mg (n=40)	90 mg (n=39)	180 mg (n=40)
Any AE	26 (65)	28 (70)	22 (56.4)	26 (65)
Infections and infestations	13 (32.5)	16 (40)	11 (28.2)	10 (25)
Musculoskeletal and connective tissue disorder	14 (35)	6 (15)	5 (12.8)	7 (17.5)
Severe AEs	1 (2.5)	1 (2.5)	1 (2.6)	0
Drug-related AEs	7 (17.5)	10 (25)	9 (23.1)	8 (20)
AEs leading to discontinuation	3 (7.5)	0	0	0
Serious infections	0	0	0	0
Serious AEs	2 (5)	0	2 (5.1)	1 (2.5)
Common AEs*				
Nasopharyngitis	3 (7.5)	6 (15)	4 (10.3)	10 (25)
Influenza	1 (2.5)	2 (5)	2 (5.1)	4 (10)
Sinusitis	1 (2.5)	0	2 (5.1)	1 (2.5)
Arthralgia	4 (10)	0	2 (5.1)	1 (2.5)
Back pain	2 (5)	3 (7.5)	1 (2.6)	2 (5.0)
Fatigue	2 (5)	2 (5)	1 (2.6)	4 (10)
Diarrhoea	0	2 (5)	1 (2.6)	3 (7.5)
Headache	3 (7.5)	5 (12.5)	3 (7.7)	4 (10)
Dizziness	0	0	2 (5.1)	1 (2.5)
Increased blood CPK	3 (7.5)	1 (2.5)	2 (5.1)	1 (2.5)
Eczema	1 (2.5)	0	2 (5.1)	0
Renal colic	0	0	2 (5.1)	0

AEs were coded using MedDRA V.19.0. The severity of AEs was graded according to RCTC V.2.0.
*Common AEs were reported in at least 5% of patients in any treatment group.
AE, adverse event; CPK, creatine phosphokinase; MedDRA, Medical Dictionary for Regulatory Activities; RCTC, Rheumatology Common Toxicity Criteria.

SPARCC total spine (median (IQR)) change from baseline in the 90 mg (–6.0 (–12.0 to –2.8); p=0.0046) and 180 mg (–3.8 (–8.0 to 1.3); p<0.05) risankizumab groups, compared with placebo at week 24. For patients who switched to escape treatment, there was no difference in change in SPARCC total spine or SI joint scores at week 12 or from weeks 12–24 between treatment groups (online supplementary table S10).

There were minimal differences between the changes from baseline in levels of β-defensin 2 and biomarkers of bone remodelling (dikkopf-1, sclerostin, BMP-7 and osteocalcin) in patients receiving risankizumab versus placebo over time. A heat map showing the percentage change from baseline to week 12 in biomarkers versus change in clinical and MRI parameters in ASAS20 responders and non-responders is provided in online supplementary figure 2.

Safety

Up to week 16, the rate of AEs was comparable between the risankizumab groups and the placebo group. Most reported AEs were of mild or moderate intensity, and there were no reports of severe infections across all groups. Three patients in the placebo group had AEs leading to the premature discontinuation of trial medication. The frequency of investigator-defined drug-related AEs was low and comparable between the risankizumab and placebo groups (table 2). The most common AEs reported with risankizumab and/or placebo included nasopharyngitis, headache and fatigue. Up to the end of the trial, the rate and profile of AEs in the risankizumab total group was consistent with the up to 16 weeks data in this trial and with other risankizumab trials (online supplementary table S11). All SAEs were deemed non-drug related by the investigator and were graded as serious due to the need for hospitalisation. Local tolerability issues at the injection site were uncommon and mostly mild, and no anaphylactic reactions or inflammatory bowel syndrome diagnoses were reported.

Overall, PK parameters and the exposure (online supplementary table S12) of risankizumab in patients with AS were comparable with patients with psoriasis treated with risankizumab in prior trials.[19 20] Antidrug antibody (ADA) incidence was 14%, with 4% testing positive at baseline before treatment (pre-existing ADA) and only 3% testing positive in the neutralising antibody assay. In most patients, ADAs were transient and of low titre (≤16). These results were similar to those reported in patients with psoriasis in a previous trial.[20] ADAs did not impact the exposure or safety of risankizumab.

DISCUSSION

AS is a complex polygenic disease showing a genetic association with the IL-23 pathway.[6 8] However, in this phase 2, proof-of-concept study, a significant improvement in the proportion of patients achieving an ASAS40 response with risankizumab at week 12, compared with placebo, was not demonstrated. Longer treatment (up to 40 weeks, by patients receiving escape treatment with 180 mg risankizumab) did not substantially improve ASAS40 attainment rates. The lack of efficacy was also confirmed by most secondary and other endpoints. While modest reductions of ASDAS-CRP were evident with risankizumab, a clinically relevant change of ≥1.1 was not achieved.[27] ASAS 5/6 at week 12 was the only secondary endpoint to demonstrate a higher response rate in each of the risankizumab treatment groups compared with placebo. The primary driver in both ASDAS-CRP and ASAS 5/6 reductions was CRP, with minimal changes in other composite endpoint parameters. Findings from MRI analysis indicated a limited impact of risankizumab at week 24 on SPARCC total spine score in patients with a clinical response. Despite this, trial discontinuations due to lack of efficacy were low.

Risankizumab was generally well tolerated in this study, and there were no new or unexpected safety signals identified.[19–22]

Findings of this study are in contrast to those reported for secukinumab, an IL-17A inhibitor that demonstrated efficacy in patients with AS.[2 16 17] In an open-label trial, ustekinumab (an IL-12/23 inhibitor) showed efficacy in 20 patients with AS through 28 weeks; however, recently, phase 3 trials in AS and non-radiographic axial spondyloarthritis were terminated for not meeting key efficacy endpoints (ClinicalTrials.gov identifiers: NCT02438787 and NCT02407223).[28] The failure of risankizumab in this study was unexpected, and the underlying reasons remain unclear. Theoretically, it is possible that risankizumab may not have sufficiently blocked levels of IL-23 in the target organ (ie, the spine and SI joints). However, this is unlikely for the following reasons: risankizumab has shown proof-of-concept in psoriasis,[19 20] PsA[22] and Crohn's disease;[21] the observed PK exposure levels were consistent with effective exposures in psoriasis; risankizumab had a dose-dependent effect on CRP; and that an evident improvement in SPARCC spine score in the 90 mg and 180 mg treatment groups was seen in patients who had remained within 24-week blinded treatment in this study. Enrolment criteria in this study excluded patients with concomitant inflammatory bowel disease or PsA; however, patients with concomitant psoriasis were permitted, but unfortunately information on this comorbidity was not collected systematically, and thus we were unable to assess whether there were any improvements in psoriatic lesions, as evidenced in previous studies of risankizumab in patients with psoriasis.

Alternatively, risankizumab may not have sufficiently blocked all relevant sources of IL-17 production. Previously, risankizumab has been shown to reduce the levels of IL-17A, IL-23A and associated transcripts in lesional skin of patients with psoriasis.[19] Although it is well characterised that one function of IL-23 is the induction and maintenance of Th17 cells,[15] it is likely that IL-23-independent sources of IL-17 are still active, such as IL-17-secreting mast cells and mucosal-associated invariant T cells.[29] In addition, in in vivo autoimmune mouse models, dual inhibition of IL-17 and IL-23 was more efficacious in reducing disease than targeting either cytokine alone,[30] confirming that the relationship between IL-23 and IL-17 is not linear. Discordant effects of IL-17 and IL-23 inhibition were previously observed in Crohn's disease, where a lack of clinical efficacy of IL-17 inhibitors and even disease exacerbation and elevated inflammatory markers (including serum CRP and faecal calprotectin) were reported,[15] whereas IL-23 inhibition has shown proof-of-concept in Crohn's disease.[21]

The results of this study challenge the notion that IL-23 is a relevant driver of AS disease pathogenesis and symptoms. This is further supported by analyses showing no overall differences in change in levels of an antimicrobial peptide and IL-23/Th17 pathway biomarker and select biomarkers of bone remodelling in patients receiving risankizumab versus placebo. The role of changes in β-defensin 2 in disease processes occurring in AS is less understood compared with psoriasis,[20 21] but the results from the current study suggest there may be differences between IL-23/Th17 pathway biomarkers associated with psoriasis and those associated with AS.

A possible limitation of this study is that the design did not include a loading dose, as has been included in some clinical studies of biological agents in AS and in phase 2 studies for risankizumab in psoriasis (treatment at weeks 0, 4 and 16)[20] and Crohn's disease (induction dosing every 4 weeks intravenously for 12 weeks);[21] therefore, the study design might have potentially contributed to the negative results. However, because there was no convincing evidence for a dose response in this study, and a single dose of 18 mg risankizumab has demonstrated efficacy in a psoriasis phase 2 trial,[20] it is unlikely that a loading dose would have improved the treatment response in AS. Studies with secukinumab have also indicated that a loading dose is not required, thus leading it to be optional in the secukinumab label.[2 31] Another limitation of the study design was that there was no comparator arm in the escape group that received four additional doses of open-label 180 mg risankizumab, making the efficacy data for the 96 patients in this group difficult to interpret.

In this proof-of-concept study, risankizumab was not effective in reducing the signs and symptoms of AS. The lack of efficacy of this IL-23 inhibitor at subcutaneous doses previously shown to be highly effective in psoriasis suggests that, despite a genetic association with the IL-23 pathway, IL-23 may not be a relevant driver of disease pathogenesis and symptoms in AS.

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Patient consent Not required.

Ethics approval The study protocol was approved by the institutional review board or ethics committee at each participating centre. The study was conducted according to the Declaration of Helsinki and the International Conference on Harmonisation guidelines. Safety data were periodically evaluated by an independent data monitoring committee. All patients provided written informed consent.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement The authors confirm that the data supporting the findings of this study are available within the article or its supplementary materials.

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EXTENDED REPORT

Work participation in spondyloarthritis across countries: analysis from the ASAS-COMOSPA study

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ABSTRACT

Objectives To explore the role of individual and country level socioeconomic (SE) factors on employment, absenteeism and presenteeism in patients with spondyloarthritis (SpA) across 22 countries worldwide.

Methods Patients with a clinical diagnosis of SpA fulfilling the ASAS classification criteria and in working age (≤65 years) from COMOSPA were included. Outcomes of interest were employment status, absenteeism and presenteeism, assessed by the Work Productivity and Activity Impairment Specific General Health questionnaire. Three multivariable models were built (one per outcome) using mixed-effects binomial (for work status) or ordinal regressions (for absenteeism and presenteeism), with country as random effect. The contribution of SE factors at the individual-level (eg, gender, education, marital status) and country-level (healthcare expenditure (HCE) per capita, Human Development Index (HDI) and gross domestic product per capita) SE factors, independent of clinical factors, was assessed.

Results In total, 3114 patients with SpA were included of which 1943 (62%) were employed. Physical function and comorbidities were related to all work outcomes in expected directions and disease activity also with absenteeism and presenteeism. Higher education (OR 4.2 (95% CI 3.1 to 5.6)) or living in a country with higher HCE (OR 2.3 (1.5 to 3.6)) or HDI (OR 1.9 (1.2 to 3.3)) was positively associated with being employed. Higher disease activity was associated with higher odds for absenteeism (OR 1.5 (1.3 to 1.7)) and presenteeism (OR 2.1 (1.8 to 2.4)). No significant association between individual-level and country-level SE factors and absenteeism or presenteeism was found.

Conclusions Higher education level and higher country SE welfare are associated with a higher likelihood of keeping patients with SpA employed. Absenteeism and presenteeism are only associated with clinical but not with individual-level or country-level SE factors.

INTRODUCTION

With a worldwide ageing population, the prevalence and consequences of chronic and debilitating diseases have been increasing.[1] Countries are challenged to invest in health and healthcare to keep their citizens active, in order to increase productivity and prolong the lifetime of efficient work. In turn, this strategy has been shown to generate wealth that can be used to improve the welfare system generating a 'positive cycle'. These societal aspirations make no exception for patients with chronic disease such as spondyloarthritis (SpA).[2]

Research on the economic impact of SpA has consistently shown that indirect costs due to sick leave and work disability are, by far, the major sources of the total SpA-related cost of illness.[3–7] Biologic drugs were able to improve absenteeism (sick leave), presenteeism (reduction on performance while at work because of health reasons)[8] and employment status.[9] However, despite optimal drug treatment, persons with SpA still experience restrictions in work participation, and a role for personal and environmental contextual factors has been suggested.[10] Also, while most studies on work outcomes have been performed in Western societies, the necessities in relation to work outcome are likely different across countries.

On this line, there is a need to better understand the variation of the different work outcomes across countries and to gain insight into the role of personal as well as country-level contextual factors, and specifically of socioeconomic (SE) characteristics.[11 12] This knowledge can be used to plan and implement better health policies for patients with SpA, and may improve work participation and reduce the economic burden. Cohorts including patients from several countries, such as the Assessment in SpondyloArthritis international Society (ASAS) Evaluation of co-morbidities in spondyloarthritis (COMOSPA) study with patients from 22 countries from all world regions, are scarce but essential to understand these effects.[13] Two studies in SpA that compared work outcome between countries suggested large differences.[3 6 14] However, as only two or three countries were compared, it was not possible to explore systematic effect related to country of residence.

Our aims were twofold: first, to assess the impact of clinical and individual SE characteristics on employment status, absenteeism and presenteeism[12] among patients with SpA at working age, while accounting for country of residence; second, to explore the potential role of specific country-level SE factors on these outcomes.

METHODS

Patients and study design

The ASAS-COMOSPA study design and procedures have been previously described.[13] Briefly, this was a

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large, cross-sectional, multicentre, international study, with 22 participating countries from four continents (Africa, America, Asia and Europe). Consecutive adult patients (≥18 years old) with a clinical diagnosis of SpA according to their rheumatologist,[15 16] and able to understand and complete questionnaires were included. For the current study, only patients of working age (≤65 years old) were included.

The study was conducted according to guidelines for good clinical practice in all countries. Written informed consent was obtained from all subjects before enrolment.

Data collection

Outcomes

Work participation, more specifically employment, absenteeism and presenteeism, were assessed according to the Work Productivity and Activity Impairment questionnaire General Health (WPAI-GH).[17]

The WPAI-GH has six questions, the first asking about the employment status (binary; yes/no). The subsequent five questions allow to calculate for the past 7 days, the percentage of time absence from the workplace (absenteeism, 0%–100%), the percentage of loss of productivity while at work (presenteeism, 0%–100%) and the percentage of overall work impairment (0%–100%) in those employed, as well as the percentage of activity impairment (0%–100%) in all patients.

For the present study, only the employment status, absenteeism and presenteeism were assessed. Because of a skewed distribution (and zero inflated for absenteeism), the last two outcomes were categorised into three (0%; >0%–20%; >20%–100%) and four categories (0%; >0%–20%; >20%–50%; >50%–100%), respectively. The number of categories and cut-points was based on preliminary analysis.

Demographic, clinical characteristics and individual SE factors

A standardised case report form was used by a local researcher or by the rheumatologist to collect the following variables: (1) individual SE factors: age, gender, education (as a categorical variable: primary education, secondary and university) and current marital status (as a categorical variable: single, married or living together, divorced and widow); (2) lifestyle: body mass index, smoking status (past and current); (3) SpA characteristics: disease duration, peripheral arthritis, enthesitis, dactylitis, human leucocyte antigen B27 (HLA-B27), SpA phenotype (axial (imaging vs clinical arm) vs peripheral) and extra-articular manifestations (inflammatory bowel disease, uveitis and psoriasis); (4) SpA activity and severity measures: Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), the Ankylosing Spondylitis Disease Activity Score (ASDAS) calculated with C reactive protein, number of swollen and tender joints (44 joint count), the presence of 'bamboo spine' and the Bath Ankylosing Spondylitis Functional Index (BASFI); (5) comorbidities: assessed by the Rheumatic Diseases Comorbidity Index (RDCI)[18 19]; (6) past and current medications: non-steroidal anti-inflammatory drugs, oral steroids, conventional synthetic and biological disease-modifying antirheumatic drugs; and (7) imaging: sacroiliitis on pelvic radiographs assessed according to the modified New York (mNY) criteria[20] and sacroiliitis on MRI defined based on the ASAS criteria,[21] both according to the treating rheumatologist.

RDCI was completed with data from the CRF with physician-reported data about stroke and patient-reported data about heart diseases, hypertension, lung diseases, stomach ulcers, cancer and fractures.

Country-level SE characteristics

The following country-level SE characteristics were obtained: (1) healthcare expenditure (HCE) per capita (source: World Bank 2013) in International dollars (Intl$) to adjust for purchasing power parities (HCE-PPP); (2) Human Development Index (HDI) (source: United Nations Development Program 2013); (3) GDP per capita (source: International Monetary Fund 2013) in int$ (GDP-PPP); (4) unemployment rate (%) of the total labour force (source: World Bank 2013; estimate from International Labour Organization).[22–26] Detailed information on SE data per country is provided in online supplementary table S1.

Statistical analyses

For analysis of work status, the total sample of patients with SpA of working age (≤65 years old) were included, while for the analysis of absenteeism and presenteeism, the subsample of employed subjects were considered. Patients reporting 100% absenteeism were excluded from the analysis of presenteeism.

Country-level SE variables were divided into tertiles, and the lowest tertiles (reference level) were compared with the medium and the highest tertiles combined.

Possible associations between all demographic, clinical factors (lifestyle, SpA characteristics, SpA activity and severity, and comorbidities), as well as individual-level SE factors, and the three work outcomes were first explored in univariable models. Variables with a p value <0.20 were selected for the multivariable models. For employment status, binomial mixed-effects regression with country as random effect (RE) was applied and ordinal mixed-effects models, also with country as RE, were used for absenteeism and presenteeism. The final models included variables that were significantly associated with the outcome of interest ($p<0.05$).

Each country-level SE variable (HCE-PPP, HDI, GDP-PPP, unemployment rate) was added, in separate models (because of collinearity), to the three multivariable models to assess their possible independent effect on work outcomes (significant if $p<0.05$), additional to the effect of demographic, clinical and individual-level SE factors.

The inferential analysis was performed on complete cases.

All analyses were performed using Stata V.14.

RESULTS

Patient characteristics

In total, 3114 patients from 22 countries (Argentina, Belgium, Canada, China, Colombia, Egypt, France, Germany, Italy, Japan, Mexico, Morocco, Netherlands, Portugal, Russia, Singapore, South Korea, Spain, Taiwan, Turkey, UK and USA) were included.

Demographic, clinical and imaging characteristics of the study population, as well as the comparison between employed and unemployed patients, are shown in table 1. In this study, 1943 patients (62%) were employed. Of note, a large proportion of patients reported a university-level of education, which was higher among employed (50%) as compared with unemployed patients (35%).

One quarter (n=508, 27%) of the employed patients had been absent from their workplaces more than 20% of the time during the previous 7 days (absenteeism), but almost half (n=803, 47%) of those who were present felt that their disease reduced their productivity (presenteeism) by more than 20% (table 1).

All work outcomes varied across countries and are presented in table 2. Employment was lowest in Colombia (28.1%) and highest in Canada (83.3%), absenteeism was lowest in South Korea (1.2%) and highest in Germany (53.1%), and presenteeism,

Table 1 Patient and disease characteristics according to employment status

Features	All (n=3114)	Employed (n=1943)	Not employed (n=1155)	P values*
Age (years), mean (SD)	40.9 (11.8)	40.1 (10.5)	42.2 (13.6)	<0.001
Disease duration (years), mean (SD)	7.6 (8.5)	7.3 (8.0)	8.2 (9.3)	0.005
Gender (male), n (%)	2047 (65.7)	1387 (71.4)	652 (56.6)	<0.001
Education, n (%)				
University	1366 (44.0)	961 (49.6)	398 (34.5)	<0.001
Secondary	1375 (44.2)	842 (43.4)	526 (45.6)	
Lower	367 (11.8)	136 (7.0)	229 (19.9)	
RDCI (0 to 8), mean (SD)	0.7 (1.0)	0.5 (0.9)	0.9 (1.2)	<0.001
ASDAS-CRP, mean (SD)	2.0 (1.1)	1.9 (1.0)	2.3 (1.1)	<0.001
BASFI (0–10), mean (SD)	3.0 (2.7) (n=3095)	2.5 (2.4) (n=1941)	4.0 (2.9) (1153)	<0.001
ASAS classification criteria				
Axial SpA, n (%)	2764 (88.8)	1727 (88.9)	1023 (88.6)	0.790
Peripheral SpA, n (%)	350 (11.2)	216 (11.1)	132 (11.4)	
Axial SpA				
Imaging arm (±clinical arm), n (%)	2546 (92.1)	1582 (91.6)	952 (93.1)	0.170
Clinical arm only, n (%)	218 (7.9) (n = 2754)	145 (8.4) (n = 1727)	71 (6.9) (n = 1023)	
Radiographic sacroiliitis (mNY), n (%)	2225 (75.2)	1357 (73.7)	859 (78.0)	0.009
Sacroiliitis on MRI (ASAS), n (%)	1204 (71.3) (n=1688)	780 (72.4) (n=1204)	419 (70.0) (n=484)	0.296
HLA-B27 positivity, n (%)	1967 (76.7) (n=2566)	1266 (77.1) (n=1967)	692 (76.0) (n=599)	0.563
Elevated CRP (ever), n (%)	1767 (60.0)	1078 (58.4)	680 (62.7)	0.020
Current medication, n (%)				
Oral steroids	335 (10.9)	183 (9.5)	152 (13.2)	0.001
NSAIDs	2130 (68.9)	1311 (67.6)	819 (71.0)	0.045
csDMARDs	1059 (34.2)	651 (33.5)	408 (35.3)	0.302
bDMARDs	1175 (37.7)	708 (36.4)	456 (39.5)	0.091

Missing <5%: employment status; disease duration; education; marital status; RDCI; BASFI; radiographic sacroiliitis; absenteeism; oral steroids; NSAIDs. Missing 5%–10%: ASDAS-CRP; CRP status. The total number of patients includes 16 patients with missing data regarding their employment status.
*Comparison between employed and unemployed (independent-samples t-test for continuous variables and χ^2 for categorical variables).
ASAS, Assessment of SpondyloArthritis international Society; ASDAS-CRP, Ankylosing Spondylitis Disease Activity Score—C Reactive Protein; BASFI, Bath Ankylosing Spondylitis Functional Index; bDMARDs, biological disease-modifying antirheumatic drugs; csDMARDs, synthetic disease-modifying antirheumatic drugs; HLA-B27, human leucocyte antigen B27; mNY, modified New York criteria; NSAIDs, non-steroidal anti-inflammatory drugs; RDCI, Rheumatic Disease Comorbidity Index; SpA, spondyloarthritis.

impact on productivity, was lowest in Japan (17.8%) and highest in Germany (42.3%).

Effect individual SE factors on work outcomes

The effect of individual SE and clinical characteristics on each of the three outcomes in the final multilevel models is shown in table 3. Patients with a higher level of education were more likely to be employed (model 1: OR 4.2 (95% CI 3.2 to 5.6)). This protective effect was not observed either for absenteeism (model 2) or presenteeism (model 3). In addition, male gender, being married or living together or being divorced (compared with being single), a better function and a lower number of comorbidities were associated with a higher likelihood of being employed (model 1) but not with absenteeism or presenteeism (model 2 and model 3).

More comorbidities and a worse function (BASFI) were associated with lower employment (model 1) and a higher odds for absenteeism (model 2) and presenteeism (model 3). In addition, higher disease activity was associated with higher odds for absenteeism (model 2) and presenteeism (model 3).

The distribution of employment across countries (unadjusted and adjusted (model 1)) is described in figure 1.

Of note, there was no significant association between classification status (axial SpA (axSpA) vs peripheral SpA (pSpA)) and any of the studied outcomes (univariable p values range: 0.19–0.99), or between the 'arms' of the axSpA criteria (imaging arm±clinical arm vs clinical arm alone) and these outcomes (univariable p values range: 0.22–0.81).

DISCUSSION

In this large international study, we have first shown the importance of individual SE factors (ie, education and gender), in addition to clinical characteristics on work participation among patients with SpA. Notwithstanding, these 'individual factors' fail to fully explain the large variability of work outcomes across countries. Our results emphasise variation in work outcomes in patients with SpA across countries and suggest that country-level SE factors also play an important role, especially the countries' wealth and the level of investment in the healthcare system.

In all final multivariable models, the random effect of country on work outcomes was still significantly greater than zero. This indicates that, after adjusting for the clinical and individual SE factors included, there are likely to be further unmeasured variables that influence work outcomes. These may be country-dependent variables that we were unable to include, such as availability and generosity of sickness benefit, or individual-level variables that vary across countries, such as the type of work and work-related stress.

Table 2 Work outcomes and therapy per country

Countries	Number of patients n (% total)	Employed patients n (% total)	Absenteeism Mean (SD) (0–100)	Presenteeism Mean (SD) (0–100)
Argentina	162 (5)	104 (64)	11.9 (26.9)	20.7 (27.6)
Belgium	31 (1)	21 (68)	8.3 (25.7)	26.5 (23.4)
Canada	36 (1)	30 (83)	8.2 (21.4)	26.6 (26.8)
China	224 (7)	131 (58)	11.1 (23.8)	38.4 (26.3)
Colombia	33 (1)	9 (28)	19.7 (21.6)	38.9 (26.7)
Egypt	205 (7)	129 (63)	15.9 (29.7)	36.5 (26.9)
France	257 (8)	163 (65)	14.1 (31.1)	26.6 (26.5)
Germany	156 (5)	86 (55)	53.1 (46.4)	42.3 (28.6)
Italy	179 (6)	143 (80)	2.0 (9.1)	22.9 (23.4)
Japan	109 (4)	85 (79)	5.6 (18.0)	17.8 (23.0)
Mexico	57 (2)	37 (66)	9.0 (18.6)	18.9 (29.7)
Morocco	94 (3)	35 (37)	34.6 (38.3)	41 (23.2)
Netherlands	125 (4)	97 (78)	2.8 (11.9)	21.8 (23.1)
Portugal	57 (2)	36 (64)	14.6 (34.4)	23.1 (28.0)
Russia	192 (6)	119 (62)	31.6 (41.7)	40.4 (23.3)
Singapore	166 (5)	135 (81)	3.3 (14.2)	26.6 (22.2)
South Korea	188 (6)	87 (47)	1.2 (4.7)	22.6 (24.7)
Spain	191 (6)	111 (58)	5.6 (21.1)	19.4 (21.8)
Taiwan	186 (6)	130 (70)	6.1 (16.2)	34.1 (25.6)
Turkey	192 (6)	103 (54)	6.5 (15.9)	30.7 (29.3)
UK	154 (5)	92 (60)	16.9 (32.0)	29.9 (28.3)
USA	120 (4)	60 (50)	9.9 (32.6)	28.1 (28.8)
Total	3114 (100)	1943 (62.7)	11.9 (27.7) (n=1900)	28.3 (26.4) (n=1773)

Absenteeism: percentage of time (0%–100%) missed from work due to a health problem; presenteeism, percentage of unproductive time (0%–100%) at work due to a health problem. Missings <5%: absenteeism; missings 5%–10%: presenteeism.

A recent systematic literature review from the Outcome Measures in Rheumatology (OMERACT) summarised the evidence on the relationship between individual-level and country-level SE factors on work status, absenteeism and presenteeism in patients with radiographic axSpA (r-axSpA).[10] Between-study differences rendered comparisons challenging. Overall, while evidence about the effect of gender and education on *work status* was conflicting, there was insufficient evidence about their role on *presenteeism and absenteeism*, and no evidence on the role of country of residence on *any work outcome*.[7 27–29]

Our study revealed that the individual SE factors are associated with employment status, but not with absenteeism and presenteeism. With regard to gender, this might not be surprising, as it likely reflects that in many countries population employment among women with an average age of 40 (SD 12) years is still lower compared with men. Of note, among those employed, gender has no influence on absenteeism or presenteeism.[30] Similarly, a positive relation between higher education or marital status was only seen for employment, but not for absenteeism and presenteeism. Our data suggest that the influence of disease activity and severity on these latter outcomes overshadows the role of personal contextual factors.

Table 4 Effect of country-level socioeconomic factors on work outcomes*

	Being employed OR (95% CI)	Absenteeism OR (95% CI)	Presenteeism OR (95% CI)
HCE-PPP per capita (low vs medium and high)	2.3 (1.5 to 3.6)	0.6 (0.3 to 1.2)	0.8 (0.5 to 1.3)
HDI (very high vs medium and high)	1.9 (1.2 to 3.3)	0.5 (0.2 to 1.0)	0.7 (0.4 to 1.3)
GDP-PPP per capita (low vs medium and high)	1.6 (1.0 to 2.7)	0.6 (0.3 to 1.1)	1.3 (0.7 to 2.1)
Unemployment (low vs medium and high)	1.3 (0.7 to 2.3)	NA	NA

*Country-level SE factors tested in the final multivariable models with clinical factors (same models from table 3).
GDP, gross domestic product; HCE, healthcare expenditure; HDI, Health Development Index; NA, not applicable.

Table 3 Sociodemographic, clinical and individual socioeconomic factors associated with work outcomes*

	Model 1 Being employed (n=3067) OR (95% CI)	Model 2 Absenteeism (n=1722) OR (95% CI)	Model 3 Presenteeism (n=1604) OR (95% CI)
Gender (male vs female)	2.3 (1.9 to 2.8)	†	†
Education level			
Secondary vs lower education	2.5 (1.9 to 3.3)	†	†
University vs lower education	4.2 (3.2 to 5.6)		
Marital status			
Married/living tog vs single	1.9 (1.6 to 2.3)	‡	‡
Divorced vs single	2.1 (1.4 to 3.2)		
Widower vs single	1.4 (0.6 to 3.3)		
RDCI (0 to 8)	0.8 (0.7 to 0.9)	1.2 (1.1 to 1.4)	1.3 (1.2 to 1.5)
ASDAS CRP	†	1.5 (1.3 to 1.7)	2.1 (1.8 to 2.4)
BASFI (0–10)	0.8 (0.8 to 0.9)	1.2 (1.1 to 1.3)	1.5 (1.4 to 1.6)
Steroids (current) (yes)	†	†	1.5 (1.1 to 2.1)

For all models the, likelihood-ratio test was significant (p<0.01). Tested variables for all outcomes in the univariable analysis: disease duration; gender; education; marital status; RDCI; ASDAS-CRP; BASFI; axial spondyloarthritis (SpA) vs peripheral SpA; ASAS axial SpA imaging arm vs clinical arm only; radiographic sacroiliitis according to the modified New York criteria; human leucocyte antigen B27 status, uveitis, psoriasis, inflammatory bowel disease; good response to non-steroidal anti-inflammatory drugs (NSAIDs); past infections; family history of SpA; presence of bamboo spine; treatment with NSAIDs; treatment with steroids; treatment with synthetic disease-modifying antirheumatic drugs; treatment with biologic disease-modifying antirheumatic drugs.
*Multivariable mixed-effect models, with country of residence as random effect.
†Not selected in the multivariable analysis (p>0.05).
‡Not selected in the univariable analysis (p>0.20).
ASDAS-CRP, Ankylosing Spondylitis Disease Activity Score—C Reactive Protein; BASFI, Bath Ankylosing Spondylitis Functional Index; RDCI, Rheumatic Disease Comorbidity Index.

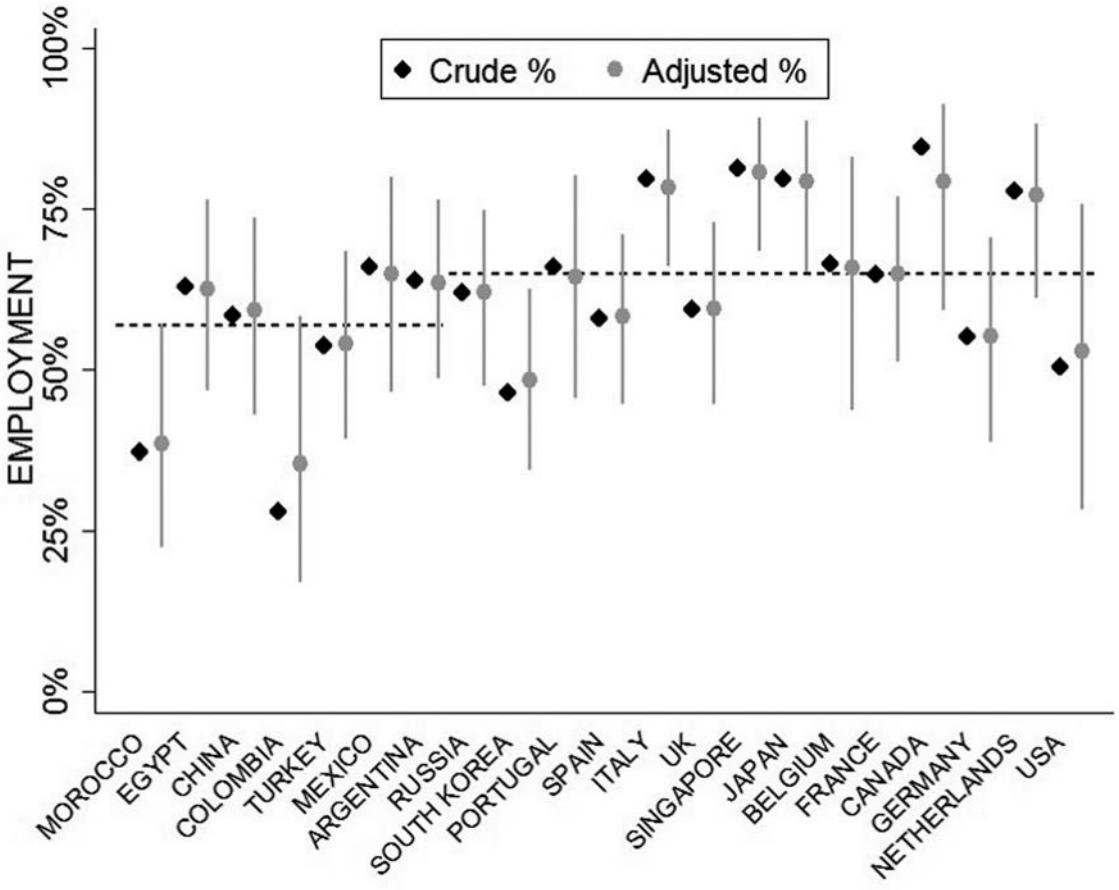

Figure 1 Percentage of employment by country (n=21; no data available on healthcare expenditure for Taiwan). Crude %: observed employment per country; adjusted %: estimated from model 1 (table 3). Countries ordered by healthcare expenditure per capita (from lowest to highest). The dashed horizontal lines represent the predicted % of employment for countries with a low healthcare expenditure (<1500 Intl$ per year per capita) and with a high health expenditure (≥1500 Intl$ per year per capita).

The aforementioned review emphasised the lack of data to quantify and understand country differences in work outcomes. Notwithstanding, this would be of importance for, among others, planning national initiatives to improve work participation in patients with chronic disease or European-level policies. Results from the *Outcomes Assessment in AS International Study* (OASIS) in patients with r-axSpA across three European countries (Belgium, France and The Netherlands) has shown differences between countries on employment and presenteeism, adjusting for individual sociodemographic and clinical characteristics.[4 29 31] Country of residence included as a categorical variable revealed to be associated with the outcomes.[4] However, the study only concerned three European countries, and the role of country-level SE characteristics was not explored when trying to explain the effect of country of residence. A study by Mau *et al* revealed that employment in the former Eastern German states was lower than in former Western German states. The higher standardised employment among patients with ankylosing spondylitis was attributed to a higher unemployment in former Eastern Germany. However, this was not formally tested in the analyses.[14]

In the current study, we have found large variations in all work outcomes across countries and showed that countries' welfare (especially HCE or HDI) is associated with a higher likelihood of being employed among patients with SpA. Associations between country-level SE status and absenteeism or presenteeism were less clear.

Wealthier and more developed countries apparently invest more in health and also in healthier workplaces, in efforts to support (chronically ill) persons to remain healthy during their working careers. Notwithstanding, even in wealthier countries, employed patients cannot avoid taking sick leave or experiencing productivity loss in case of higher disease activity.

It should be noted that the determinant factor for reaching statistical significance may be the number of included countries, which, though impressive (n=22), statistically speaking is not so high and may therefore lead to power issues when analysing country-level variables. In other words, the effects found, namely the positive association between higher HCE or higher HDI and higher likelihood of employment, may be an underestimation of the real effect of country-level SE factors on work outcomes in SpA. Similar results have already been seen in rheumatoid arthritis (RA). The Comorbidities in Rheumatoid Arthritis (COMORA) international study assessed the same work outcomes as in COMOSPA but in patients with RA across 17 countries. Similar to our results, this study has also shown that there are substantial differences in work outcomes among patients from different countries, and that this difference is independent of clinical and individual SE factors.[32] Patients with RA, living in a country with a lower economic wealth and a lower human development of countries had a higher chance to have no employment, higher absenteeism, but paradoxically lower presenteeism. Our results were identical for employment. However, no association could be found for the other two outcomes.

To the best of our knowledge, this is the first study to assess the effect of both individual-level and country-level SE factors in a worldwide setting and in patients within the full spectrum of SpA. Of note, there was no association between the 'SpA phenotype' (axSpA vs pSpA) and work-related outcomes, as previously found in a smaller Swedish study.[33] In addition, being mNY-positive had no effect on work participation and we also found no measurable differences in work outcomes between those fulfilling the clinical and imaging 'arms' of the ASAS axSpA classification criteria. It has been consistently shown that patients with r-axSpA and nr-axSpA have similar disease burden[34] and respond similarly to therapy.[35] In addition, the 'clinical arm' of the axSpA criteria has been shown to belong to the 'SpA Gestalt' as much as the imaging 'arm' contrary to what has been initially claimed.[36 37] Our results yield further evidence in favour of the full 'SpA spectrum', by showing, for the first time, similar work outcomes across the different 'sub-groups' of the disease spectrum.

Consistent with other studies, the association between BASFI and comorbidities with work outcomes is confirmed.[7 27 29 33] Since fewer studies are available on presenteeism and absenteeism, it is important to highlight the additional, and expected, strong role of ASDAS in sick leave and presenteeism during the last 7 days.

Our study has some limitations worth noticing. First, being a cross-sectional study the direction of the associations found cannot be determined, especially for comorbidities and for the role of disease activity on employment. However, this is the largest yet worldwide study performed to determine factors associated with work outcomes in the full spectrum of SpA and provides relevant data to inform future longitudinal studies. Second, some countries may be under-represented due to their small sample size (eg, Belgium, Canada and Colombia). Thus, caution should be taken when extrapolating our results to specific countries. However, all available data could efficiently be taken into account in our analysis using sophisticated multilevel analytical methods. With this method, there is no need for between-countries stratification and the resulting loss of statistical power. Third, although we have used data from the well-known COMOSPA study, information bias cannot be completely ruled out. In fact, we found a larger-than-expected number of patients with university-level education (1375 or 44%). Fourth, even though consecutive patients were included and the baseline characteristics are representative of a SpA population, selection bias cannot be excluded, and the patients may therefor not fully reflect the real SpA population. Fifth, there are no work-related variables that are likely to be important predictors of work participation

In conclusion, we have shown, using international data, the relevance of the much-overlooked individual-level and country-level SE factors on work participation in patients with SpA. Investing in the healthcare system leads to better work outcomes irrespective of the patient's individual characteristics including disease activity and therapy. Although longitudinal data are warranted, our results suggest that health policies taking both individual-level and country-level SE factors into account may, more effectively, promote work participation among patients with SpA.

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EXTENDED REPORT

Measurement properties of the ASAS Health Index: results of a global study in patients with axial and peripheral spondyloarthritis

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ABSTRACT

Objectives To evaluate construct validity, interpretability, reliability and responsiveness as well as determination of cut-off points for good and poor health within the original English version and the 18 translations of the disease-specific Assessment of Spondyloarthritis international Society Health Index (ASAS HI) in 23 countries worldwide in patients with spondyloarthritis (SpA).

Methods A representative sample of patients with SpA fulfilling the ASAS classification criteria for axial (axSpA) or peripheral SpA was used. The construct validity of the ASAS HI was tested using Spearman correlation with several standard health outcomes for axSpA. Test–retest reliability was assessed by intraclass correlation coefficients (ICCs) in patients with stable disease (interval 4–7 days). In patients who required an escalation of therapy because of high disease activity, responsiveness was tested after 2–24 weeks using standardised response mean (SRM).

Results Among the 1548 patients, 64.9% were men, with a mean (SD) age 42.0 (13.4) years. Construct validity ranged from low (age: 0.10) to high (Bath AnkylosingSpondylitisFunctioning Index: 0.71). Internal consistency was high (Cronbach's α of 0.93). The reliability among 578 patients was good (ICC=0.87 (95% CI 0.84 to 0.89)). Responsiveness among 246 patients was moderate-large (SRM=−0.44 for non-steroidal anti-inflammatory drugs, −0.69 for conventional synthetic disease-modifying antirheumatic drug and −0.85 for tumour necrosis factor inhibitor). The smallest detectable change was 3.0. Values ≤5.0 have balanced specificity to distinguish good health as opposed to moderate health, and values ≥12.0 are specific to represent poor health as opposed to moderate health.

Conclusions The ASAS HI proved to be valid, reliable and responsive. It can be used to evaluate the impact of SpA and its treatment on functioning and health. Furthermore, comparison of disease impact between populations is possible.

INTRODUCTION

Spondyloarthritis (SpA) is characterised by inflammation and new bone formation in the axial skeleton and joints.[1] Patients with SpA suffer from axial and peripheral symptoms resulting in pain, spinal stiffness, sleep problems and fatigue.[2–4] Peripheral manifestations (arthritis, dactylitis or enthesitis) and extra-articular manifestations such as uveitis, psoriasis and inflammatory bowel disease may add to the burden of disease in a substantial number of patients but are less well studied.[5 6] The course of disease varies, but many patients experience functional disability and limitation in activities and social participation. The influence of the disease on health-related quality of life and functional status has been well characterised in patients with ankylosing spondylitis (AS), and to a lesser extent for patients with non-radiographic axial SpA (nr-axSpA), but there are little data relating to patients with peripheral SpA (pSpA).[4 6]

The Assessment of Spondyloarthritis international Society Health Index (ASAS HI) has been developed to measure functioning and health in patients with SpA with the aim of defining and comparing the impact of the disease and health in this patient group.[7] Initial phases in the development of the ASAS HI focused on investigating functional impairments from the patients' perspective using both qualitative and quantitative approaches.

The biopsychosocial model of disease proposed by the International Classification of Functioning, Disability and Health (ICF) was used as the basis for the development of the ASAS HI. The ICF is accompanied by a classification of categories, called factors, that allow description of functioning, disability and health in individuals in a systematic and inclusive way.[8] The comprehensive ICF Core Set for AS is a disease-specific selection of the ICF factors that are typical and relevant for AS, and has served as the underlying construct of the ASAS HI since the whole range of functioning, disability and health of patients with AS was captured.[9] Patients, rheumatologists and methodologists were involved

in the further reduction of categories using qualitative and quantitative methods and resulting finally in the ASAS HI.[7] The 17 dichotomous items of the ASAS HI address aspects of pain, emotional functions, sleep, sexual functions, mobility, self-care and community life representing a wide spectrum of different levels of functioning, disability and health in patients with SpA. The sum score of the ASAS HI ranges from 0 to 17, with a lower score indicating a better health status. Preliminary validity and feasibility (time of completion) have already been assessed in a field test during the final steps of the development phase.[7] Cognitive debriefing was undertaken in patients with AS and nr-axSpA and patients with peripheral manifestations aiming at assessment of a broad impact of health on all patients with SpA.[7 10] The ASAS HI was originally developed in parallel in English-speaking countries (Australia, Canada, Ireland, UK, USA), and it has later been translated and cross-culturally adapted into 18 languages worldwide.[10]

The objective of the current paper is to evaluate construct validity, interpretability, reliability and responsiveness as well as determination of cut-off points for good and poor health within the original English version and the 18 translations of the disease-specific ASAS HI in 23 countries worldwide.

METHODS

Study design

A cross-sectional international observational study with a longitudinal component for reliability and responsiveness of the ASAS HI was performed in 23 countries during 2014 and 2015.

Patients

A representative sample of patients with SpA fulfilling the ASAS classification criteria for either axial (axSpA) or pSpA were recruited.[11 12] Each centre was asked to recruit a sample of patients, 80% of whom were to have axSpA and 20% pSpA with no more than 10% of all recruits having coexistent psoriasis. Of the axSpA subset, 40% were to have nr-axSpA and 60% AS. There was a target of 50–100 recruits per country to reach an overall sample size of 1700 patients. The aim was to include patients with a broad range of disease severity and a variety of treatments. Patients with severe concomitant diseases that may influence their functional status were excluded from participation together with patients who were unable to understand the objectives of the study or the various questionnaires. Centres were asked to include at least 25% of their sample in the reliability arm and 25% in the responsiveness arm. All centres received approval from their local ethics committee. Written informed consent was obtained from all respondents prior to the start of their participation.

Data collection

Demographic and clinical information was collected including age, gender, predominant presentation, presence of extra-articular manifestations, years of education and employment. C reactive protein levels, imaging results and current medications were also recorded. Physician' s judgement of patients' overall functioning and health was assessed by a single global question ("Please score the overall status of the subject's signs and symptoms and the functional capacity of the subject") on a zero to 10 numerical rating scale (NRS) (10 representing severe impairment) and a Likert scale ("How do you rate the health of your patient today?") on a 4-point scale ranging from very poor to very good. Physician's opinion on the level of disease activity was recorded by answering the question "How active was the spondyloarthritis of your patient during the last week?".

Patients completed a series of self-reported questionnaires: ASAS HI,[7] Bath Ankylosing Spondylitis Disease Index (BASDAI),[13] Bath Ankylosing Spondylitis Functioning Index (BASFI),[14] EuroQol five dimensions questionnaire (EQ-5D-5L index and thermometer),[15] Short Form Survey Instrument 36-Item (SF-36),[16] Hospital Anxiety and Depression Scale (HADS),[17] work productivity and activity impairment questionnaire (WPAI)[18] and pain and spinal pain NRS (0–10 NRS; 10 representing severe pain). Patient's opinion on the level of disease activity was recorded by a single patient global question ("How active was your rheumatic disease on average during the last week?") on a NRS 0–10 and on the health status ("How do you rate your health today?") on a 4-point Likert scale ranging from very poor to very good. Based on collected data, the Ankylosing Spondylitis Disease Activity Score (ASDAS) sum score was calculated and patients were categorised into ASDAS status groups.[19 20] EQ-5D index was calculated using the value set for UK except for France, Germany, Netherlands, Spain, Thailand and USA for which country-specific value sets were used.

ASAS Health Index

The ASAS HI contains 17 items (dichotomous response option: 'I agree' and 'I do not agree') addressing different aspects of functioning. A sum score is being calculated by summing up all responses to 'I agree' given a total ASAS HI score ranging from 0 to 17—with a lower score indicating a better and a higher score indicating an inferior health status (see also user's manual for handling missing items; online supplementary file 1).[7]

Variables were collected at baseline and longitudinally in stable patients (reliability arm) or in patients who required a therapeutic change because of high disease activity (responsiveness arm) (flow chart and patients' disposition in online supplementary file 2). Longitudinal assessments were performed in patients who were in a stable disease state (reliability arm) or in patients who required a therapeutic change because of high disease activity (responsiveness arm). Patients in the reliability arm were eligible for the analyses when they considered themselves in a stable disease state while on stable treatment (no change in non-steroidal anti-inflammatory drugs (NSAIDs) over the preceding week, with no change in conventional synthetic disease-modifying antirheumatic drug (csDMARD) or tumour necrosis factor inhibitor (TNFi) therapy over the last 4 weeks). Patients were invited to complete the questionnaire at home after an interval of 4–7 days to evaluate reproducibility. Patients in the responsiveness arm required therapeutic change initiated due to high disease activity. The therapeutic change could include initiation of NSAIDs, a csDMARD or a TNFi. Patients were reassessed 12–24 weeks (for NSAIDs 2–24 weeks) after the treatment change had been implemented. The patients with longitudinal assessments (reliability and responsiveness) were asked to answer a global question at the second assessment and respond as to whether their condition was stable, improved or had worsened compared with baseline assessments. Only those patients reporting improvement in response to the global change question were analysed to assess responsiveness. Results of the validation process and the psychometric properties of the ASAS HI were presented at various ASAS meetings. Votes were taken from ASAS members to confirm the thresholds of ASAS HI.

Statistics

COSMIN recommendations were followed to test and report measurement properties.[21] Psychometric properties were

Table 1 Patient characteristics, values of health status and composite indices at baseline and for reliability and responsiveness assessment

Patient characteristics	Baseline (n=1548)	Reliability, first visit (n=578)	Reliability, second visit (n=578)	Responsiveness first visit (n=246)*	Responsiveness second visit* (n=246)
Age (years)	42.0 (13.4)		45.3 (13.7)		37.2 (12.2)
Male, n (%)	1005 (64.9)		372 (64.4)		152 (61.8)
Symptom duration (years)	14.5 (11.4)		16.6 (11.9)		10.9 (9.5)
Extraspinal manifestation, current, n (%)					
Arthritis	301 (19.4)		99 (17.1)		43 (17.4)
Dactylitis	50 (3.2)		9 (1.6)		11 (4.5)
Enthesitis	261 (16.9)		80 (13.8)		44 (17.9)
Extra-articular manifestation, current, n (%)					
Uveitis	53 (3.4)		17 (2.9)		5 (2.0)
IBD	65 (4.2)		32 (5.5)		7 (2.8)
Skin psoriasis	110 (7.1)		51 (8.8)		3 (1.2)
HLA-B27 positive, n (%)	994 (77.0)		350 (73.9)		107 (71.9)
CRP (mg/L), sample n=1353	9.8 (16.01)	6.8 (10.2)	–	16.7 (22.6)	6.7 (11.4)
Elevated CRP (≥0.5 mg/L), n (%)	765 (49.4)	256 (44.3)	–	100 (64.5)	48 (31.0)
Current NSAID treatment	994 (64.2)		364 (63.0)		119 (76.8)
Current csDMARD treatment	402 (26.2)		166 (28.7)		35 (22.6)
Current TNFi treatment	591 (38.2)		240 (41.5)		23 (14.8)
ASAS HI (0–17)	6.7 (4.3)	6.2 (4.2)	6.0 (4.2)	8.2 (3.9)	5.7 (4.0)
ASDAS	2.5 (1.2)	2.6 (1.1)	–	3.3 (1.0)	1.9 (1.0)
BASDAI	4.1 (2.5)	3.7 (2.3)	3.4 (2.1)	5.4 (2.1)	3.0 (2.1)
BASFI	3.3 (2.8)	3.1 (2.7)	3.1 (2.7)	4.1 (2.7)	2.4 (2.3)
Pain, NRS 0–10	4.4 (2.9)	3.8 (2.6)	3.6 (2.4)	6.1 (2.4)	3.0 (2.1)
Physician global, NRS 0–10	3.7 (2.3)	3.1 (2.0)	–	5.6 (2.1)	2.4 (1.7)
Patient global, NRS 0–10	4.5 (2.8)	3.8 (2.5)	3.6 (2.3)	5.9 (2.4)	2.3 (1.7)
Well-being last week	4.4 (2.8)	3.8 (2.6)	3.5 (2.3)	5.8 (2.5)	2.4 (2.0)
PASS yes	801 (51.7)	368 (63.7)	401 (69.4)	45 (29.0)	130 (83.9)
HADS anxiety	17.6 (3.9)	18.0 (3.8)	18.3 (3.8)	16.6 (3.9)	18.5 (3.6)
HADS depression	15.3 (3.5)	15.9 (3.5)	15.7 (3.5)	14.4 (3.4)	16.0 (3.4)
EQ-5D VAS (0–100 mm)	61.6 (22.7)	63.9 (22.6)	65.3 (22.6)	57.5 (20.2)	67.3 (22.1)
EQ-5D (pooled)†	0.67 (0.2)	0.7 (0.2)	0.7 (0.2)	0.7 (0.2)	0.7 (0.2)
SF-36 PSC	38.9 (10.5)	40. 4 (10.2)	40.6 (10.2)	34.4 (9.3)	41.3 (9.5)
SF-36 MSC	47.0 (11.5)	48.6 (11.2)	48.5 (11.2)	43.5 (10.9)	48.7 (11.0)
WPAI, presenteeism	29.2 (26.0)	23.4 (23.4)	21.9 (21.1)	39.3 (27.4)	23.6 (21.9)
WPAI, absenteeism‡	16.0 (32.3)	11.68±27.5	10.5 (27.3)	31.0±42.7	10.9±27.3

Values are presented as mean (SD) or absolute number (%). Percentages are % of available data. Fewer than 5% of the data were missing, except for HLA-B27 with 16.6%, CRP with 8.7% and EQ-5D with 9.7% at baseline visit and ASDAS (in responsiveness arm) with 7.3%. CRP and physician global were not measured in the reliability arm at the second visit.

*Only data analysed from those patients who stated that they improved during the time interval.

†The phrase *EQ pooled* means that EQ-5D-5L analysis was based on the five-level value set for UK except for France, Germany, Netherlands, Spain, Thailand and USA for which the country-specific value set was used .

‡Calculated for employed patients (n=961); see online supplementary file 4.

ASAS HI, ASAS Health Index; ASDAS, Ankylosing Spondylitis Disease Activity Score; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; CRP, C reactive protein; csDMARD, conventional synthetic disease-modifying antirheumatic drug; EQ-5D, Euro Quality of Life 5 Dimensions; HADS, Hospital Anxiety Depression Scale; HLA, human leucocyte antigen; IBD, inflammatory bowel disease; MSC, mental component summary score; NRS, numerical rating scale; NSAID, non-steroidal antirheumatic drug; PASS, Patient Acceptable Symptom State; PSC, physical component summary score; SF-36, Short Form 36; TNFi, tumour necrosis factor inhibitor; VAS, Visual Analogue Scale; WPAI, Work Productivity and Impairment Scale.

examined according to the OMERACT filter.[22] Descriptive statistics were used to characterise the sample. According to the COMSIN checklist, interpretability is being summarised as information about percentage of missing items and description of how missing items were handled as well as distribution of the (total) ASAS HI score including floor and ceiling effects. Distributions of scores were examined for identification of floor and ceiling effects. Construct validity was evaluated against other health outcomes (including patient and physician global assessment, ASDAS, BASDAI, BASFI, HADS, WPAI, SF-36 summary values (physical component summary score (PCS) and mental component summary score (MCS), EQ-5D) in a cross-sectional analysis using Spearman correlation. Prior to the analysis, we hypothesised magnitude and direction of correlations, and correlation were considered low if ≤0.30, moderate if >0.30 and ≤0.50, high if >0.50 and <0.80, and very high if ≥0.8.[23] Internal consistency was evaluated using Cronbach's α coefficient (adequate: ≥0.70). Test–retest reliability was assessed by intraclass correlation coefficient (ICC) (two-way model, single measure) with a 95% CI. An ICC of ≥0.8 was considered

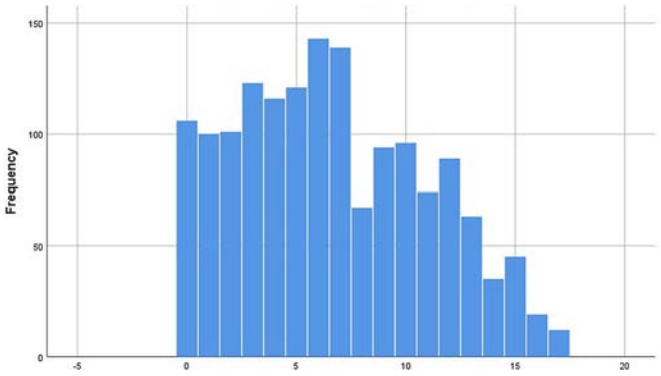

Figure 1 Score distribution (0–17) of the ASAS Health Index (ASAS HI) at baseline.

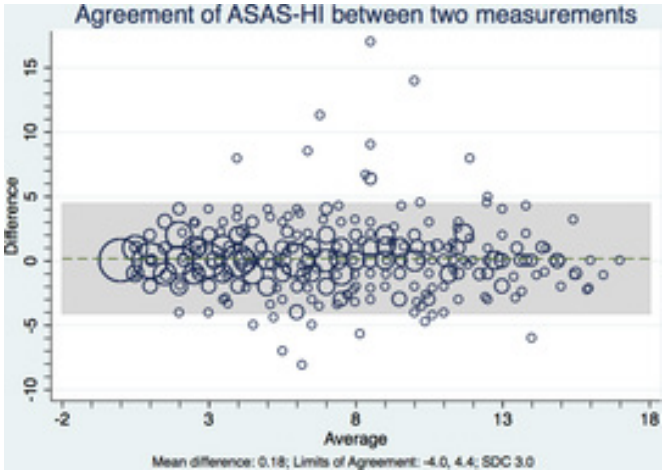

Figure 2 Bland and Altman plot. The differences between total sum score of ASAS Health Index (ASAS HI) at two time points were plotted against the mean of the two values together with the smallest detectable change (SDC).

to indicate excellent reliability. Agreement across the scale of the ASAS HI was visualised by Bland and Altman plot. Measurement error was assessed by analysing the smallest detectable change (SDC) based on the 95% limits of agreement by using the formula: SDC=1.96×SD of the mean difference in ASAS HI of the two assessments in the reliability sample/√2.[24] Responsiveness was tested with standardised response mean (SRM) after 2–24 weeks depending on the type of medication. SRM was assessed by using the following formula: SRM=ASAS HI mean difference/SD of ASAS HI mean difference. A SRM <0.4 was considered to represent a low effect, 0.4–0.79 a moderate effect and ≥0.8 a large effect. The discriminant ability of the ASAS HI was assessed by calculating ASAS HI mean scores for predefined status groups (ASDAS status groups (inactive, moderate, high and very high), BASDAI and BASFI thresholds (<2.0, 2.0–3.99, 4.0–5.99, ≥6.0)) by analysis of variance. To distinguish between relevant health states (an additional relevant aspect of interpretability), two different methods were applied: fixed 90% specificity and the closest point to (0,1).[25 26] We used the patient global assessment at predefined levels (<3 and >6 on NRS and cut-off between good and poor on Likert scale) as external constructs for 'poor', 'moderate' and 'good' health status. We used a global rating of change question (Likert scale) as external construct to assess change perceived by the patient. A cut-off between 'improved' versus 'no change' or 'worse' was used to determine minimal clinically important improvement. Final choice was based on a consensus during the ASAS meeting in June 2017 (74 participants, 100% agreement). A p value ≤0.05 was considered significant. Statistical analyses were performed using SPSS V.23.

Table 2 Spearman correlation between ASAS Health Index scores and other PRO

	Hypothesis	Spearman correlation	Confirmation*
Pain	High	0.60	Yes
Spinal pain	High	0.54	Yes
Patient global	High	0.57	Yes
Physician global	Moderate	0.49	Yes
ASDAS	High	0.61	Yes
BASDAI	High	0.70	Yes
BASFI	High	0.71	Yes
HADS anxiety	Moderate	−0.55	No
HADS depression	Moderate	−0.57	No
EQ-5D VAS (0–100 mm)	High	0.45	No
EQ-5D	High	−0.72	Yes
SF-36 PSC	High	−0.73	Yes
SF-36 MSC	Moderate	−0.59	No
WPAI presenteeism	Moderate	0.60	No
WPAI absenteeism	Moderate	0.38	Yes
Well-being last week	High	0.61	Yes

*Column indicates whether hypothesis generated prior to analysis about magnitude and direction of correlation was confirmed in the specific variable.
ASDAS, Ankylosing Spondylitis Disease Activity Score; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; EQ-5D, Euro Quality of Life 5 Dimensions; HADS, Hospital Anxiety Depression Scale; MSC, mental sum component; PSC, physical sum component; SF-36, Short Form 36; VAS, Visual Analogue Scale; WPAI, Work Productivity and Impairment Scale.

RESULTS

Sample characteristics

In total, 1593 patients participated in the international validation study (sample size per country varied between 15 and 130) (see online supplementary file 3). Of these, 1548 had analysable data (45 patients were excluded because of major incomplete data): 64.9% were men, mean age 42.0 (SD 13.4) years, mean symptom duration 14.5 (11.4) (table 1). There were 1292 (83.5%) patients with axSpA (375 patients (29.0%) with nr-axSpA and 917 (71.0%) with AS) and 256 (16.5%) patients with pSpA. Patients had, on average, moderate disease activity as measured by ASDAS and BASDAI, with 64.2% treated with NSAIDs and 38.2% were treated with TNFi (table 1; additional detailed patients' characteristics of the whole cohort are presented in online supplementary file 4). As expected, the patients in the responsiveness sample have a higher level of disease activity at baseline.

Psychometric properties of the ASAS HI

Interpretability

The mean total score in the population sampled for the ASAS HI was 6.7 (SD 4.3). A total score was calculated for respondents in which not more than 20% of the data were missing (see also user's manual published in online supplementary file 1). Numbers of missing values were limited and occurred between 0.1% and 0.3% (online supplementary file 5). Floor (percentage of the respondents who had the lowest possible (total) score)

Table 3 Discriminant ability of the ASAS Health Index (ASAS HI) with respect to disease activity and physical functioning

Disease activity					F test	P values
ASDAS thresholds	Inactive (n=245)	Moderate (n=283)	High (n=500)	Very high (n=289)		
ASAS HI	2.9 (3.1)	5.1 (3.5)	7.3 (3.6)	10.4 (3.5)	230.	<0.001
BASDAI thresholds	<2.0 (n=372)	2.0–3.9 (n=405)	4.0–5.9 (n=347)	≥6.0 (n=414)		
ASAS HI	2.8 (2.9)	5.2 (3.1)	7.8 (3.3)	10.5 (3.4)	421.4	<0.001
Functioning					**F test**	**P values**
BASFI thresholds	<2.0 (n=633)	2.0–3.9 (n=322)	4.0–5.9 (n=258)	≥6.0 (n=323)		
ASAS HI	3.7 (3.1)	6.5 (3.1)	8.6 (3.4)	11.2 (3.1.6)	438.0	P<0.001

*All values given as mean (SD).
ASDAS, Ankylosing Spondylitis Disease Activity Score; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index.

or ceiling effects (percentage of the respondents who had the highest possible (total) score) of the ASAS HI in this analysis were acceptable (6.9% and 0.8%, respectively) (figure 1).

Construct validity

Construct validity showed Spearman correlation coefficient ranging from moderate (WPAI absenteeism: 0.38) to high (BASFI: 0.71 or SF-36 PSC 0.73).

As hypothesised, the ASAS HI had high correlation with patient global (r=0.57), pain (r=0.60), spinal pain (r=0.54), SF-36 MCS (r=0.59), HADS (r=−0.55 and −0.57), BASFI, ASDAS (r=0.61), presenteeism (r=0.60), BASDAI, BASFI, EQ-5D and SF-36 PCS (r>0.70). The correlation of ASAS HI with physician global (r=0.49) and absenteeism (r=0.38) was moderate (table 2). Of note, correlation of ASAS HI with age was weak (r=0.10). Hypothesis about magnitude and direction of correlation was confirmed in 68.7% of variables.

Internal consistency

ASAS HI scores showed a high Cronbach's α of 0.93. Internal consistency of ASAS HI did not vary much across different disease groups (0.93 for AS, 0.94 for nr-axSpA and 0.91 for pSpA).

Reliability and measurement error

A total of 770 patients had a second assessment for reliability. Of these, 192 patients had to be excluded because of missing data (n=54), patients not being stable (n=74) or second assessment performed outside the time frame (n=64). Finally, 578 (75.1%) patients who considered themselves to be in a stable state were analysed (table 1). The mean (SD) baseline ASAS HI was 6.2 (4.2) and the second ASAS HI was 6.0 (4.2). Reliability was excellent with an ICC of 0.87 (95% CI 0.84 to 0.89) and ICCs were comparably high in all disease subtypes (AS 0.87 (95% CI 0.84 to 0.89); nr-axSpA 0.89 (95% CI 0.85 to 0.93); pSpA 0.83 (95% CI 0.75 to 0.88)). Bland-Altman plot shows a good agreement between ASAS HI sum score at first and second assessment. No systematic differences in sum score for the two measurement time points were found. Calculation of the limits of agreement (and the SDC) was based on the assumption that reliability was homoscedastic over the entire range of ASAS HI although this was not completely the case as the variation was somewhat more pronounced in the middle of the range (figure 2). The SDC was calculated as 3.0, which corresponds to the minimum change beyond measurement error that can be detected in an individual patient over time.

Responsiveness

A total of 353 patients were allocated to the sensitivity to change arm because of initiation of a new treatment. Also, 107 patients had to be excluded from the 353 initial patients because of missing data (n=47), patients deteriorating during time interval (n=12), patients not reporting a change in their disease state (n=47) and second assessment performed outside of the time frame (n=1). Finally, 246 (69.7%) estimated themselves to have improved between visits and were analysed. Seventy-eight patients started NSAIDs, 41 patients a csDMARD and 127 patients TNFi. The SRM was −0.44 for NSAIDs (moderate), −0.69 for csDMARDs (moderate) and −0.85 for TNFi (large).

Discriminant ability

The ASAS HI discriminated well between patients with different disease activity states (measured by ASDAS and BASDAI) and function (measured by BASFI) (table 3). The groups with greater disease activity and more impaired functioning had higher mean ASAS HI scores (indicating impaired health) than those with lower disease activity.

Table 4 Analysis of cut-off values for ASAS Health Index (ASAS HI) scores to define health status

ASAS HI cut-offs and external criterion	n (P+N)	90% SP (SE/SP)	(0,1) (SE/SP)	AUC
Cut-off between 'good' and 'moderate' functioning				
Patient global, Likert very good/good versus all others	1531 (624+907)	3.0 (49.5/90.0)	5.7 (73.5/75.9)	0.81
Patient global, NRS <3	1533 (435+1098)	2.3 (46.2/90.5)	5.0 (76.5/71.9)	0.80
Cut-off between 'moderate' and 'poor' functioning				
Patient global, Likert very poor/poor versus all others	1531 (304+1227)	12.0 (39.5/90.5)	7.4 (79.3/67.6)	0.80
Patient global, NRS >6	1533 (425+1108)	11.7 (34.3/89.6)	7.4 (71.3/69.7)	0.76

AUC, area under the curve; NRS, numerical rating scale; P+N, number of positive+negative results according to the external criterion; SE, sensitivity; SP, specificity; 90% SP, cut-off according to the 90% specificity criterion; (0,1), cut-off according to the closest point to (0,1) criterion.

Table 5 Discriminant ability of the health status groups

Health state (number, % patients)	Good ≤5.0 (n=553, 36%)	Moderate <5.0 to <12.0 (n=755, 49%)	Poor ≥12.0 (n=235, 15%)
ASAS HI	2.1 (1.5)	7.8 (2.0)	13.7 (1.5)
BASFI	1.2 (1.5)	3.8 (2.5)	6.3 (2.3)
BASDAI	2.1 (1.6)	4.8 (2.1)	6.6 (1.9)
ASDAS	1.7 (0.9)	2.6 (2.1)	3.7 (1.1)
SF-36 PSC	47.6 (7.1)	35.7 (8.8)	28.7 (6.6)
EQ-5D	0.8 (0.1)	0.6 (0.2)	0.4 (0.2)

*Values given as mean (SD) otherwise indicated.
ASAS HI, ASAS Health Index; ASDAS, Ankylosing Spondylitis Disease Activity Score; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; EQ-5D, Euro Quality of Life 5 Dimensions; PSC, physical sum component; SF-36, Short Form 36.

Cut-off values for interpreting health status based on ASAS HI scores

Final cut-offs for ASAS HI scores to distinguish poor versus moderate, and moderate versus good health are presented in table 4. All analysed scenarios with application of different external anchors and different methodological approaches are presented in online supplementary file 6. In order to balance sensitivity and specificity, a threshold of ASAS HI, which differentiated patients with 'good/very good' health from those with 'moderate' health state, was identified as being 5.0. In contrast, the 90% specificity criterion was considered to be the most clinically relevant threshold of ASAS HI for 'moderate' versus 'poor/very poor' health identified as a score of 12.0 or above.

Attempts to define a clinically important improvement proved an elusive target since scores were too heterogeneous. We therefore recommend using the SDC value of 3.0 to determine change in ASAS HI in individual patients and present the percentage of patients with a change of ≥3.0.

Applying these thresholds within the validation cohort, we were able to show that the three defined health status groups within ASAS HI could discriminate with respect to both disease activity, functioning and health measures (table 5). The two cut-off values delineating the three health statuses were agreed on after discussion and voting by 74 ASAS members during their European League Against Rheumatism meeting 2017 (74 approval, 0 decline, 0 abstention).

DISCUSSION

The manuscript presents the psychometric properties of the original English ASAS HI and its different translations, as obtained in a large international cohort. We show that the ASAS HI is a valid, reliable and responsive measure of functioning and health in patients with SpA on a global level. Interpretability was good as has been shown for different aspects. In this paper, we report the values for the entire cohort and country-specific results will be published separately in the language of the specific country. Generally, the results were similar in the various countries (data not shown).

Since the ASAS HI contains only 17 items with a dichotomous response option addressing all important aspects of patient complaints, administration of the questionnaire is feasible as it has been shown in a previous field test.[10] The calculation to obtain a single sum score is simple and quick to undertake. Floor effect was acceptable with almost no ceiling effect observed in our study. The scores have good face validity and the ASAS HI exhibited excellent correlation with other measures covering a range of health outcomes. Analysis of construct validity demonstrated a strong association between ASAS HI sum score and both disease activity and functional disability, indicating that the ASAS HI is measuring a broader concept than just disease activity or physical functioning. In addition, the high correlation between ASAS HI and patient global assessment as well as generic health measures (such as SF-36) suggest that patients do not make substantial distinctions between disease-specific and more generally worded questionnaires. We noted in our cohort a weaker correlation between ASAS HI and physician global as well as discordance between physician and patient global scores at baseline. However, the discordance between patient responses and physician response is very small and not comparable with those reported in literature.[27 28]

We were able to show that the ASAS HI is applicable in all patients with SpA irrespective of the disease subgroup. Similar results in internal consistency between AS, nr-axSpA and pSpA provide support for the use of these questionnaires in the whole group of SpA. This is an important finding as the ASAS HI was originally developed in patients with AS. However, use of the ASAS HI in patients with pSpA should be carefully checked and its applicability should be further investigated to gain more insights into this subgroup of patients.

There is a debate about which measure is suitable for assessing responsiveness. Our choice is SRM, which is not recommended according to the COSMIN guidelines.[21] However, SRM is one of the widely used responsiveness measures and there is also critique published in the literature about this part of the COSMIN guidelines.[29] One of the arguments is that the SRM is more reflecting the magnitude of the event than providing information on the measure. Indeed, we do show that the SRM is better for start of biological DMARDs than for NSAIDs. However, providing the SRM is a useful information for researchers who want to use the ASAS HI as an outcome measure in a trial.

This study has clear strengths and weaknesses. Strengths include the involvement of 23 countries with 18 country-specific translations with different cultures and socioeconomic backgrounds within the validation process.[10] Thus, the domains of functioning and health assessed in the questionnaire are likely to be relevant across countries and cultures. However, qualitative research about this issue is lacking. One relative weakness of this international validation study may be considered the small sample size in some countries, especially in the longitudinal arm. However, the results of the study do show that the psychometric properties are robust and meaningful. The ASAS HI can be used in clinical trials to evaluate the impact of SpA and its treatment on overall functioning and health in patients with SpA and also to compare disease impact in cohorts and populations. Further research is needed to address the question whether and how the ASAS HI is applicable in daily routine care to guide treatment decisions.

In conclusion, the ASAS HI proved to be a valid, interpretable, reliable and responsive questionnaire to assess overall functioning and health in this global international validation study including 19 languages.

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EXTENDED REPORT

Changing patterns in clinical–histological presentation and renal outcome over the last five decades in a cohort of 499 patients with lupus nephritis

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ABSTRACT

Objectives To evaluate changes in demographic, clinical and histological presentation, and prognosis of lupus nephritis (LN) over time.

Patients and methods We studied a multicentre cohort of 499 patients diagnosed with LN from 1970 to 2016. The 46-year follow-up was subdivided into three periods (P): P1 1970–1985, P2 1986–2001 and P3 2002–2016, and patients accordingly grouped based on the year of LN diagnosis. Predictors of patient and renal survival were investigated by univariate and multivariate proportional hazards Cox regression analyses. Survival curves were compared using the log-rank test.

Results A progressive increase in patient age at the time of LN diagnosis ($p<0.0001$) and a longer time between systemic lupus erythematosus onset and LN occurrence ($p<0.0001$) was observed from 1970 to 2016. During the same period, the frequency of renal insufficiency at the time of LN presentation progressively decreased ($p<0.0001$) and that of isolated urinary abnormalities increased ($p<0.0001$). No changes in histological class and activity index were observed, while chronicity index significantly decreased from 1970 to 2016 ($p=0.023$). Survival without end-stage renal disease (ESRD) was 87% in P1, 94% in P2% and 99% in P3 at 10 years, 80% in P1 and 90% in P2 at 20 years ($p=0.0019$). At multivariate analysis, male gender, arterial hypertension, absence of maintenance immunosuppressive therapy, increased serum creatinine, and high activity and chronicity index were independent predictors of ESRD.

Conclusions Clinical presentation of LN has become less severe in the last years, leading to a better long-term renal survival.

INTRODUCTION

Lupus nephritis (LN) is a frequent and severe manifestation of systemic lupus erythematosus (SLE) and is characterised by a relapsing and remitting clinical course.[1–4] Renal involvement occurs at the time of SLE diagnosis or during the course of the disease in up to two-thirds of patients.[5 6] Clinical presentation varies from asymptomatic urinary abnormalities to chronic irreversible renal insufficiency.[7] Although renal involvement is still considered a strong predictor of death and end-stage renal disease (ESRD),[8 9] both patient and renal survival have significantly improved in the last few decades[10–13] and the rate of renal flares has considerably decreased over time as well.[3] The improvement in LN prognosis has been attributed to many factors including the better understanding of SLE pathogenesis, new treatment options and strategies, and improved management of hypertension, infections and other comorbidities.[14]

To the best of our knowledge, no studies have evaluated whether changes in demographic, clinical and histological features at the time of LN presentation have occurred over the last decades and whether these changes have had an influence on the disease management and outcome.

The objective of our study was to examine the changes in demographic, clinical and histological features at the time of LN onset in a large cohort of patients during a 46-year follow-up. We looked at changes in LN prognosis during the course of the follow-up and searched for the prognostic factors associated with patient and renal outcomes.

PATIENTS AND METHODS

Four hundred and ninety-nine patients were included in this retrospective study of prospectively collected data. Inclusion criteria were American College of Rheumatology criteria-based diagnosis of SLE[15] and biopsy-proven LN performed between January 1970 and December 2016. Patients were followed in four Italian referral centres: Renal Divisions of Ospedale Maggiore Milano, San Carlo Hospital Milano and University of Parma, and Rheumatology Unit of Padova University. Since the 1980s, according to the good clinical practice, patients undergoing renal biopsy in Italy signed informed consent that includes the consent for using clinical data for scientific purposes, while in previous years no consent was required for this type of studies. The study was approved by the local ethics committees. The 46-year follow-up was subdivided into three periods (P), 15 years each: P1 from January 1970 to December 1985, P2 from January 1986 to December 2001 and P3 from January 2002 to December 2016, and patients

accordingly grouped based on the year of LN diagnosis. Detailed data on the source population and study design are reported in table 1 and online supplementary text S1. Notably, 70.3% of the overall source population had biochemical and/or urinary abnormalities of lupus nephritis. The high proportion of patients with LN is due to the fact that three of the four centres participating in this study were Nephrology Units.

All patients received a renal biopsy that was classified according to the International Society of Nephrology/Renal Pathology Society (IRS/RPS) classification criteria.[16] Since 2003, all renal biopsies performed before 2002 were reclassified according to the same IRS/RPS classification criteria by the clinicians and pathologists based on written reports of light microscopy and immunofluorescence or the re-evaluation of slides, where necessary. Activity and chronicity indices were calculated according to the score proposed by Austin *et al*.[17] Estimated glomerular filtration rate (eGFR) was calculated according to the Cockcroft and Gault formula based on gender, serum creatinine, age and body weight of the patients. Normal renal function was defined as serum creatinine ≤1 mg/dL and eGFR >60 mL/min that correspond to the definition of CKD 1 and 2. Proteinuria was measured by benzethonium chloride on the urine collected over 24 hours expressed as grams per 24 hours. Arterial hypertension was defined as the mean of three consecutive measurements

Table 1 Description of source population and features of patients included in the study

	Overall	P1	P2	P3	P values
(A) Source population					
All patients with SLE, N	793	162	249	382	–
Patients with LN, N (%)	557 (70.2)	124 (76.5)	174 (69.8)	259 (67.8)	ns
Patients with renal biopsy, N (%)*	499 (89.6)	106 (85.5)	158 (90.8)	235 (90.7)	ns
Patients without renal biopsy, N (%)*	58 (6.1)	18 (14.5)	16 (9.2)	24 (9.3)	ns
Lost to follow-up, N (%)	21 (3.7)	2 (1.2)	6 (2.4)	13 (3.4)	ns
(B) Clinical features of patients with renal biopsy					
	Overall 499 patients	P1 106 patients	P2 158 patients	P3 235 patients	
Gender, female, N (%)	427 (85.6)	99 (93.4)	139 (88)	189 (80.4)	0.004
Age at SLE diagnosis, years	28.11±12.0	27±10.3	26.3±11.2	29.8±13	0.01
Age at LN diagnosis, years	31.4±12.5	28.4±10.4	29±11.5	34.4±13.3	0.001
Disease duration before LN diagnosis, years	3.3±5.3	1.3±1.3	2.6±4.5	4.6±6.3	<0.0001
Follow-up duration, years	12.7±9.8	20.5±13	15.8±7.8	6.8±4.3	
Weight, kg	61.7±12.2	57.4±10.4	62±11.2	63.3±13.1	ns
Hypertension, N (%)	240 (48.2%)	56 (52.8%)	77 (48.7%)	107 (45.9%)	ns
Serum creatinine, mg/dL	1.2±1.1	1.8±1.8	1.2±0.8	1.0±0.7	<0.0001
Creatinine clearance, mL/min	86.3±41	72.2±45.1	83.7±36.6	94.1±40.2	0.0001
Proteinuria, g/24 hours	4.1±3.7	3.6±2.7	4.5±4.0	4.1±3.9	ns
Urinary erythrocytes/HPF	27.7±45.7	18.6±18.6	24.2±24.3	34.1±61.9	0.01
Serum albumin, g/dL	3.0±0.7	2.7±0.7	3.0±0.7	3±0.7	0.005
Haematocrit, %	33.5±6.2	33.3±7.3	33.8±5.5	33.4±6	ns
White blood cells/10^3/mL	6252±3223	6258±2842	6180±2888	6299±3603	ns
Platelets/109/L	240 302±96 198	230 422±103 282	252 193±97 365	236 641±91 640	ns
C3, mg/dL	62.1±25.4	65.1±22.6	58.7±25.4	63.1±26.3	ns
C4, mg/dL	13.7±14.3	20.7±20.2	14.7±15.8	10.2±8	0.001
Anti-dsDNA, positive N (%) (NA 25)	414 (87.3)	82 (93.6)	128 (85.3)	204 (90.3)	ns
Urinary abnormalities	203 (40.7)	28 (26.4)	60 (38)	115 (48.9)	<0.0001
Nephrotic syndrome	174 (34.9)	32 (30.2)	59 (37.3)	83 (35.4)	ns
Nephritic syndrome	92 (18.4)	31 (29.2)	32 (20.3)	29 (12.4)	0.0001
Rapidly progressive renal insufficiency	30 (9.0)	15 (14.2)	7 (3.9)	8 (3.4)	<0.0001
Histological classes, N (%)					
II	22 (4.4)	5 (4.8)	4 (2.5)	13 (5.5)	ns
III†	115 (23.1)	23 (21.9)	28 (17.8)	64 (27.2)	ns
IV†	267 (53.7)	56 (53.3)	91 (58)	120 (51.1)	ns
V	93 (18.7)	21 (20)	34 (21.7)	38 (16.2)	ns
VI	2 (0.4)	1 (0.9)	1 (0.6)	0 (0)	ns
Activity index	6.4±4.9	6.2±4.9	6.6±4.9	5.9±4.5	ns
Chronicity index	2.0±2.2	2.6±2.5	2.0±2.2	1.6±2	0.0023

(A) Number of patients with SLE followed in the four centres (three Nephrology Units and one Rheumatology Unit) and number of patients with clinical diagnosis of lupus nephritis who underwent or did not undergo renal biopsy, overall and subdivided according to the different periods. (B) Clinical features at the time of lupus nephritis diagnosis in patients who underwent renal biopsy, overall and according to the three different periods. P values refer to t-test, Kruskal-Wallis test or χ^2 test (with 2 df), according to the type and distribution of variables.

*Percentages refer to the number of patients who received renal biopsy (n=557).

†Class III+V: overall, four patients; P1, three patients; P2, one patient, P3, no cases. Class IV+V: overall, 31 patients; P1, 2 patients, P2, 8 patients; P3, 21 patients.

P, period; P1: 1970–1985; P2: 1986–2001; P3: 2002–2016.

C3/C4, complement components; HPF, high-power field; LN, lupus nephritis; NA, not available; N, number; ns, not significant; SLE, systemic lupus erythematosus.

of systolic blood pressure >140 mm Hg and/or diastolic blood pressure >90 mm Hg in sitting position. Data on death were obtained from hospital charts for patients who died in hospital and through information obtained from relatives for other patients.

Definitions

Clinical syndromes at presentation were defined as follows:

- Isolated urinary abnormalities: normal renal function, proteinuria <3.5 g/24 hours and >0.5 g/24 hours, and/or microscopic haematuria (urinary red blood cells >5/high-power field (HPF)) after having excluded non-renal causes;
- Nephrotic syndrome: normal renal function, proteinuria >3.5 g/24 hours and serum albumin <3.5 g/dL;
- Acute nephritic syndrome: acute renal dysfunction (serum creatinine >1 mg/dL and eGFR <60 mL/min), macroscopic or severe microscopic haematuria (urinary red blood cells >20/HPF) and/or erythrocyte casts, arterial hypertension and variables degrees of proteinuria;
- Rapidly progressive renal insufficiency: rapid deterioration of renal function leading to CKD stage 3 to 5 within a few weeks, with oliguria, arterial hypertension and severe haematuria.
- Renal states at last observation were defined as follows: complete renal remission, serum creatinine <1 mg/dL with eGFR >60 mL/min, proteinuria <0.5 g/day and inactive urinary sediment; partial renal remission, serum creatinine <1 mg/dL with eGFR >60 mL/min and proteinuria <3.5 g/day and ≥0.5 g/day; CKD, serum creatinine >1.0 mg/dL with eGFR <60 mL/min and inactive urinary sediment, confirmed by at least three determinations; ESRD, the need of renal replacement therapy; Poor renal outcome, CKD or ESRD.

Statistical analysis

Mean±SD or median and IQR were used for descriptive statistics, according to variable distribution. Temporal trends of clinical parameters were tested through Pearson or Spearman correlation analysis, according to parametric or non-parametric variable distribution. Survival curves were drawn using the Kaplan-Meier estimate and compared using the log-rank test. Univariate and multivariate proportional hazards Cox regression analyses were used to investigate the prognostic value of continuous and binary (dichotomised) variables. Patients lost to follow-up were 2/106 (1.9%) in P1, 6/158 (3.8%) in P2 and 13/235 (5.5%) in P3. These low numbers of patients and the lack of a significant clinical deterioration at their last available follow-up suggest that censoring due to loss to follow-up was likely to be minimal and non-informative. The statistical package S-Plus was used to analyse sample data.[18]

RESULTS

Demographic characteristics

Four hundred and ninety-nine patients (427 women, 85.6%) were included in the study; they were followed for a median period of 10.6 years (IQR 4–18). All but 51 (10.2%) patients were Caucasian. Demographic, clinical and histological features of the cohort at the time of LN diagnosis are reported in table 1. The cohort was subdivided into three groups according to the year of LN diagnosis: group 1 included 106 patients (21%) diagnosed with LN in P1; group 2 encompassed 158 patients (32%) diagnosed with LN in P2; group 3 comprised 235 patients (47%) diagnosed with LN in P3.

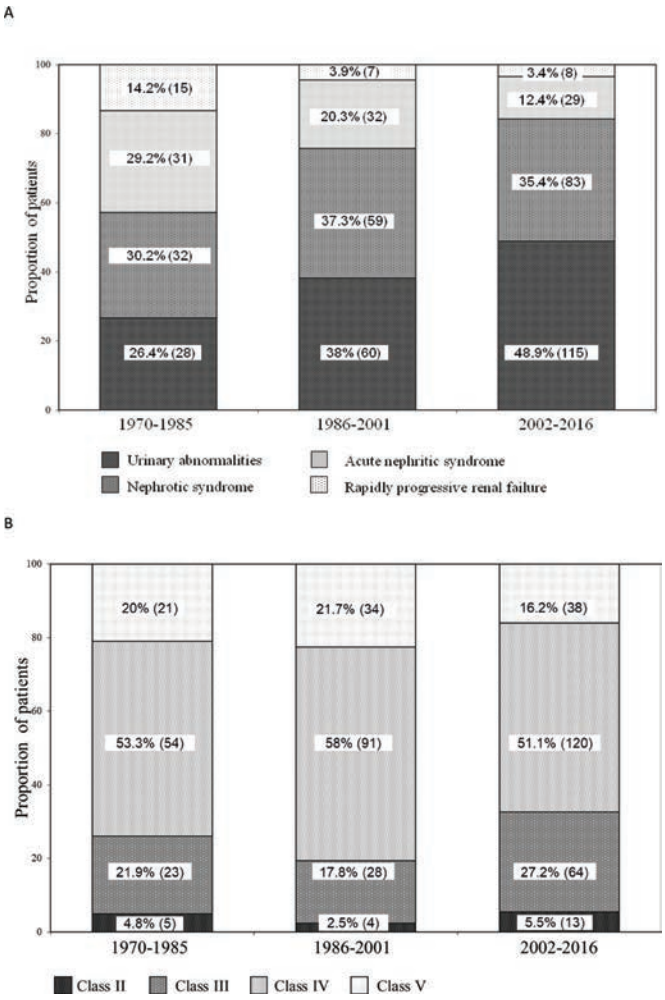

Figure 1 (A) Clinical syndrome at presentation of lupus nephritis in three different periods. (B) Histological classes at renal biopsy in three different periods.

The number of male patients progressively increased over the three periods: 6.6% in P1, 12% in P2 and 19.6% in P3 (p=0.004). The lag time between SLE and LN diagnosis (p<0.0001) progressively increased from 1970 to 2016. The mean age at the time of LN occurrence increased from 28.4±10.4 in P1 to 29±11.5 in P2, and to 34.4±13.3 in P3 (p<0.001).

Clinical and histological presentation

The mean values of serum creatinine progressively decreased overtime: 1.8±1.8 mg/dL in P1, 1.2±0.8 mg/dL in P2 and 1.0±0.7 mg/dL in P3 (p<0.0001). Consistently, a significant decrease in the frequency of acute nephritic syndrome (p=0.0001) and rapidly progressive renal insufficiency (p=0.0001) was observed, together with a significant increase in the prevalence of isolated urinary abnormalities from the first to the third period (p<0.001) (figure 1A). The rate of nephrotic syndrome presentation was similar in the three periods. Creatinine serum levels, eGFR, proteinuria and urinary red blood cells in patients with the different clinical syndromes at the time of LN diagnosis by the three periods are reported in online supplementary table S1.

No differences in the percentage of histological classes in the three periods were observed (table 1 and figure 1B). Interestingly, an increase in mixed forms (class III+IV and IV+V) from P1 (4.7% of cases) to P2 (12.6%) and P3 (17.4%) (p=0.006) was

Table 2 Induction and maintenance therapy, and outcomes in all patients and according to the three different periods

	Overall 499 patients	P1 106 patients	P2 158 patients	P3 235 patients	P values
Methylprednisolone pulses, N (%)	351 (70.3)	63 (67.7)	120 (83.9)	168 (73.7)	0.01
Immunosuppressive drugs, induction					
None, N (%)	66 (13.2)	28 (29)	26 (17.9)	12 (5.4)	<0.0001
Cyclophosphamide, N (%)	258 (51.7)	49 (51)	95 (65.5)	114 (51.3)	0.016
Azathioprine, N (%)	42 (8.4)	15 (15.6)	18 (12.4)	9 (4.0)	<0.0001
Mycophenolate, N (%)	79 (15.8)	0	4 (2.7)	75 (33.8)	<0.0001
Others*, N (%)	17 (3.4)	3 (3.1)	2 (1.4)	12 (5.4)	ns
Immunosuppressive drugs, maintenance					
None, N (%)	140 (28)	66 (68.7)	50 (34)	24 (10.9)	<0.0001
Cyclophosphamide, N (%)	7 (1.4)	1 (1)	5 (3.4)	1 (0.45)	ns
Azathioprine, N (%)	152 (30.4)	27 (28)	58 (39)	67 (30.6)	ns
Mycophenolate, N (%)	143 (28.6)	1 (1)	22 (15.1)	120 (54.8)	<0.0001
Others*, N (%)	18 (3.6)	0	11 (7.5)	7 (3.2)	ns
Outcomes†					
Partial renal remission, N (%)	122 (25.5)	7 (6.9)	43 (28.1)	72 (32.1)	<0.0001
Complete renal remission, N (%)	246 (51.4)	41 (49.6)	74 (48.4)	131 (58.5)	0.01
CKD, N (%)	31 (6.4)	8 (7.9)	13 (8.5)	10 (4.5)	<0.0001
ESRD, N (%)	42 (8.8)	25 (24.8)	14 (9.1)	3 (1.3)	<0.0001
Death, N (%)	37 (7.7)	20 (19.8)	9 (5.9)	8 (3.6)	<0.0001

P, period; P1: 1970–1985; P2:1986–2001; P3: 2002–2016. P values refer to χ^2 test with 2 df.
*'Others' includes ciclosporin A, methotrexate, rituximab.
†Outcome was available in 478 patients (P1, 101 patients; P2, 153 patients; P3, 224 patients).
CKD, chronic kidney disease; ESRD, end-stage renal disease.

noted. Activity index did not significantly change over the three periods either when all the classes were considered (table 1) or when patients with class III (4.95±2.9 in P1, 5.6±3.1 in P2 and 5.9±4.5 in P3, p=ns) and class IV (9.4±4.9 in P1, 9.4±3.7 in P2 and 9.4±3.8 in P3, p=ns) were separately analysed. Conversely, chronicity index significantly decreased (p=0.0023) from P1 to P3 (table 1).

Treatment

More than two-thirds of patients in each period were treated with methylprednisolone pulses as induction therapy. In P1, 29% of patients received corticosteroids alone for induction therapy in comparison with 17.9% in P2% and 5.4% in P3 (p<0.0001). Immunosuppressive drugs were added to corticosteroids for maintenance therapy in 30.5% of patients in P1, 65.5% in P2% and 89.1% in P3 (p<0.0001). The immunosuppressive drugs used in induction and maintenance therapy during the three periods are reported in table 2. More than 50% of patients in each period received cyclophosphamide as induction therapy (online supplementary table S2). A decrease in the use of azathioprine as induction therapy from P1 to P3 was counterbalanced by an increase in the use of mycophenolate mofetil (MMF). As far as maintenance therapy is concerned, the proportion of patients receiving azathioprine remained stable in the first two periods and decreased in the third period (p<0.0001), while MMF use significantly increased in the last period compared with the previous ones (p<0.0001). Notably, the proportion of patients who were not treated with induction therapies progressively decreased over time (p<0.0001).

Renal outcome and predictors of renal survival

Outcome was available in 478 patients (95.8%) (table 2). At last observation, complete renal remission was observed in 49.6% of patients in P1, 48.4% in P2% and 58.5% in P3 (p=0.01) (table 2). CKD and ESRD occurred in 7.9% and 24.8% of patients in P1, in 8.5% and 9.1% in P2 and in 4.5% and in 1.3% in P3, respectively (p<0.0001 for all comparisons). Twenty patients in P1 died (19.8%), in comparison with 9 (5.9%) in P2 and 8 (3.6%) in P3 (p<0.0001). The CKD-free survival at 10 and at 20 years was 75% and 66% in P1, 85.5% and 80.2% in P2%, and 91.5% in P3, respectively (p=0.0069) (figure 2A). The ESRD-free survival at 10 and at 20 years were respectively 87% and 80% in P1, 94% and 90% in P2%, and 99% in P3, respectively (p=0.0019) (figure 2B). Predictors of CKD and ESRD at univariate analyses are reported in table 3.

At multivariate analysis, carried out in the entire cohort, several factors at the time of the diagnosis of LN were independently associated with poor renal outcomes (CKD or ESRD) including baseline serum creatinine, high activity and chronicity index, arterial hypertension and the absence of maintenance immunosuppressive therapy (table 4). In addition, male gender, older age and high serum creatinine were predictors of death (table 4).

DISCUSSION

Our study outlines the most significant changes observed during the last five decades in demographic, clinical and histological features of LN at presentation. These results were drawn from a large multicentric cohort of patients followed in four Italian referral centres from 1970 to 2016. In order to identify changes in LN presentation, the whole observational time was subdivided into three periods, 15 years each.

Historically, from 1970 to 1985 (P1) corticosteroid monotherapy was progressively replaced by combination treatment of corticosteroids with either azathioprine or cyclophosphamide probably due to the results of a pooled analysis that showed the superiority of combined immunosuppressive regimens over corticosteroids alone.[19] Intravenous methylprednisolone pulses were also largely used in this period.[20 21] From 1986 to 2001 (P2), high-dose intravenous cyclophosphamide was commonly used as

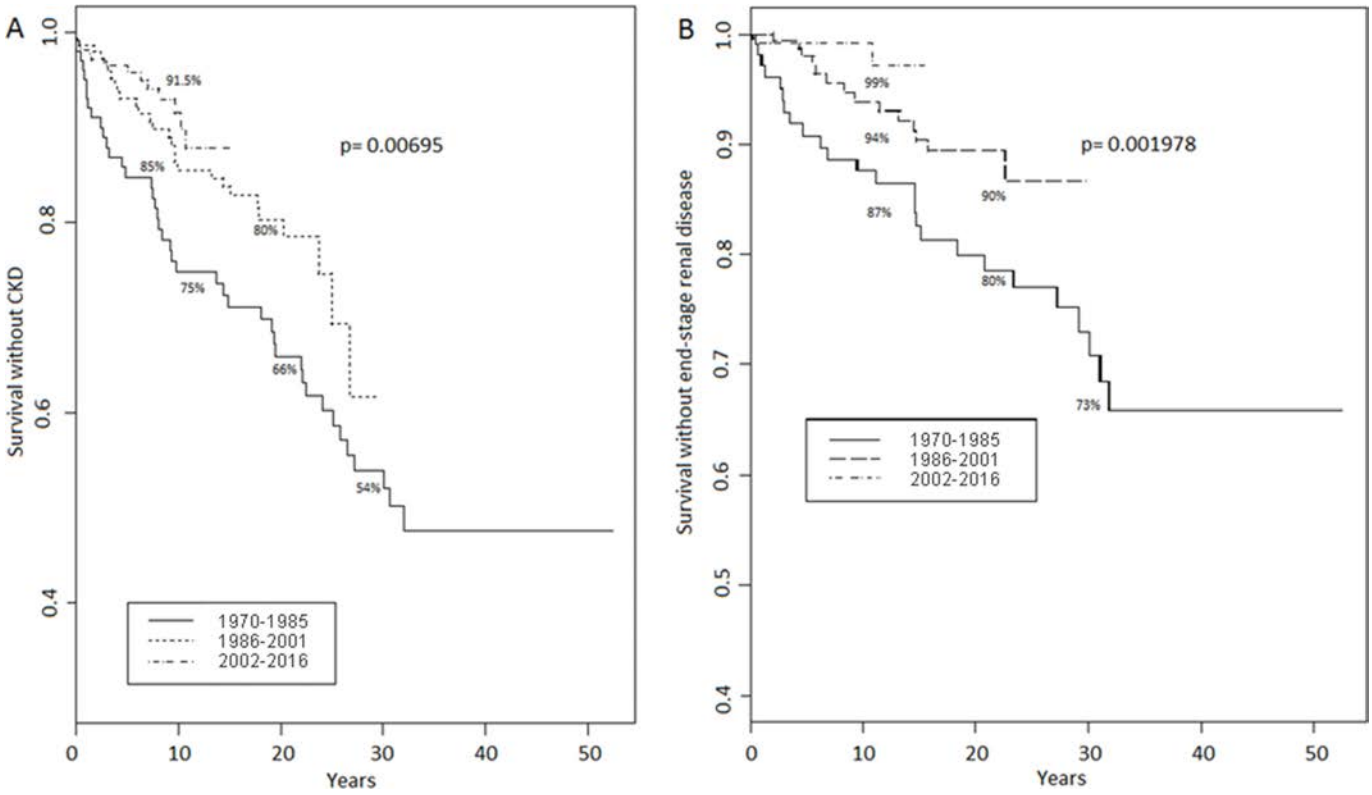

Figure 2 (A) Survival without chronic kidney disease (CKD) in three different periods. (B) Survival without end-stage renal disease in three different periods.

induction and maintenance therapy following the positive results of long-term controlled trials carried out at the National Institutes of Health.[22] In the same period, the use of a combined oral immunosuppressive regimen as maintenance therapy became progressively more popular.[23] Interestingly, the proportion of our patients who received steroids alone as induction therapy decreased from 29% in P1% to 18% in P2 and further declined to 5% in P3. Finally, from 2002 to 2016 (P3), the evidence that MMF has a similar efficacy compared with cyclophosphamide in the induction phase and is more effective than azathioprine in the maintenance phase led to an increase in the use of MMF for induction as well as for maintenance therapy.[24–26]

The age of our patients at LN diagnosis progressively increased from 1970 to 2016 and LN developed progressively later after the onset of SLE. These changes may result from an earlier diagnosis of SLE, which leads to a closer surveillance of LN over time and, in turn, allows the identification of mild disease phenotypes, as well as from the earlier and more appropriate therapeutic intervention that includes the extensive use of antimalarial drugs,[27 28] MMF[29 30] and biological drugs[31 32] capable of hindering the development of LN.

The most interesting and innovative observation of our study is the progressively milder clinical presentation of LN from P1 to P3. Presentation with isolated urinary abnormalities significantly increased from 25% in P1 to about 50% in P3. This finding was accompanied by the progressive decrease in the frequency of renal insufficiency at presentation, while the percentage of nephrotic syndrome did not significantly change over time. The decreased severity in clinical presentation from 1970 to 2016 is in keeping with the progressive decline in serum creatinine at the time of LN diagnosis, which is one of the most important predictors of renal adverse outcome in short-term and long-term follow-up.[17 33 34]

Nevertheless, the distribution of the renal histological classes was similar in the three periods regardless of clinical presentation. Class IV accounted for more than 50% of cases in all periods, followed by class III in 25%, class V in around 20% and class II in a minority of patients. There was a significant increase from P1 to P3 in mixed classes (class III+Vand class IV+IV) that are considered to be associated with the worst prognosis in some [35 36] but not all studies.[37 38] Activity index remained unchanged from P1 to P3 either when we considered all histological classes or class III and IV separately. These data are consistent with the discrepancy between clinical and histological severity of LN at presentation reported in previous studies.[7] Proliferative forms of LN were observed even in the absence of urinary abnormalities,[39 40] suggesting that a certain amount of time is required for histological lesions to give rise to clinical manifestations. On the other hand, the early diagnosis of renal involvement in recent years can account for the lower severity of clinical presentation, which is in accordance with the significant progressive decrease in the chronicity index from P1 to P3. Moreover, in the last decades, the indication to renal biopsy has become wider due to the decrease in post-biopsy complications, which has led to perform renal biopsy in a number of patients with less severe urinary abnormalities. The increasing number of class III and class IV LN diagnosed with isolated urinary abnormalities, yet with high activity index (unchanged over the three periods), has important implications in clinical practice. Indeed, this result emphasises once again the importance of renal biopsy in defining the prognosis and tailoring therapeutic approaches to LN. Notably, high activity and chronicity indexes were independent predictors of ESRD and CKD at multivariate analysis. Due to the decreasing trend of LN presentation with severe renal dysfunction, these histopathological variables remain a valuable tool aiding the physician in defining prognosis and taking treatment decisions in all patients.[41]

Table 3 Univariate Cox proportional hazard regression analysis among the clinical characteristics at presentation of lupus nephritis for end-stage renal disease and chronic kidney disease

	Univariate analysis ESRD			Univariate analysis CKD		
	RR	95% CI	P values	RR	95% CI	P values
Year of LN diagnosis	0.941	0.914 to 0.967	**<0.0001**	0.964	0.945 to 1.058	**0.00017**
Male gender	1.84	0.810 to 4.188	0.14	1.53	0.824 to 2.836	0.18
Age at diagnosis of LN	0.998	0.969 to 1.027	0.9	1.01	0.987 to 1.026	0.5
Duration of SLE before diagnosis of LN	0.925	0.835 to 1.024	0.13	0.961	0.906 to 1.019	0.19
Histological classes: II+V vs III+IV	3.01	1.067 to 8.456	**0.037**	1.79	0.987 to 3.251	0.055
Activity index*	1.15	1.085 to 1.26	**<0.0001**	1.11	1.065 to 1.167	**<0.0001**
Chronicity index*	1.39	0.935 to 1.531	**<0.0001**	1.3	1.197 to 1.414	**<0.0001**
Urinary abnormalities+nephrotic syndrome vs nephritic syndrome+rapidly progressive renal insufficiency	3.19	2.202 to 4.620	**<0.0001**	2.35	1.88 to 2.943	**<0.0001**
Log serum creatinine†	5.03	3.52 to 7.26	**<0.0001**	3.72	2.838 to 4.838	**<0.0001**
Creatinine clearance	0.967	0.864 to 1.082	**<0.0001**	0.974	0.967 to 0.981	**<0.0001**
Proteinuria g/24 hours	1.04	0.969 to 1.110	0.28	1.03	0.979 to 1.083	0.24
Urinary erythrocytes	0.996	0.984 to 1.008	0.56	1.002	0.997 to 1.006	0.46
Serum albumin	0.551	0.36 to 0.84	0.0058	0.716	0.53 to 0.96	0.026
Arterial hypertension	8.35	3.277 to 21.177	**<0.0001**	4.15	2.480 to 6.900	**<0.0001**
Haematocrit	0.91	0.875 to 0.946	**<0.0001**	0.926	0.899 to 0.953	**<0.0001**
White blood cell count	1	1.000 to 1.000	**<0.0001**	1	1.000 to 1.000	**0.008**
Platelet count	1	1.000 to 1.000	0.33	1	1.000 to 1.000	0.07
C3	0.993	0.979 to 1.005	0.26	0.997	0.988 to 1.005	0.5
C4	0.998	0.977 to 0.995	0.8	0.997	0.982 to 1.011	0.68
Methyprednisolone pulses/oral prednisolone	1.01	0.45 to 2.26	0.97	0.913	0.530 to 1.571	0.74
Immunosuppressive induction therapy	2.23	1.079 to 4.623	**0.03**	0.724	0.420 to 1.244	0.24
Immunosuppressive maintenance therapy	0.693	0.34 to 1.41	0.31	0.857	0.53	1.38

*For any unit increase in activity or in chronicity index.
†For any unit increase in log serum creatinine.
Significant P values are given in bold.
C3/C4, complement components; CKD, chronic kidney disease; ESRD, end-stage renal disease; LN, lupus nephritis; SLE, systemic lupus erythematosus.

Arterial hypertension was another important predictor of both ESRD and CKD.[34 42–44] Thus, the effective control of blood pressure is of paramount importance in the management of LN. In keeping with previous reports,[45–48] male gender was associated with worse renal outcome in our cohort; however, according to a recent critical review of the literature, there is limited evidence supporting the worse prognosis in male than in female patients.[49]

Table 4 Predictors of chronic kidney disease, end-stage renal disease and death at multivariate Cox proportional hazards regression analysis

	Coefficient	RR	95% CI	P value
Dependent variable: chronic kidney disease				
Logarithm of serum creatinine	0.8708	2.39*	1.57 to 3.65	<0.0001
Activity index	0.0611	1.06†	1 to 1.13	0.038
Chronicity index	0.1188	1.13†	1.01 to 1.26	0.034
Hypertension	1.4243	4.16	2.15 to 8.03	<0.0001
No immunosuppressive drugs for maintenance	0.7341	2.08	1.14 to 3.82	0.018
Dependent variable: end-stage renal disease				
Logarithm of serum creatinine	1.0001	2.72*	1.5 to 4.92	0.00095
Male gender	1.2057	3.34	1.25 to 8.93	0.016
Activity index	0.0936	1.1†	1.02 to 1.19	0.02
Chronicity index	0.2545	1.29†	1.11 to 1.49	0.00069
Hypertension	1.7835	5.95	1.99 to 17.75	0.0014
No immunosuppressive drugs for maintenance	1.1106	3.04	1.37 to 6.74	0.0063
Dependent variable: death				
Logarithm of serum creatinine	0.6355	1.8*	1.1 to 3.25	<0.0001
Male gender	1.0584	2.88	1.17 to 7.1	<0.0001
Older age	0.0711	1.07‡	1.04 to 1.11	<0.0001

Clinical characteristics at presentation of lupus nephritis were analysed as independent variables.
*For any unit increase in log serum creatinine.
†For any unit increase in activity or in chronicity index.
‡For any increase in 1 year of age.
RR, relative risk.

We observed that the proportion of male patients progressively increased over time, but we have no explanation for the increase in number of men diagnosed in the last decades and we think that this preliminary result needs to be confirmed in large multicentre studies. Another interesting result of our study is the significant and progressive improvement of renal survival from P1 to P3, which confirms previous data[10–13] and is probably the result of a wider indication to renal biopsy and improved treatment of LN over the last decades.[48]

We are aware of a number of limitations of this study. It is a retrospective study of prospectively collected data and no information is provided on the number of patients who achieved remission after induction therapy, the duration of remission, the number of flares and the need of repeated renal biopsy. The majority of our patients were Caucasian; hence, the results may not be applied to other ethnic groups.

In conclusion, the clinical presentation at the time of kidney biopsy for suspected LN has apparently become less severe in the last years and is now characterised by an increase in isolated urinary abnormalities and a decrease in renal insufficiency. However, a concomitant decrease in histological active lesions was not observed. This emphasises once again the importance of performing renal biopsy in the management of LN. The progressive improvement in renal survival in our cohort is the result of a comprehensive approach, which includes a prompt diagnosis of renal involvement, a wider indication to renal biopsy, treatment based on renal biopsy and increased clinical experience in the management of LN.

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Contributors GM, AD and RAS contributed to the conception and design of the work, interpreted the data, drafted and revised the manuscript for important intellectual content. SQ and LS contributed to the statistical analysis. PGV, MG, DG, GC, FR, MZ and MLU followed up patients and contributed to the acquisition of data. PM, FP and AV critically revised the final work. All the authors approved the final version of the manuscript and gave their agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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EXTENDED REPORT

Prediction of progression of interstitial lung disease in patients with systemic sclerosis: the SPAR model

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ABSTRACT

Objectives To identify the predictive clinical characteristics and establish a prediction model for the progression of mild interstitial lung disease (ILD) in patients with systemic sclerosis (SSc).

Methods Patients with SSc from two independent prospective cohorts were included in this observational study. All patients fulfilled the 2013 American College of Rheumatology/European League Against Rheumatism criteria, had mild ILD at baseline diagnosed by High-Resolution Computed Tomography (HRCT), available baseline and ≥1 annual follow-up pulmonary function tests and no concomitant pulmonary hypertension or airflow obstruction. ILD progression was defined as a relative decrease in forced vital capacity (FVC)%≥15%, or FVC%≥10% combined with diffusing capacity for carbon monoxide %≥15% at 1-year follow-up. Candidate predictors for multivariate logistic regression were selected by expert opinion based on clinical significance. A prediction model for ILD progression was established in the derivation cohort and validated in the multinational validation cohort.

Results A total of 25/98 and 25/117 patients with SSc showed ILD progression in the derivation cohort and the validation cohort, respectively. Lower SpO_2 after 6 min walk test (6MWT) and arthritis ever were identified as independent predictors for ILD progression in both cohorts. The optimal cut-off value of SpO_2 after 6MWT for predicting ILD progression was determined as 94% by receiver operating characteristic curve analysis. The derived SPAR model combining both predictors (SPO_2 and ARthritis) increased the prediction rate from 25.5% to 91.7% with an area under the curve (95% CI) of 0.83 (0.73 to 0.93).

Conclusions The evidence-based SPAR prediction model developed in our study might be helpful for the risk stratification of patients with mild SSc-ILD in clinical practice and cohort enrichment for future clinical trial design.

INTRODUCTION

Systemic sclerosis (SSc) is a heterogeneous autoimmune disease, characterised by vascular damage, inflammation and fibrosis of skin and various visceral organs.[1] Interstitial lung disease associated with SSc (SSc-ILD) is a common complication and leading cause of death in SSc.[2 3] Nowadays, treatment options are still limited and challenging. The European League Against Rheumatism (EULAR) recommended that cyclophosphamide (CYC) should be considered for the immunosuppressive treatment of SSc-ILD. Mycophenolate mofetil (MMF) has shown similar effects to CYC.[4–6] However, due to their known toxicity, overall mild to moderate and short-lasting effects, CYC and MMF are generally administered only to selected cases with risk for ILD deterioration.[4]

Previous studies explored baseline predictors for lung progression and mortality in SSc-ILD. Diffuse cutaneous subset, presence of antitopoisomerase-I antibodies, decreased baseline forced vital capacity (FVC) and diffusing capacity for carbon monoxide (DLCO) were reported to be significantly associated with lung progression in patients with SSc, while positive anticentromere antibody (ACA) was protective.[7–13] However, different definitions of lung deterioration were applied. When patients with SSc-ILD were preselected as the study population, only extensive lung disease was predictive for both lung progression and mortality in previous studies.[14–16] Older age, decreased baseline FVC, short-term pulmonary function trends and exercise peripheral oxygen saturation (SpO_2) were shown to be predictive for mortality in patients with SSc-ILD.[14 15 17–22] However, these results were limited by small sample size, lack of validation and insufficient adjustment for potential confounders.[23] Moreover, few extrapulmonary factors were assessed for prognostic value in SSc-ILD.

The natural disease course of SSc-ILD is highly heterogeneous. Some patients with minimal ILD will remain stable while others could deteriorate rapidly. Clinicians would like to start active treatment earlier for those who are at risk of lung progression. However, currently no data are available to distinguish between progressive and stable patients when mild ILD is diagnosed. These patients might be easily overlooked and undertreated due to better pulmonary function tests (PFTs) often within normal values.[24] This defines an urgent clinical need for the identification of baseline predictors of lung progression in patients with mild SSc-ILD. A clinically applicable model to identify patients at risk for progression of mild SSc-ILD should become even more meaningful in view of the multiple ongoing phase II/III prospective randomised placebo-controlled clinical trials with targeted therapies in SSc, which might open new treatment options for SSc and SSc-ILD in the near future.[25]

The objectives of the current study were (1) to identify baseline clinical characteristics that can

predict the progression of mild SSc-ILD at 1-year follow-up in a derivation cohort; (2) to establish a simple-to-use and practical prediction model by combining independent predictors for lung progression in mild SSc-ILD; (3) to validate the derived prediction model in a multinational validation cohort.

METHODS

Study cohorts

Patients were included from the longitudinal cohort with prospectively collected data from the University Hospital Zurich (derivation cohort). Data from three other SSc expert centres (Oslo, Paris, Berlin) combined as a validation cohort were also included.

All included patients fulfilled the following criteria: diagnosis of SSc according to the 2013 American College of Rheumatology/EULAR (ACR/EULAR) classification criteria,[26] diagnosis of mild ILD by HRCT at baseline visit, available PFTs at baseline and annual follow-up visits (annual defined as 12±3 months), no concomitant pulmonary hypertension according to right heart catheterisation or echocardiography as judged by the local investigators, no evidence of substantial airflow obstruction defined as forced expiratory volume in 1 s/FVC<70%.

Clinical data

Demographic and clinical parameters including age, gender, disease duration, SSc antibodies, PFTs, 6 min walk test (6MWT), arthritis status and modified Rodnan Skin Score (mRSS) at baseline were obtained from all included centres. The parameters in each local database were collected following the European Scleroderma Trials and Research recommendations (for details about oximetry assessment see online supplementary material).[3]

Assessment of ILD

PFTs were obtained at baseline and annual follow-up visits in all patients. HRCT lung images were available at baseline in all included patients. Reticular pattern abnormalities and superimposed ground-glass opacities defined as equivalent to fibrosis were assessed semi-quantitatively as previously described.[12 15 27] Pulmonary fibrosis at baseline was expressed as percentage of total lung volumes, an extent of <20% pulmonary fibrosis was considered as mild ILD and an extent of pulmonary fibrosis ≥20% was defined as extensive ILD (see online supplementary material).

Study end point

The end point of this study, progression of mild SSc-ILD, was defined present if either of the following parameters was fulfilled at 1-year follow-up: a relative decrease in FVC% predicted ≥15%, or relative decrease in FVC% predicted ≥10% combined with DLCO% predicted ≥15%. Patients fulfilling these criteria were classified as progressors while the others were non-progressors. If more than one follow-up visit was available, the visit fulfilling the above criteria for progression was chosen as the follow-up visit and the visit 1 year before as the baseline visit.

We defined the thresholds of PFTs' decline for the end point based on the criteria recommended for idiopathic pulmonary fibrosis trials by the American Thoracic Society/European Respiratory Society and previous clinical trials in SSc-ILD.[5 6 28] The evolution period of 1 year has been chosen since it is the routine follow-up interval for patients with SSc in clinical practice and also frequently used in clinical trials for SSc-ILD.

Statistical analysis

Candidate predictors were selected using the nominal group technique by SSc experts (OD, A-MH-V, YA, ES, SY, BM, RD, MOB, WW), who were asked to suggest clinically meaningful variables with face validity based on previous studies and feasibility (see online supplementary material). The following hypothesis was tested in this study: one or more of six candidate predictors including ILD-associated variables (FVC, DLCO, SpO_2 after 6MWT) and extrapulmonary variables (mRSS, arthritis ever, disease duration) can independently predict the progression of mild SSc-ILD. Potential confounding demographic and SSc-associated variables (age, gender, anti-Scl-70 positivity, ACA positivity) were adjusted in the multivariate analysis.

Baseline characteristics were described and compared between progressors and non-progressors by univariate analyses followed by Bonferroni correction. The independent sample t-test, Mann-Whitney U test, X^2 test and Fisher's exact test were conducted, as appropriate.

Relationships between the candidate predictors and the occurrence of ILD progression were investigated using multivariate logistic regression. Each significant continuous parameter was then converted to categorical variable by determining the optimal cut-off value using receiver operating characteristic (ROC) curve analysis. Multiple imputation was used to address missing data in the validation cohort and the pooled cohort. The logistic regression models from both the original dataset and the multiply imputed dataset were presented.

The risk prediction model was established by combining independent predictors. Discriminatory performance by applying ROC curve analysis (eg, area under the curve (AUC), sensitivity and specificity) and predictive performance (eg, positive and negative predictive values) of the prediction model were examined. The prediction model was also separately tested in patients stratified by disease duration and immunosuppressive treatment at baseline.

All above-mentioned statistical analyses were conducted in both the derivation cohort and validation cohort. Significance was defined as $p<0.05$. The statistical analysis was performed using SPSS V.23.

RESULTS

Study populations

A total of 98 patients with SSc with mild ILD at baseline who met the inclusion criteria were analysed in the derivation cohort. Of those, 25 (25.5%) patients showed ILD progression at 1-year follow-up (classified as progressors). Patients were predominantly female (79.6%) with a mean age of 57.3 years. The median disease duration was 4.7 years (IQR 1.9–7.4). Thirty patients (30.6%) had diffuse cutaneous involvement. The mean FVC (% predicted) was 101.9% and the mean DLCO (% predicted) was 76.5%. The detailed demographic and clinical characteristics at baseline are provided in table 1.

In the validation cohort, 25 of 117 (21.4%) patients with SSc with mild ILD at baseline were ILD progressors at 1-year follow-up. The description of baseline characteristics and univariate comparisons are summarised in table 2.

Follow-up of lung function

The available follow-up (time between baseline and latest follow-up visit) was similar for progressors and non-progressors (derivation cohort: 3.3±1.4 vs 3.0±1.1 years, p=0.330; validation cohort: 3.4±2.1 vs 4.7±3.4 years, p=0.168). However, progressors had significantly lower FVC values at latest follow-up

Table 1 Patients' demographic and clinical characteristics at baseline in the derivation cohort

Characteristics	Derivation cohort (n=98)	Progressors (n=25)	Non-progressors (n=73)	P values
Demographic				
Age (year)	57.3±12.9	61.6±12.7	55.9±12.8	0.058
Male sex	20 (20.4)	7 (28.0)	13 (17.8)	0.275
Disease duration (year)*	6.1±5.6	6.1±6.6	6.1±5.3	0.702
Smoker ever	30 (30.6)	10 (40.0)	20 (27.4)	0.238
Extrapulmonary				
Anti-Scl-70 positive	39 (39.8)	10 (40.0)	29 (39.7)	0.981
ACA positive	24 (24.5)	4 (16.0)	20 (27.4)	0.253
Anti-CCP positive	3/86 (3.5)	1/23 (4.3)	2/63 (3.2)	1.000
Diffuse cutaneous subset	30 (30.6)	10 (40.0)	20 (27.4)	0.238
mRSS (unit)	7.6±8.3	10.1±8.9	6.8±8.0	0.046
Digital ulcers ever	46 (46.9)	15 (60.0)	31 (42.5)	0.129
Arthritis ever†	27 (27.6)	15 (60.0)	12 (16.4)	<0.001
Joint contractures	40 (40.8)	15 (60.0)	25 (34.2)	0.024
ESR>20 mm/1 hour	33/97 (34.0)	11 (44.0)	22/72 (30.6)	0.222
CRP elevation	25 (25.5)	7 (28.0)	18 (24.7)	0.741
Lung-associated				
FVC (% predicted)	101.9±17.6	102.9±16.7	101.5±18.0	0.736
DLCO (% predicted)	76.5±17.1	73.2±15.0	77.6±17.7	0.268
Ground glass opacification	40 (40.8)	9 (36.0)	31 (42.5)	0.570
Honey combing	3 (3.1)	1 (4.0)	2 (2.7)	1.000
Dyspnoea NYHA≥2	42 (42.9)	15 (60.0)	27 (37.0)	0.045
6 min walk distance (m)	524.0±99.3	483.6±105.6	537.8±93.8	0.037
SpO_2 before 6MWT (%)	96.8±1.6	96.0±2.2	97.1±1.2	0.022
SpO_2 after 6MWT (%)	94.7±3.4 (n=97)	92.4±3.8	95.5±2.8 (n=72)	<0.001
Oxygen desaturation (%)	2.1±2.9 (n=97)	3.6±3.2	1.6±2.6 (n=72)	0.002
Active disease (VAI>3)‡[33]	15 (15.3)	5 (20.0)	10 (13.7)	0.522
Immunosuppressive therapy§	41 (41.8)	16 (64.0)	25 (34.2)	0.009

Data are presented as mean±SD for continuous variables and number (frequency) (%) for categorical variables.
*Disease duration was calculated as difference between the date of the baseline visit and the date of the first non-Raynaud's symptom of the disease as reported by the patients.
†Arthritis ever was defined as one or more tender and swollen joints as judged by the treating physician ever existed currently or before the baseline visit.
‡Active disease was defined as scored >3 by calculating European Scleroderma Study Group (EScSG) disease activity indices for SSc proposed by Valentini *et al*.[33]
§Immunosuppressive therapy was defined as treatment with prednisone in doses >10 mg/day or any immunosuppressant (cyclophosphamide, methotrexate, azathioprine, mycophenolate, rituximab and tocilizumab) at baseline.
6MWT, 6 min walk test; ACA, anticentromere antibody; anti-CCP, anticyclic citrullinated peptide; anti-Scl-70, antitopoisomerase-I antibodies; CRP, C reactive protein; DLCO, diffusing capacity for carbon monoxide; ESR, erythrocyte sedimentation rate; FVC, forced vital capacity; mRSS, modified Rodnan Skin Score; NYHA, New York Heart Association; SpO_2, peripheral capillary oxygen saturation; VAI, Valentini Activity Index.

compared with non-progressors (derivation cohort: 82.1±14.7 vs 102.2±20.0, p<0.001; validation cohort: 81.4±20.2 vs 98.2±18.5, p=0.001) and more FVC decline since baseline (online supplementary table S1).

Univariate and multivariate analysis

Results of univariate comparison of baseline parameters between progressors and non-progressors are summarised in tables 1 and 2 for the derivation and validation cohort, respectively. In the derivation cohort, progressors had higher mRSS, incidence of arthritis ever, joint contractures and dyspnoea New York Heart Association grade ≥2. Worse results for 6MWT including 6MWD, SpO_2 before 6MWT, SpO_2 after 6MWT and oxygen desaturation were also observed in progressors. By using Bonferroni correction, the modified critical p value (α) was determined as 0.002. Significantly higher incidence of arthritis ever and lower SpO_2 after 6MWT were found in progressors compared with non-progressors. Arthritis was first reported 2.7 years in average before baseline among the 27 patients who ever had arthritis; oxygen desaturation after 6MWT was first observed 0.6 year in average before baseline among the 28 patients who had oxygen desaturation. There was no significant difference in FVC or DLCO. Similar results were found in the validation cohort.

The logistic regression model including all six candidate predictors identified lower SpO_2 after 6MWT (OR=0.77, 95% CI 0.64 to 0.94, p=0.009) and arthritis ever (OR=6.84, 95% CI 2.19 to 21.33, p=0.001) as independent predictors for progression of mild SSc-ILD at 1 year (figure 1A, for details see online supplementary table S2).

The same multivariate logistic regression model was tested in the validation cohort. Lower SpO_2 after 6MWT (OR=0.65, 95% CI 0.47 to 0.90, p=0.009) and arthritis ever (OR=5.27, 95% CI 1.29 to 21.44, p=0.020) were confirmed as independent predictors for ILD progression. Similar results were obtained using multiply imputed datasets (figure 1B,C for details see online supplementary table S2).

We then combined the derivation cohort and the validation cohort into a pooled cohort. In order to adjust for possible confounding effects of age, gender and profile of antibodies on ILD progression, a new multivariate logistic regression model was conducted in the pooled cohort, which confirmed lower SpO_2 after 6MWT (OR=0.75, 95% CI 0.64 to 0.89, p=0.001) and arthritis ever (OR=7.91, 95% CI 3.11 to 20.13, p<0.001)

Table 2 Patients' demographic and clinical characteristics at baseline in the validation cohort

Characteristics	Validation cohort (n=117)	Progressors (n=25)	Non-progressors (n=92)	P values
Demographic				
Age (year)	53.7±14.6	55.4±13.6	53.3±14.9	0.569
Male sex	19 (16.2)	7 (28.0)	12 (13.0)	0.121
Disease duration (year)*	7.6±6.6 (n=107)	7.2±6.9 (n=23)	7.7±6.5 (n=84)	0.609
Extrapulmonary				
Anti-Scl-70 positive	43/115 (37.4)	9 (36.0)	34/90 (37.8)	0.871
ACA positive	22/115 (19.1)	4 (16.0)	18/90 (20.0)	0.779
Anti-CCP positive	6/92 (6.5)	2/19 (10.5)	4/73 (5.5)	0.600
Diffuse cutaneous subset	57 (48.7)	12 (48.0)	45 (48.9)	0.935
mRSS (unit)	9.1±9.4 (n=116)	9.6±9.9 (n=24)	9.0±9.4	0.761
Arthritis ever†	35 (29.9)	14 (56.0)	21 (22.8)	0.001
ESR>20 mm/1 hour	32/114 (28.1)	6 (24.0)	26/89 (29.2)	0.608
CRP elevation	32/116 (27.6)	7 (28.0)	25/91 (27.5)	0.958
Lung-associated				
FVC (% predicted)	97.6±16.5	100.0±16.4	97.0±16.5	0.287
DLCO (% predicted)	66.7±15.1	60.5±13.2	68.4±15.3	0.020
Ground glass opacification	39/102 (38.2)	5/22 (22.7)	34/80 (42.5)	0.091
Honey combing	13/98 (13.3)	2/21 (9.5)	11/77 (14.3)	0.728
Dyspnoea NYHA≥2	47 (40.2)	11 (44.0)	36 (39.1)	0.660
6 min walk distance (m)	505.3±95.1 (n=93)	472.9±106.3 (n=20)	514.2±90.6 (n=73)	0.164
SpO_2 before 6MWT (%)	97.9±1.3 (n=88)	97.2±1.5 (n=20)	98.1±1.1 (n=68)	0.009
SpO_2 after 6MWT (%)	96.3±2.3 (n=80)	94.3±2.4 (n=18)	96.9±1.9 (n=62)	<0.001
Oxygen desaturation (%)	1.5±1.7 (n=80)	2.7±1.7 (n=18)	1.1±1.6 (n=62)	<0.001
Immunosuppressive therapy‡	45 (38.5)	12 (48.0)	33 (35.9)	0.269

Data are presented as mean±SD for continuous variables and number (frequency) (%) for categorical variables.
*Disease duration was calculated as difference between the date of the baseline visit and the date of the first non-Raynaud's symptom of the disease as reported by the patients.
†Arthritis ever was defined as one or more tender and swollen joints as judged by the treating physician ever existed currently or before the baseline visit.
‡Immunosuppressive therapy was defined as treatment with prednisone in doses >10 mg/day or any immunosuppressant (cyclophosphamide, methotrexate, azathioprine, mycophenolate, rituximab and tocilizumab) at baseline.
6MWT, 6 min walk test; anti-Scl-70, antitopoisomerase-I antibodies; ACA, anticentromere antibody; anti-CCP, anticyclic citrullinated peptide; CRP, C reactive protein; DLCO, diffusing capacity for carbon monoxide; ESR, erythrocyte sedimentation rate; FVC, forced vital capacity; NYHA, New York Heart Association; mRSS, modified Rodnan Skin Score; SpO_2, peripheral capillary oxygen saturation.

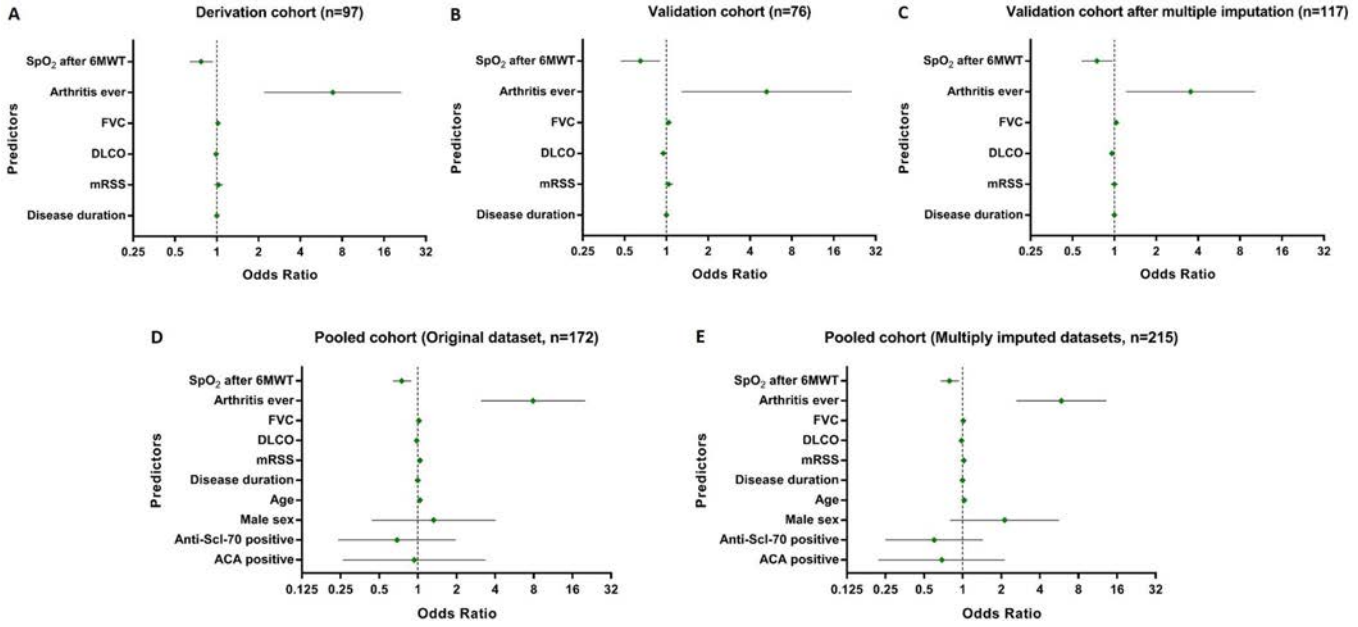

Figure 1 Multivariate logistic regression models in the derivation cohort, the validation cohort and the pooled cohort (both original and multiply imputed datasets in the validation cohort and the pooled cohort, adjusted for age, gender, anti-Scl-70 positive, ACA positive in the pooled cohort). 6MWT, 6 min walk test; anti-Scl-70, antitopoisomerase-I antibodies; ACA, anticentromere antibody; DLCO, diffusing capacity for carbon monoxide; FVC, forced vital capacity; mRSS, modified Rodnan Skin Score; SpO_2, peripheral capillary oxygen saturation.

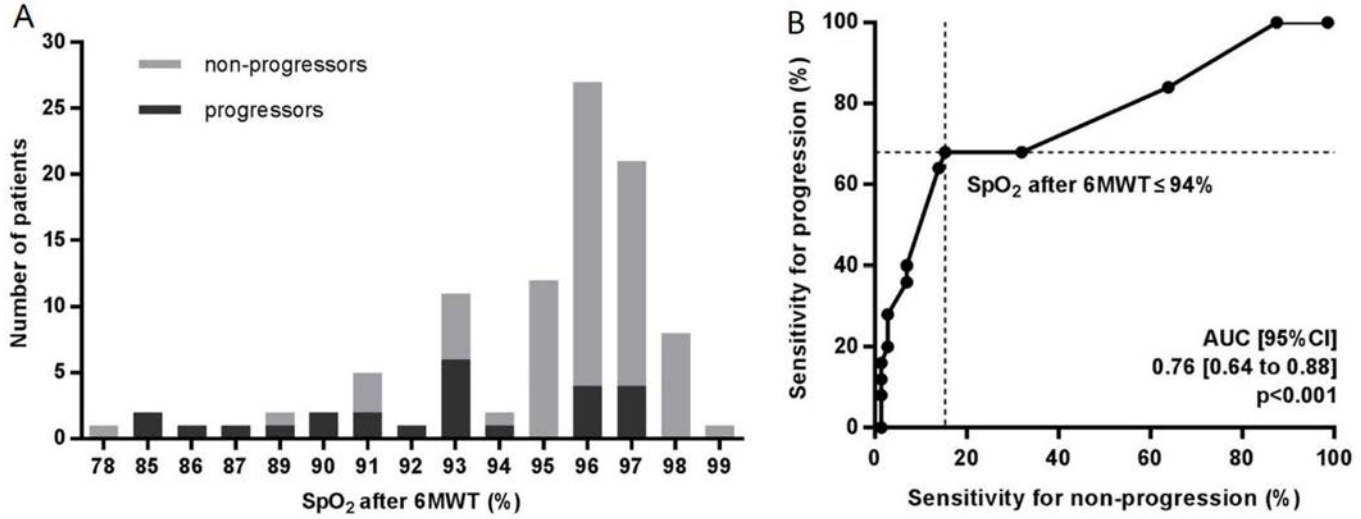

Figure 2 (A) Percentage of progressors and non-progressors per SpO_2 after 6MWT in the derivation cohort. (B) Sensitivity analysis for ILD progression depending on different cut-off values for SpO_2 after 6MWT in the derivation cohort. 6MWT, 6 min walk test; AUC, area under the curve; ILD, interstitial lung disease; SpO_2, peripheral capillary oxygen saturation.

as significant predictors. Similar results were also obtained using multiply imputed datasets (figure 1D,E for details see online supplementary table S3).

Identification of the optimal cut-off value for SpO_2 after 6MWT

A ROC curve analysis was then performed that identified 94% as the best cut-off value for SpO_2 after 6MWT to distinguish progressors from non-progressors. For predicting ILD progression, SpO_2 after 6MWT≤94% had a sensitivity of 68.0% and a specificity of 84.7%, together with an AUC of 0.76 (95% CI 0.64 to 0.88) (p<0.001) in the derivation cohort (figure 2). The subsequent ROC curve analysis also identified 94% as the best cut-off value for SpO_2 after 6MWT (AUC (95% CI)=0.81 (0.70 to 0.92)) in the validation cohort, with a sensitivity of 55.6% and a specificity of 93.5% (p<0.001).

Prediction models of progression of mild SSc-ILD

We then established prediction models by combining the two categorical predictors. Altogether, four models were tested and compared with discriminatory and predictive performance (table 3). Model 3 increased the prediction success rate from 25.5% in the whole unselected cohort to 91.7% in the optimised enrichment cohort. for example, among the 12 patients who fulfilled both the SpO_2 after 6MWT≤94% and arthritis ever criterion, 11 had ILD progression.

In a simplified scoring system, presence of both SPO_2 after 6MWT≤94% and ARthritis ever were set to 1, while both SPO_2 after 6MWT>94% and ARthritis never were set to 0, giving a SPAR score ranging from 0 to 2. We tested the discriminatory and predictive performances of the SPAR prediction model in both derivation and validation cohorts. The AUCs for the SPAR score were 0.83 (95% CI 0.73 to 0.93) (p<0.001) in the derivation cohort and 0.82 (95% CI 0.70 to 0.94) (p<0.001) in the validation cohort, respectively. The successful prediction rate for ILD progression increased with an increase in SPAR score in both cohorts. The prediction rate with a SPAR score of 0 was 7.4%/6.3%, with a score of 1 it was 32.3%/36.0%, and with a score of 2 it was 91.7%/85.7% in the derivation/validation cohorts, respectively (table 4).

Subgroup analyses of disease duration and immunosuppressive treatment

In order to investigate whether the predictive value of the SPAR model on ILD progression could be influenced by disease duration and immunosuppressive treatment at baseline, we tested the discriminatory and predictive performances of the SPAR model in the subgroups of patients with short (≤5 years) and long (>5 years) disease duration, treated and untreated patients in the derivation and validation cohort, respectively. The SPAR model could still significantly distinguish ILD progressors and non-progressors in any subgroup (for details see online supplementary table S4–S5).

DISCUSSION

Among patients with mild SSc-ILD, those prone to faster deterioration are more likely to benefit from active therapeutic interventions than those prone to stabilisation. Here, we present predictors of rapid lung worsening in patients with SSc with mild ILD and identify a simple prediction model (SPAR

Table 3 Prediction models of progression of mild SSc-ILD in the derivation cohort

Model	Included predictors	PPV (prediction success)	NPV (%)	Sensitivity (%)	Specificity (%)	AUC (95% CI)	P values
1	SpO_2 after 6MWT≤94%	17/28 (60.7%)	88.4	68.0	84.7	0.76 (0.65 to 0.88)	<0.001
2	Arthritis ever	15/27 (55.6%)	85.9	60.0	83.6	0.72 (0.59 to 0.84)	0.001
3	SpO_2 after 6MWT≤94% and arthritis ever	11/12 (91.7%)	83.5	44.0	98.6	0.71 (0.58 to 0.85)	0.002
4	SpO_2 after 6MWT≤94% or arthritis ever	21/43 (48.8%)	92.6	84.0	69.4	0.77 (0.66 to 0.87)	<0.001

6MWT, 6 min walk test; AUC, area under the curve; ILD, interstitial lung disease; NPV, negative predictive value; PPV, positive predictive value; SpO_2, peripheral capillary oxygen saturation; SSc, systemic sclerosis.

Table 4 Sensitivities, specificities, PPVs and NPVs of the SPAR score in the derivation and validation cohorts

SPAR score cut-offs	Derivation cohort (n=97)		Validation cohort (n=80)	
	0 and ≥1	≤1 and 2	0 and ≥1	≤1 and 2
Sensitivity (%)	**84.0**	44.0	**83.3**	33.3
Specificity (%)	69.4	**98.6**	72.6	**98.4**
PPV (%)	48.8	**91.7**	46.9	**85.7**
NPV (%)	**92.6**	83.5	**93.8**	83.6
AUC (95% CI)	0.83 (0.73 to 0.93)		0.82 (0.70 to 0.94)	

The discriminatory and predictive performances of the SPAR score on ILD progression were tested in the derivation and validation cohorts, respectively. e.g. In the derivation cohort, 84.0% of ILD progressors had the SPAR score=1 or 2 (sensitivity), 98.6% of non-progressors had the SPAR score=0 or 1 (specificity), 91.7% of patients with SPAR score=2 actually had ILD progression (PPV), 92.6% of patients with SPAR score=0 actually didn't have ILD progression (NPV).
AUC, area under the curve; NPV, negative predictive value; PPV, positive predictive value .

model) combining SpO_2 and arthritis, both readily accessible clinical characteristics, to predict ILD progression at the 1-year follow-up from real-life data in two independent SSc cohorts from multiple centres.

We chose to focus on mild ILD as the study population, since this subgroup frequently has a relatively normal PFTs value and will be recognised as progressors only when lung volumes drop dramatically 1 year later. Therefore, such progressors could have a better chance to be monitored more carefully in daily practice if a prediction model is available.

Although a moderate definition of ILD deterioration (either FVC decline ≥10% or DLCO decline ≥15%) was commonly used in previous observational studies and clinical trials,[9 15 29–31] we set a stricter definition. That is because we assumed patients with mild ILD would have better lung function than overall patients with SSc-ILD. Thus, a greater decline should be more clinically meaningful when related to worse outcome and survival. The baseline PFT values reported in our study were better than previous studies and clinical trials, which confirmed that our cohorts indeed had mild functional SSc-ILD as assessed by HRCT.[5 6 19 20] Although there is possibility that some patients with smaller changes in PFTs than in our definition ultimately deteriorate to clinically severe cases, our data showed that progressors had significantly lower FVC values and more FVC decline than non-progressors during a similar follow-up period, indicating that we successfully captured the real long-term 'ILD progressor' population in our study.

It should be noted that exercise SpO_2 and presence of arthritis were identified as significant predictors rather than some known risk factors for progression such as PFTs. A possible explanation could be that these known risk factors have been derived from cohorts with established, more extensive ILD. While they might be important predictors in these advanced cases, they might be less important in earlier and mild cases. Indeed, FVC was within the normal range in our cohort, and thus might not be sensitive enough for the prediction of progression in mild SSc-ILD.

Exercise-induced peripheral oxygen saturation was significantly lower in progressors and proved to be predictive for ILD progression, consistent with previous results for overall mortality.[22] This finding indicates that the insufficient pulmonary physiological reserve might be ahead of lung function volumes' decline in mild SSc-ILD, which suggests that measurement of oxygen saturation during 6MWT might be a more sensitive parameter than PFTs for predicting the progression of SSc-ILD.

The current study also identified arthritis ever as an independent predictor of mild SSc-ILD progression. This association was not found in a previous study,[7] which might be explained by two major differences in our study design. Only patients with ILD extent <20% lung involvement on HRCT were included in our cohort, which was different from the cohort selection of overall SSc-ILD or patients with SSc in previous studies. In addition, we have chosen 'ever happened' rather than 'current' arthritis. The presence of anti-CCP was low in our cohorts (3.5%~6.5%), and showed no significant difference between progressors and non-progressors, indicating that overlap syndrome with rheumatoid arthritis could hardly influence our findings.

There are several strengths of our study. First, we validated our results in a multinational cohort including patients from three centres. Second, we collected serial lung function tests and extensive clinical data in both derivation and validation cohorts. Third, the results were derived from the real-life data, which could probably guide the daily practice. Finally, we excluded the patients with pulmonary hypertension and substantial airflow obstruction, which could also impair the lung function, to make our cohort more consistent.

There are also limitations of our study. First, our data were derived from several local databases. Despite extensive quality control, there were still missing data as common for real-life registries. Those parameters with >50% missing values (eg, history of smoking) could not be considered for multivariate analysis even by imputation methods. Hence, we might have missed some potential predictors. Second, peripheral oxygen saturation was obtained by finger oximetry in some centres. In order to avoid biases by Raynaud phenomenon and peripheral vasculopathy, forehead oximetry would have been more reliable.[32] However, measuring exercise SpO_2 by finger oximetry in SSc-ILD reflected arterial oxygen saturation (SaO_2) and provided a meaningful prognostic value in previous studies.[22] Third, one should not misinterpret the relatively high percentage of lung progression in our study, because we had a specific cumulative rather than cross-sectional selection of progressors. Finally, although patients from three international centres had been included as a validation cohort in our study, external validation with qualified data in other international cohorts should be performed to confirm our findings.

In conclusion, our study defined lower SpO_2 after 6MWT and arthritis ever as independent baseline predictors for progression of mild SSc-ILD at 1-year follow-up. The derived evidence-based SPAR model might help physicians to identify patients at risk for progressive lung fibrosis, which might have implications for therapeutic strategies in clinical practice.

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Contributors Study conception and design: OD, WW, SJ, A-MH-V, YA, ES, SY, BM, RD, MOB. Acquisition of data: WW, OD, SJ, A-MH-V, HF, YA, ES. Analysis and interpretation of data: WW, OD, SJ. Drafting the article: WW, OD, SJ. Revising the article: A-MH-V, YA, SY, ES, HF, MOB, BM, RD. All authors have finally approved the submitted version to be published.

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Competing interests OD has consultancy relationship and/or has received research funding from Actelion, Bayer, Boehringer Ingelheim, ChemomAb, espeRare foundation, Genentech/Roche, GSK, Inventiva, Italfarmaco, Lilly, Medac, MedImmune, Mitsubishi Tanabe Pharma, Novartis, Pfizer, Sanofi, Sinoxa and UCB in the area of potential treatments of scleroderma and its complications, and a patent mir-29 for the treatment of systemic sclerosis licensed. YA has received grants from BMS, Genentech-Roche, Inventiva, Sanofi and consulting fees from Actelion, Bayer, Biogen, Boehringer, Genentech-Roche, Galapagos, Inventiva, Medac, Pfizer, Sanofi and Servier, about the treatment of systemic sclerosis. BM has received research funding in the area of systemic sclerosis and related conditions from AbbVie, Protagen, EMDO, Novartis, German SSc Society, Pfizer, Roche, Actelion, MSD, OPO Foundation and a patent mir-29 for the treatment of systemic sclerosis licensed. WW, SJ, MOB, RD, HF, SY, ES and A-MH-V have nothing to disclose.

Patient consent Obtained.

Ethics approval Each participating centre has obtained approval from the local ethics committee for including a patient's data in the SSc cohort after the patient has given written informed consent.

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EXTENDED REPORT

Improved survival with renal transplantation for end-stage renal disease due to granulomatosis with polyangiitis: data from the United States Renal Data System

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ABSTRACT

Background Renal transplantation is the optimal treatment for selected patients with end-stage renal disease (ESRD). However, the survival benefit of renal transplantation among patients with ESRD attributed to granulomatosis with polyangiitis (GPA) is unknown.

Methods We identified patients from the United States Renal Data System with ESRD due to GPA (ESRD-GPA) between 1995 and 2014. We restricted our analysis to waitlisted subjects to evaluate the impact of transplantation on mortality. We followed patients until death or the end of follow-up. We compared the relative risk (RR) of all-cause and cause-specific mortality in patients who received a transplant versus non-transplanted patients using a pooled logistic regression model with transplantation as a time-varying exposure.

Results During the study period, 1525 patients were waitlisted and 946 received a renal transplant. Receiving a renal transplant was associated with a 70% reduction in the risk of all-cause mortality in multivariable-adjusted analyses (RR=0.30, 95% CI 0.25 to 0.37), largely attributed to a 90% reduction in the risk of death due to cardiovascular disease (CVD) (RR=0.10, 95% 0.06–0.16).

Discussion Renal transplantation is associated with a significant decrease in all-cause mortality among patients with ESRD attributed to GPA, largely due to a decrease in the risk of death to CVD. Prompt referral for transplantation is critical to optimise outcomes for this patient population.

INTRODUCTION

Granulomatosis with polyangiitis (GPA) is a small vessel vasculitis associated with renal involvement (ie, glomerulonephritis) in up to 70% of patients. Among patients with renal disease, 20%–25% develop end-stage renal disease (ESRD).[1] Renal transplantation is the standard of care for selected patients with ESRD attributed to GPA (ESRD-GPA) given prior studies describing safe and successful transplantation in this population.[2–4] GPA and ESRD are associated with premature death, often due to cardiovascular disease (CVD), but the impact of renal transplantation on mortality in this population is unknown.[5–7]

A prior study demonstrated that renal transplantation is associated with a 68% reduction in the risk of death among patients with ESRD waitlisted for a renal transplant.[8] The majority of patients included in that study had ESRD attributable to causes other than glomerulonephritis, such as hypertension and diabetes. However, overall health, comorbidities and life expectancy may differ significantly between patients with ESRD due to GPA and patients with ESRD due to diabetes, hypertension or other causes.[9 10]

We used a national registry of patients with ESRD-GPA to determine the impact of renal transplantation on survival.

PATIENTS AND METHODS

Data source and study population

The United States Renal Data System (USRDS) is a national registry of patients with ESRD, representing an estimated 94% of patients who receive dialysis or kidney transplantation. Patients who refuse replacement therapy, die prior to enrolment or receive transient dialysis for acute renal failure may not be enrolled. Attending nephrologists are required by law to submit a Medical Evidence Report, which includes the cause of ESRD according to the International Classification of Diseases, ninth revision (ICD-9) codes within 45 days of a patient starting a new ESRD treatment.

To assess the impact of transplantation on survival (primary analysis), we included all patients who fulfilled the following criteria: (1) ESRD attributed to GPA (ICD-9: 446.4); (2) initiated haemodialysis or peritoneal dialysis between 1 January 1995 and 31 December 2014; and (3) waitlisted for a renal transplant between 1 January 1995 and 31 December 2015. We excluded patients who were pre-emptively transplanted without being waitlisted or without receiving haemodialysis or peritoneal dialysis. We restricted our analysis to those patients waitlisted for a renal transplant to minimise confounding by indication given that, generally, younger and healthier patients with higher socioeconomic status and social support are more likely to be waitlisted for a renal transplant.[8]

Covariates

The following information was extracted from the USRDS and used as covariates, exposures or outcomes: demographics (eg, age, sex, self-reported race); body mass index; relevant comorbidities (eg, diabetes, hypertension, coronary artery disease);

initial ESRD therapy modality; waitlisting date; transplant status; date of renal transplant; vital status; date of death; and primary cause of death. Relevant comorbidities reported in the Medical Evidence Report at the onset of ESRD were used to calculate a weighted comorbidity score developed specifically for USRDS data.[11]

Statistical analysis

The date on which the patient was first waitlisted for a renal transplant was used as the start of follow-up. We determined mortality rates (/1000 patient-years) by allocating time spent prior to a renal transplant to the group of patients who did not receive a renal transplant; 95% CIs for mortality rates were estimated using least-squares means. To avoid immortal time bias, we performed a pooled logistic regression in which first renal transplantation was treated as a time-varying exposure; this approach approximates that of a time-dependent Cox regression.[12] Age was used as the time scale. We compared the relative risk (RR) of all-cause mortality and cause-specific mortality (ie, CVD, infection and other causes) among patients who received a renal transplant during the study period to those who did not, after adjusting for relevant covariates. Given the limited number of outcomes of interest in some subgroup analyses, we adjusted for comorbidities using a weighted comorbidity score.[11] We performed subgroup analyses, evaluating differences in all-cause mortality with and without transplantation according to sex, age group at ESRD onset and year of ESRD onset. For year of ESRD onset subgroup analyses, the cohort was divided into two subcohorts based on the year of ESRD (1995–2004 and 2005–2014). Patients with ESRD onset during a respective time period who were not waitlisted during that time period were excluded from this subgroup analysis. Cumulative incidence functions were used to compare overall and cause-specific mortality between patients who did and did not receive a transplant during the study period.

All p values were two-sided with a significance threshold of <0.05. Statistical analyses were performed using SAS V.9.4.

Sensitivity analysis

We performed sensitivity analyses to verify the results of our primary analysis. To further address potential confounding by indication (or contraindication) that may occur when patients become too sick or otherwise unsuitable for a renal transplant, we censored patients at the time they were inactivated or removed from the transplant waitlist.[13] We expected this to attenuate our results since it introduces informative censoring (ie, censoring for a factor in the causal pathway between being waitlisted and dying). In a separate analysis, we censored patients who received living donor transplant at the time of transplantation since these patients may differ from other patients on the waitlist with regard to potential unmeasured confounding factors.

Data use

The data reported here have been supplied by the USRDS. The interpretation and reporting of these data are the responsibility of the authors and in no way should be seen as official policy or interpretation of the US government.

RESULTS

Baseline characteristics

Between 1995 and 2014, 5929 patients were diagnosed with ESRD attributed to GPA; of these, 1525 patients were waitlisted for a renal transplant during the study period (table 1). The average age at the time of being waitlisted for a renal transplant was 49.5 (±16.4) years. The majority of patients were male (59%) and white (86%). Hypertension was the most common comorbidity (67%). Haemodialysis was the most common (86%) first modality of renal replacement therapy. Of the 1525 patients waitlisted for a renal transplant, 946 received a renal transplant during the study period. The average age at the time of transplant was 48.4 (±17.0) years. Deceased donor transplantations were performed in 56% of cases, and living donor transplantations were performed in 44% of cases.

Table 1 Baseline features of patients with end-stage renal disease due to granulomatosis with polyangiitis (ESRD-GPA)

	GPA-ESRD	Waitlisted	Transplanted during study period
N	5929	1525	946
Age at ESRD, years (%)			
<40	12.6%	27.9%	33.9%
40–49	9.9%	18.2%	19.2%
50–59	16.9%	26.6%	24.4%
≥60	60.6%	27.3%	22.4%
Male (N, %)	3367 (57%)	906 (59%)	560 (59%)
Body mass index	26.8 (±6.7)	27.0 (±6.5)	26.7 (±6.4)
Race			
White	5305 (90%)	1312 (86%)	825 (87%)
Black	385 (7%)	132 (9%)	72 (8%)
Other	239 (4%)	81 (5%)	51 (5%)
Hispanic	536 (9%)	191 (13%)	106 (11%)
Comorbidities			
Diabetes	852 (14%)	134 (9%)	68 (7%)
Hypertension	4014 (68%)	1010 (67%)	602 (64%)
COPD	507 (9%)	48 (3%)	20 (2%)
CAD	686 (12%)	67 (5%)	42 (5%)
PVD	303 (5%)	35 (2%)	21 (2%)
CHF	859 (15%)	94 (6%)	43 (5%)
CVA	265 (5%)	36 (2%)	24 (3%)
Other cardiac disease	151 (3%)	20 (2%)	16 (2%)
Tobacco	227 (4%)	48 (3%)	20 (2%)
Cancer	306 (5%)	38 (3%)	20 (2%)
Comorbidity score	1.1 (±1.8)	0.5 (±1.2)	0.4 (±1.1)
First modality			
Transplant*	128 (2%)	–	–
Haemodialysis	5328 (90%)	1305 (86%)	798 (84%)
Peritoneal dialysis	450 (8%)	220 (14%)	148 (16%)

*Patients transplanted prior to dialysis were excluded from the transplantation analyses.
CAD, coronary artery disease; CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; CVA, cerebrovascular attack; MPA, microscopic polyangiitis; PVD, peripheral vascular disease.

All-cause mortality

During follow-up, 438 patients died. Among those who received a renal transplant (n=946), there were 199 deaths (table 2). Among those who did not receive a renal transplant (n=579), there were 239 deaths. The mortality rate (95% CI) among those who received a renal transplant was 29.3 (25.5–33.6)/1000 patient-years in contrast to 65.5 (57.7–74.3)/1000 patient-years among those who did not receive a renal transplant ($p<0.001$). In multivariable-adjusted analyses, renal transplantation was associated with a 70% reduction in the risk of death (figure 1; RR=0.30, 95% CI 0.25 to 0.36).

Table 2 All-cause mortality according to transplant status among waitlisted patients with end-stage renal disease (ESRD) due to granulomatosis with polyangiitis

	N	Total follow-up	Deaths	Mortality rate (/1000 patient-years, 95% CI)	Unadjusted RR (95% CI)	Age-adjusted, sex-adjusted, enrolment-adjusted RR (95% CI)*	Fully adjusted RR (95% CI)†
Overall	1525	10456	438	41.9 (38.1 to 46.0)			
Transplanted	946	6804	199	29.3 (25.5 to 33.6)	0.39 (0.33 to 0.46)	0.30 (0.25 to 0.36)	0.30 (0.25 to 0.36)
Not transplanted	579	3651	239	65.5 (57.7 to 74.3)	Ref	Ref	Ref
Age							
<40 years	448	3481	71	20.4 (16.2 to 25.7)			
Transplanted	344	2816	39	13.9 (10.1 to 19.0)	0.32 (0.20 to 0.50)	0.22 (0.14 to 0.35)	0.19 (0.12 to 0.31)
Not transplanted	104	666	32	48.1 (34.0 to 68.0)	Ref	Ref	Ref
40–49 years	284	2119	75	35.4 (28.2 to 44.4)			
Transplanted	188	1453	35	24.1 (17.3 to 33.6)	0.33 (0.22 to 0.52)	0.22 (0.14 to 0.37)	0.25 (0.15 to 0.41)
Not transplanted	96	667	40	60.0 (44.0 to 81.8)	Ref	Ref	Ref
50–59 years	407	2723	130	47.7 (40.2 to 56.7)			
Transplanted	232	1679	57	34.0 (26.2 to 44.0)	0.43 (0.31 to 0.60)	0.23 (0.16 to 0.34)	0.21 (0.14 to 0.32)
Not transplanted	175	1045	73	69.9 (55.5 to 87.9)	Ref	Ref	Ref
≥60 years	417	2302	168	73.0 (62.7 to 84.9)			
Transplanted	213	1256	74	58.9 (46.9 to 74.0)	0.62 (0.47 to 0.82)	0.35 (0.26 to 0.48)	0.38 (0.27 to 0.52)
Not transplanted	204	1046	94	89.9 (73.4 to 110.0)	Ref	Ref	Ref
Sex							
Male	923	6268	274	43.7 (38.8 to 49.2)			
Transplanted	577	4292	127	29.6 (24.9 to 35.2)	0.38 (0.31 to 0.48)	0.28 (0.23 to 0.36)	0.30 (0.23 to 0.37)
Not transplanted	346	1976	147	74.4 (63.3 to 87.5)	Ref	Ref	Ref
Female	633	4358	170	39.0 (33.6 to 45.3)			
Transplanted	400	2911	78	26.8 (21.5 to 33.5)	0.39 (0.29 to 0.51)	0.33 (0.25 to 0.45)	0.33 (0.24 to 0.45)
Not transplanted	233	1447	92	63.6 (51.8 to 78.0)	Ref	Ref	Ref
*Calendar year of ESRD onset**							
1995–2004	560	2217	96	43.3 (35.5 to 52.9)			
Transplanted	347	1290	25	19.4 (13.1 to 28.7)	0.16 (0.10 to 0.27)	0.16 (0.10 to 0.26)	0.17 (0.10 to 0.29)
Not transplanted	213	897	71	79.1 (62.7 to 99.9)	Ref	Ref	Ref
2005–2014	890	4230	121	28.6 (23.9 to 34.2)			
Transplanted	478	2097	27	12.9 (8.8 to 18.8)	0.24 (0.16 to 0.37)	0.25 (0.16 to 0.38)	0.25 (0.16 to 0.38)
Not transplanted	412	2121	94	44.3 (36.2 to 54.3)	Ref	Ref	

*Excluded patients who were not waitlisted prior to the end of time period censoring.
†Fully adjusted models adjust for sex, age, ESRD year, comorbidity score, white/non-white, first modality.
RR. relative risk.

Our results were similar across the subgroups, including sex, age at ESRD onset and year of ESRD onset (table 2). As expected, the greatest benefit was identified in those patients under the age of 40 years (RR=0.19, 95% CI 0.12 to 0.31). There was a slight attenuation in the mortality benefit associated with transplantation in the most recent decade (2005–2014) compared with the preceding decade (1995–2004).

Cause-specific mortality

In multivariable-adjusted analyses, patients who received a transplant had a 90% lower risk of death due to CVD than non-transplanted patients (RR=0.10, 95% 0.06–0.16; table 3, figure 2). There were also significant reductions in the risk of death due to infection (RR=0.55, 95% CI 0.31 to 0.97) and other causes (RR=0.48, 95% CI 0.29 to 0.79). The most frequent other causes identified included withdrawal from dialysis (n=19, 14% of other causes) and malignancy (n=24, 9% of other causes). Because of the few number of malignancies, fully adjusted analyses were not possible but in age-adjusted and sex-adjusted analyses, there was no difference in the risk of malignancy (RR=1.44, 95% CI 0.59 to 3.51).

Sensitivity analyses

To verify the findings in our primary analysis, we performed two sensitivity analyses. First, we censored patients who were inactivated or removed from the waitlist (n=192). Common reasons for being inactivated or removed included being too sick or medically unsuitable for a renal transplant. The survival benefit associated with transplantation was attenuated in this analysis (RR=0.54, 95% CI 0.44 to 0.66) which was expected because we introduced informative censoring (ie, those who were more likely to die were preferentially eliminated from the non-transplanted group). Second, we censored living donor transplant recipients and found a similar survival benefit as in our primary analysis (RR=0.45, 95% CI 0.37 to 0.55).

DISCUSSION

In this nationwide study of patients with ESRD due to GPA, renal transplantation was associated with a dramatic reduction in the risk of death, especially due to CVD. Previous studies had reported good graft and patient survival in smaller cohorts of renal transplant recipients who had ESRD due to antineutrophil cytoplasmic antibody-associated vasculitis (AAV).[3 4 10 14]

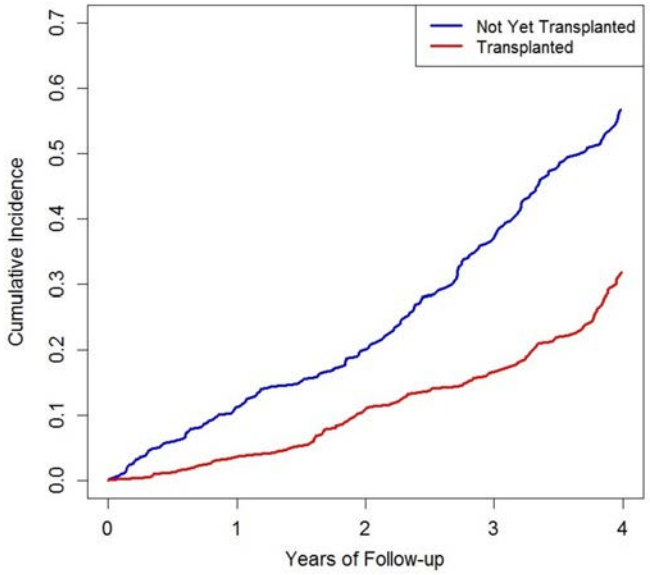

Figure 1 Cumulative incidence of all-cause death according to transplant status among waitlisted patients with end-stage renal disease due to granulomatosis with polyangiitis.

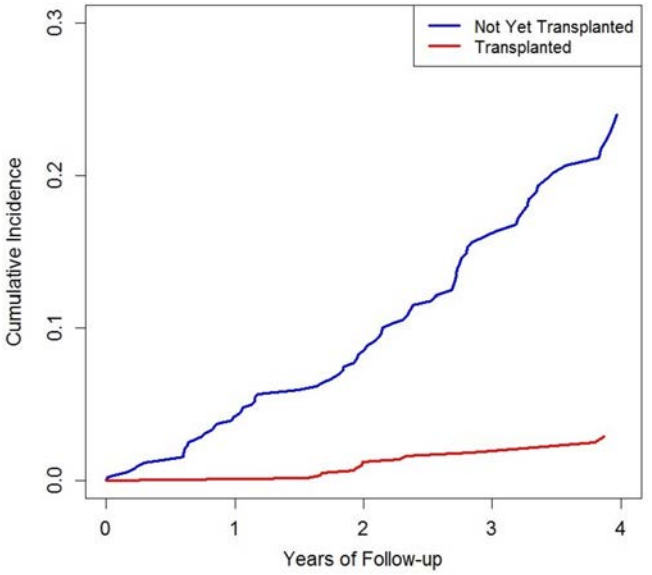

Figure 2 Cumulative incidence of death due to cardiovascular disease according to transplant status among waitlisted patients with end-stage renal disease due to granulomatosis with polyangiitis.

However, no study has evaluated the relative survival benefit of renal transplantation in this population.

Wolfe *et al* first reported the significant survival benefit associated with renal transplantation in patients with ESRD from a variety of causes, the minority of whom had glomerulonephritis.[8] Indeed, patients with ESRD resulting from GPA are often different from patients with ESRD due to diabetes or hypertension, especially with regard to comorbidities and other organ involvement by GPA that may impact their potential to be waitlisted and overall survival with or without a renal transplant.[9] Moreover, patients with GPA typically have a history of immunosuppression and may be immunosuppressed at the time of renal transplantation which could impact outcomes following renal transplantation. Despite these differences, we found that the survival benefit of renal transplantation among patients with GPA was similar to that previously described in the general population of patients with ESRD.[8 15] In those studies, RR of death due to CVD associated with transplantation was not reported. The impact of contemporary post-transplant immunosuppression may influence the risk of GPA flares which might prevent further organ damage and improve overall survival.

Moreover, our findings indicate that much of the improvement in mortality is due to a reduction in death due to CVD. CVD is the most common cause of death in ESRD, in general, and transplantation is known to decrease the risk of CVD death in this general population.[16] Due to differences in methods, we cannot directly compare our findings with regard to CVD mortality to prior studies.[16] However, to our knowledge, this is the first study in AAV to demonstrate that a specific intervention can significantly reduce the risk of death through an impact on cardiovascular death. Previous studies have found an increased risk of CVD among patients with AAV but it is unclear what portion of CVD in this population is mediated by chronic kidney disease, steroid exposure and/or the inflammatory state associated with AAV.[7] Post-transplant immunosuppression may also have a beneficial effect with regard to CVD risk. The impact of CVD risk reduction strategies in AAV has not been previously studied.

This study has important implications for the management of AAV (eg, GPA) patients with advanced renal involvement. First, providers, regardless of specialty (eg, rheumatology, nephrology, pulmonary), should consider referring patients

Table 3 Cause-specific mortality according to transplant status among waitlisted patients with end-stage renal disease due to granulomatosis with polyangiitis

	Deaths	Unadjusted RR (95% CI)	Age-adjusted, sex-adjusted, enrolment-adjusted RR (95% CI)	Fully adjusted RR (95% CI)
Cardiovascular death				
Transplanted	23	0.13 (0.08 to 0.20)	0.11 (0.07 to 0.18)	0.10 (0.06 to 0.16)
Not transplanted	97	Ref	Ref	Ref
Infection death				
Transplanted	24	0.49 (0.28 to 0.84)	0.52 (0.29 to 92)	0.55 (0.31 to 0.97)
Not transplanted	29	Ref	Ref	Ref
Other death				
Transplanted	152	0.46 (0.27 to 0.76)	0.46 (0.27 to 0.77)	0.48 (0.29 to 0.79)
Not transplanted	113	Ref	Ref	Ref

RR, relative risk.

to a renal transplant centre for evaluation and do so early in their disease course. Pre-emptive transplantation performed before any dialysis is required is associated with improved outcomes relative to even a 6-month period of dialysis.[16] Of note, patients can accrue time on the transplantation waiting list once the estimated glomerular filtration rate (eGFR) is <20 mL/min/1.73 m^2; in other words, a patient does not need to be on dialysis to be waitlisted for a renal transplant. However, many transplant centres recommend referrals of patients whose eGFR is >20 (eg, 25–30 mL/min/1.73 m^2), to allow sufficient time for recipient and live donor evaluations and to increase the chance that a pre-emptive transplant can be arranged. Moreover, there are few absolute contraindications to organ transplantation and referring potential candidates to a transplant centre is necessary to determine a patient's candidacy for renal transplantation, regardless of age or comorbidities (eg, malignancy, CVD).

Second, CVD is a common cause of death among waitlisted patients, suggesting that CVD risk assessment and modification may further improve survival in this population. Notably, a prior randomised controlled trial comparing statin therapy versus placebo in patients with ESRD on haemodialysis found no benefit associated with statin therapy.[17] It is unclear if these results can be extrapolated to patients with ESRD attributable to immune-mediated conditions (eg, GPA) or those on the waitlist. Future studies might evaluate factors responsible for the dramatic reduction in the risk of death due to CVD following renal transplantation. Possible explanations include physiological differences between filtration through a functioning kidney as opposed to across a dialysis membrane[18] and/or differences in the management of patients prior to and after a renal transplant.

There are several strengths of this study related to our data source and study design. In particular, the USRDS is a nationwide registry that captures nearly all patients with ESRD in the USA. The diagnosis of GPA was made and reported by attending nephrologists as per legal requirements associated with documentation for medical benefits associated with having ESRD. Previous studies have used similar methods.[3 19] We also designed our study to limit the potential biases associated with confounding by indication as well as immortal time bias by restricting our analysis to waitlisted patients and treating renal transplantation as a time-varying exposure, respectively.[8] Generally, people waitlisted for a renal transplant share many common features (eg, generally good overall health, younger age, higher socioeconomic status and strong social support) which would otherwise be potentially impactful confounders in a study that included all patients with ESRD.

Our study has certain limitations. The USRDS enrols patients when they reach ESRD but does not include details regarding the history of GPA. As such, we cannot address how certain factors, such as time between GPA onset and transplantation, ANCA type and titre or immunosuppression exposure, may affect outcomes. The ICD-9 code for MPA is not specific for that condition, and we therefore only included GPA patients with ESRD in this study. While the ICD-9 code for GPA is more specific, validated diagnostic criteria could not be applied to confirm each diagnosis. Additionally, we do not have details regarding ANCA titres in these patients at the time of renal transplantation. The impact of positive ANCA testing at the time of transplantation and duration of remission prior to transplantation on transplant outcomes remains controversial.[20 21] Generally, it is recommended that vasculitis be in remission at the time of transplantation (with or without a negative ANCA test) and this remission has lasted for at least 12 months prior to transplantation.[20]

In summary, in this national cohort study of patients with ESRD due to GPA, we found that renal transplantation is associated with a significant survival benefit, largely due to a dramatic reduction in the risk of death due to CVD. Routine management of GPA patients with advanced chronic kidney disease should include an evaluation for renal transplant eligibility.

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Competing interests ZSW has received funding from a Scientist Development Award from the Rheumatology Research Foundation, a Fund for Medical Discovery Award from the Executive Committee on Research at Massachusetts General Hospital, and an NIH Loan Repayment Award.

Patient consent Not required.

Ethics approval The IRB has determined that this activity does not meet the definition of human subjects research. The investigators conducting this research will not obtain data through an intervention or interaction with individual subjects or identifiable private information about living individuals. This study was exempted from the Partner's HealthCare Institutional Review Board because it only used deidentified data.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement Data collected by the United States Renal Data System (USRDS) are extensive and maintained by the National Institutes of Health. Details regarding the data collected can be reviewed at this website: https://www.usrds.org/

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EXTENDED REPORT

Urinary epidermal growth factor predicts renal prognosis in antineutrophil cytoplasmic antibody-associated vasculitis

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ABSTRACT

Introduction The current study aimed to investigate the association between urinary epidermal growth factor (uEGF) and renal disease severity and outcomes in patients with antineutrophil cytoplasmic antibody-associated vasculitis (AAV).

Methods Intrarenal *EGF*mRNA expression was extracted from transcriptomic data of microdissected tubulointerstitial compartments of kidney biopsies of patients with AAV. uEGF was measured in 173 patients with AAV in active stage and 143 in remission, and normalised to urine creatinine excretion (uEGF/Cr). The association between uEGF/Cr (or *EGF*mRNA) and clinical–pathological parameters was tested using linear regression analysis. The ability of uEGF/Cr to predict renal outcomes was analysed using Cox's regression analysis.

Results In patients with AAV, intrarenal *EGF*mRNA expression was significantly associated with estimated glomerular filtration rate (eGFR)($\log_2$) at time of biopsy (β=0.63, p<0.001). The level of uEGF/Cr was significantly higher in patients in remission than in patients with active disease, both when looking at patients with sequential measurements (2.75±1.03vs 2.08±0.98, p<0.001) and in cross-sectional comparison. uEGF/Cr level was positively associated with eGFR($\log_2$) at time of sampling in both active and remission stage (β=0.60, p<0.001; β=0.74, p<0.001, respectively). Patients with resistant renal disease had significantly lower uEGF/Cr levels than responders (1.65±1.22vs 2.16±1.26, p=0.04). Moreover, after adjusting for other potential predictors, uEGF/Cr was independently associated with composite endpoint of end-stage renal disease or 30% reduction of eGFR (HR 0.61, 95% CI 0.45 to 0.83, p=0.001).

Conclusion Lower uEGF/Cr levels are associated with more severe renal disease, renal resistance to treatment and higher risk of progression to composite outcome in patients with AAV.

INTRODUCTION

Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is a group of life-threatening autoimmune diseases, comprising microscopic polyangiitis, granulomatosis with polyangiitis and eosinophilic granulomatosis with polyangiitis.[1] The kidney is one of the most commonly involved organs in AAV. With the initiation of immunosuppressive therapy, the prognosis of AAV has been improved greatly. However, approximately 25% of patients with AAV are still resistant to the induction therapy and 20%–25% of patients with AAV progress to end-stage renal disease (ESRD).[2 3] It is therefore imperative to find non-invasive biomarkers predicting renal response to treatment and the deterioration of renal function in patients with AAV.

Epidermal growth factor (EGF) is a peptide growth factor. Several studies have shown that urinary EGF (uEGF) is decreased in a variety of renal diseases including diabetic nephropathy,[4] acute kidney injury,[5] IgA nephropathy (IgAN),[6] congenital ureteropelvic junction obstruction[7] and chronic kidney disease (CKD) in children.[8] More importantly, it has been shown that uEGF has the ability to predict the development of diabetic nephropathy[9] and IgAN.[6] In the present study, we investigated the association between intrarenal *EGF* mRNA and kidney function, and the association between uEGF and renal outcomes, including the renal response to treatment and renal disease progression in patients with AAV.

MATERIALS AND METHODS

Tubulointerstitial *EGF* mRNA expression in patients with AAV

Human renal biopsies were collected in a multicentre study, the European Renal cDNA Bank (ERCB), after informed consent was obtained, according to the guidelines of the respective local ethics committees. Transcriptome analysis was performed on microdissected tubulointerstitial compartments of kidney biopsies of patients with AAV procured for molecular analysis from ERCB. The healthy controls biopsies were from living donor transplant biopsies. The details of biopsies handling have been described elsewhere.[10 11] Briefly, immediately after renal biopsy, a minimum of 10% of the renal biopsy specimen was separated and stored in RNA preservative. Under a stereomicroscope, specimens were divided into glomeruli and tubulointerstitial compartments by manual microdissection. RNA was isolated following standard protocols. Tubulointerstitial *EGF* mRNA expression data were extracted from profiles of patients with AAV (table 1). Samples were hybridised on Affymetrix GeneChip U133 plus 2.[12] Normalised expression data were $\log_2$-transformed.

Patients and urine samples

One hundred and seventy-three patients with active AAV, diagnosed from January 2001 to December 2016, were enrolled in this study. Urine samples

Table 1 Basic demographic data and clinical information for ERCB patients whose intrarenal *EGF* mRNA level are available

Characteristics	(n=21)
Sex (M/F)	12/9
Target antigen of ANCA	13 PR3/7 MPO/1 both
Age (years) (mean±SD)	58.5±14.1
eGFR (mL/min/1.73 m^2) (mean±SD)	46.6±31.5
Urinary protein (g/24 hours) (mean±SD)	1.57±1.52
Treatment (steroids or immunosuppressant)	14 yes/3 no/4 unknown

ANCA, antineutrophil cytoplasmic antibody; eGFR, estimated glomerular filtration rate; MPO, myeloperoxidase; PR3, proteinase 3.

from these patients were collected on the day of renal biopsy and before commencement of immunosuppressive treatment. Urine samples from 143 patients with AAV, who achieved complete or partial remission after immunosuppressive therapy (see definitions below), were collected at their regular ambulatory visits. Among the aforementioned patients with AAV, there were 60 patients who had urine samples of both active and remission stage. Altogether, 256 patients with AAV were enrolled in this study. All these patients were diagnosed at Peking University First Hospital and met the Chapel Hill Consensus Conference criteria of AAV.[1] Patients with secondary vasculitis or other coexisting renal diseases, such as antiglomerular basement membrane nephritis, lupus nephritis and IgA nephropathy, were excluded. We also acquired 27 urine samples of age-matched and gender-matched healthy donors. The urine samples of all participants were centrifuged at 600*g* for 5 min and stored at −80°C until tested. The research was done in compliance with the Declaration of Helsinki and approved by the ethics committee of our hospital. Written informed consent was obtained from each participant.

Treatment

The protocols of induction and maintenance treatment were described previously.[13–15] More details are provided in the online supplementary text.

Clinical evaluation

Disease activity was assessed by the Birmingham Vasculitis Activity Score (BVAS).[16] Remission was defined as the "absence of disease activity attributable to active disease qualified by the need for ongoing stable maintenance immunosuppressive therapy (complete remission)" or "at least 50% reduction of disease activity score and absence of new manifestations (partial remission)". Treatment resistance was defined as unchanged or increased disease activity in patients with acute AAV after 4 weeks of treatment with standard therapy or a reduction of less than 50% in the disease activity score after 6 weeks of treatment, or chronic persistent disease defined as the presence of at least one major item or three minor items on the disease activity score list after >12 weeks of treatment.[17]

The renal response to treatment, evaluated after initiation of immunosuppressive therapy, was judged according to the following criteria, as described previously[18–20]: (1) complete recovery of renal function was indicated by normalisation of renal function and resolution of haematuria; (2) partial recovery of renal function was indicated by stabilisation or improvement of renal function, with serum creatinine ≥133 μmol/L but dialysis-independent; and (3) treatment failure was indicated by progressive decline in kidney function with persistence of active urinary sediment despite immunosuppressive therapy.

The estimated glomerular filtration rate (eGFR) was calculated using the adaptation of the four-factor Modification of Diet in Renal Disease study equation formula.[21]

Detection of uEGF

uEGF concentration was measured with Human EGF Immunoassay Quantikine ELISA according to the manufacturer's instructions (R&D Systems). Samples (1:100 dilution for disease samples, 1:150 dilution for healthy control samples) and standards were run in duplicate. Meanwhile, three quality controls with high, medium and low concentrations using Quantikine Immunoassay Control Group 4 (R&D Systems) were included in each plate to control for interplate variance. Absorbance was read at 450 nm with the ELISA plate reader, and results were calculated with ELISACalc software. The concentration of the quality controls measured was in the acceptable ranges and interplate coefficients of variation of the quality controls were less than 15%. Urine creatinine was measured by kinetic Jaffe reaction using Quantikine Creatinine Assay Kit (R&D Systems). Finally, the level of uEGF was normalised to urine creatinine excretion, referred to uEGF/Cr. Log_2-transformed uEGF/Cr value was used for all analyses.

Renal histopathology

The renal biopsy specimens were routinely examined by light microscopy, direct immunofluorescence and electron microscopy. Biopsies were independently scored by two pathologists blinded to the clinical data, according to the previously described standardised protocol for scoring renal biopsies of patients with AAV,[22–24] with some minor modifications. The presence of crescents, sclerotic glomeruli and fibrinoid necrosis were calculated as the percentage of the total number of glomeruli in the biopsy. The severity of interstitial fibrosis and tubular atrophy (IFTA) was evaluated semiquantitatively and scored on a scale from 0 to 3: grade 0, no IFTA; grade 1, <25%; grade 2, 25–50%; and grade 3, >50%.[25]

Outcome measurement

Two parameters were employed to evaluate renal progression of AAV[26]: (1) the eGFR slope and (2) a composite endpoint of ESRD or 30% reduction of eGFR from the beginning of maintenance therapy. To calculate the eGFR slope over time, we fitted linear mixed-effects model to the outpatient eGFR records at the beginning of maintenance therapy for patients followed up for more than 12 months and had at least three eGFR records in remission stage. The endpoint events of patients were collected before 31 December 2017.

Statistical analysis

The continuous data were expressed as mean±SD or median and IQR. Categorical data were described by absolute frequencies and percentages. Comparison of clinical characteristic parameters between 173 patients in active stage and 143 patients in remission was assessed using a linear mixed model, with group as fixed effect, patients as a random effect and age, gender and other clinical parameters as dependent variables, respectively. A linear mixed model was also used to compare repeated measurements in 60 paired data. The model included uEGF/Cr as a dependent variable, group as a fixed effect and patient as a random effect. eGFR was added as covariate for eGFR adjustment. To compare independent continuous variables, Student's t-test or Mann-Whitney U test was used as appropriate. Differences of qualitative results were compared using χ^2 test. Linear

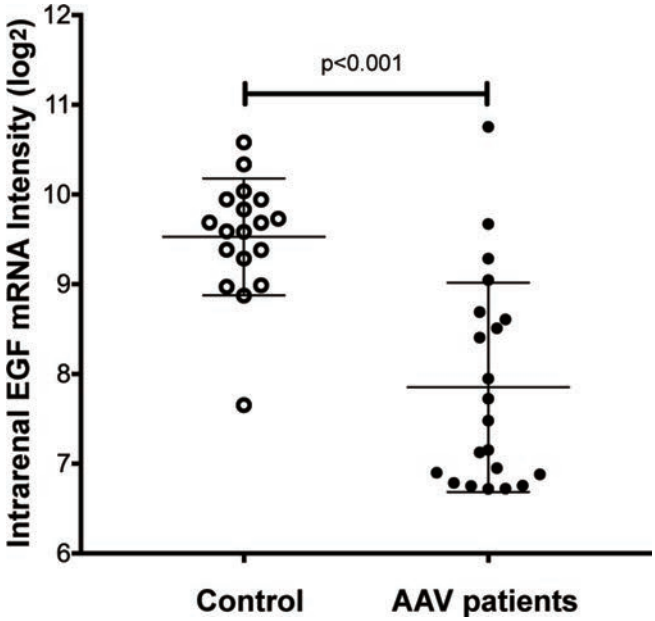

Figure 1 *EGF* mRNA is significantly lower in patients with AAV (n=21) in comparison with controls (n=18). Horizontal lines represent mean±SD. AAV, antineutrophil cytoplasmic antibody-associated vasculitis; EGF, epidermal growth factor.

regression analysis with unstandardised regression coefficients (β) as effect measure was performed to analyse the association between uEGF/Cr (or *EGF* mRNA) and other clinical parameters, in which uEGF/Cr (or *EGF* mRNA) acted as dependent variable, and eGFR (log_2 transformed), IFTA grade, BVAS, C reactive protein (CRP) as well as eGFR slope acted as independent variables, respectively.

The association between potential risk variables and the composite endpoint were analysed using Cox proportional hazards regression, first with one potential predictor at a time, second including all the potential predictors simultaneously. Patients with missing data were excluded from the analysis. Time-dependent receiver operating characteristic (ROC) curve analysis was conducted to evaluate the prognostic value of uEGF/Cr for the composite endpoint. In addition, we examined nested Cox proportional hazards models to evaluate the additive effect of uEGF/Cr on traditional predictive markers for renal progression. The goodness of fit and improved prediction ability of the additional parameters was assessed by R^2, likelihood ratio tests and Akaike information criterion. Analysis was performed with SPSS statistical software package (SPSS V.24.0; IBM) and SAS (V.9.3). Differences were considered significant if p value <0.05.

RESULTS

Intrarenal *EGF* mRNA in patients with AAV compared with living donors and association with eGFR

To investigate whether EGF is associated with kidney impairment, we extracted intrarenal *EGF* mRNA expression data in samples from patients with AAV from the ERCB (table 1) and from healthy living donors. Figure 1 illustrates reduced *EGF* steady-state mRNA levels in the tubulointerstitial compartment in samples of patients with AAV relative to healthy living donor samples (7.85±1.17 vs 9.53±0.65, p<0.001, by Student's t-test). In addition, linear regression analysis showed that tubulointerstitial *EGF* mRNA level was significantly associated with eGFR(log_2) at time of biopsy (β=0.63, p<0.001).

Table 2 General data of patients with AAV for measuring urinary EGF

Parameters	Active stage	Remission stage	P values
No of patients*	173	143	–
Age (mean±SD)	58.73±13.66	59.84±11.82	0.341
Gender (M/F)	66/107	61/82	0.417
Type of ANCA (MPO/PR3/negative)	162/11/0	53/4/86	–
Scr (μmol/L) (mean±SD)	338.50±262.82	171.93±117.31	<0.001
eGFR (mL/min/1.73 m^2) (mean±SD)	28.93±26.63	44.66±21.23	<0.001
Urinary protein (g/24 hours)† (mean±SD)	1.67±1.49	–	–
BVAS (mean±SD)	18.24±6.12	0.65±0.83	<0.001
ESR (mm/1 hour)‡ (mean±SD)	60.90±37.90	32.35±26.63	<0.001
Total crescents (%) (mean±SD)	55.07±28.38		
Cellular crescents	19.13±19.41	–	–
Cellular–fibrous crescents	26.18±20.75	–	–
Fibrous crescents	9.76±16.38	–	–
IFTA score			
Grade 0 (n, %)	13 (8%)	–	–
Grade 1 (n, %)	44 (25%)	–	–
Grade 2 (n, %)	102 (59%)	–	–
Grade 3 (n, %)	14 (8%)	–	–
Treatment			
Induction therapy§ (plus PE only/MP only/PE+MP/neither PE nor MP)	10/73/21/69	–	–
Maintenance therapy (AZA/MMF/LEF)	–	88/31/24	–

*Sixty patients with paired data.
†Five patients with missing data for urinary protein.
‡Two patients with missing data for ESR.
§All patients in active stage received corticosteroids in combination with cyclophosphamide.
AAV, antineutrophil cytoplasmic antibody-associated vasculitis; ANCA, antineutrophil cytoplasmic antibody; AZA, azathioprine; BVAS, Birmingham Vasculitis Activity Score; EGF, epidermal growth factor; eGFR, estimated glomerular filtration rate; ESR, erythrocyte sedimentation rate; IFTA, interstitial fibrosis and tubular atrophy; LEF, leflunomide; MMF, mycophenolate mofetil; MP, methylprednisolone pulse therapy; MPO, myeloperoxidase; PE, plasma exchange; PR3, proteinase 3; Scr, serum creatinine; –, not available.

The levels of uEGF/Cr between patients with active disease and patients in remission

The general data of patients with AAV are listed in table 2. For patients who had only one urine sample collection (113 patients in active stage and 83 patients in remission), the level of uEGF/Cr in active stage was significantly lower than that in remission (2.04±1.41 vs 2.63±1.31, p<0.001, by Student's t-test) and was also significantly lower than healthy controls (2.04±1.41 vs 4.34±0.76, p<0.001, by Student's t-test) (figure 2A).

Of the 60 patients with AAV with sequential urine samples of both active stage and remission, the level of uEGF/Cr was significantly higher in remission than those in the active stage (2.75±1.03 vs 2.08±0.98, p<0.001, by linear mixed model). After adjusting for eGFR value, the level of uEGF/Cr in patients in remission was still significantly higher than that in the active stage (mean (95% CI): 2.62 (2.40 to 2.84) vs 2.21 (2.00 to 2.43), p=0.008). Forty-two out of 60 patients had an increase (group A), while 18 patients had a decrease (group B), in the level of

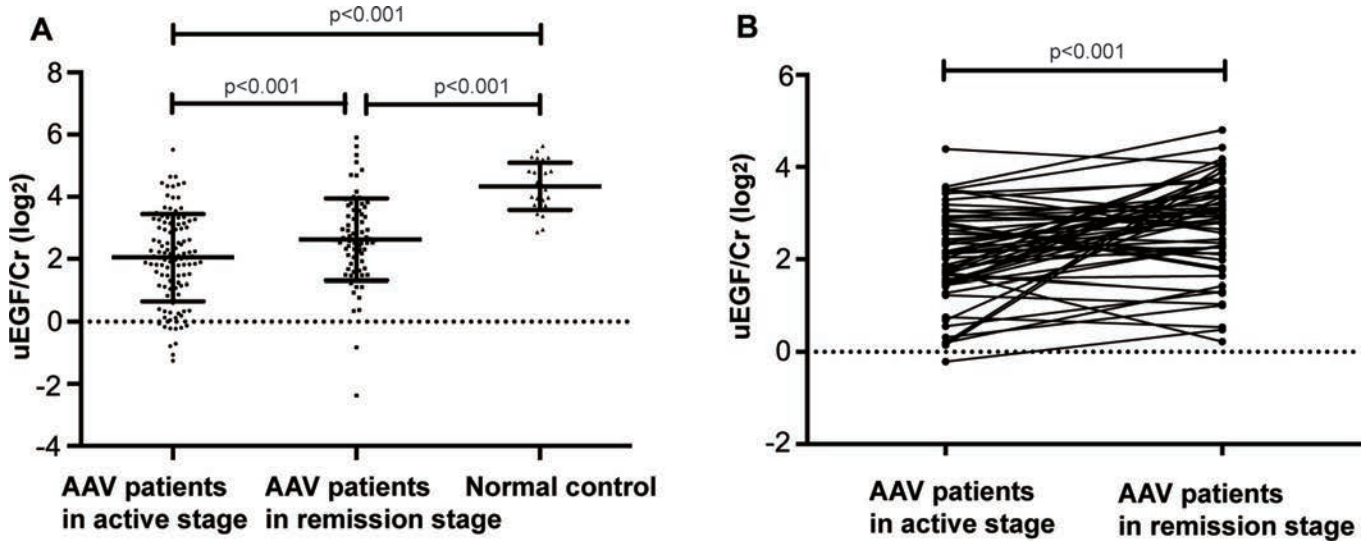

Figure 2 uEGF/Cr levels in patients with AAV. (A) The levels of urinary EGF/Cr(log_2) in active stage, in remission and normal controls. Horizontal lines represent mean±SD. (B) Change of uEGF/Cr levels in 60 patients with AAV with sequential urine samples of active stage and remission. AAV, antineutrophil cytoplasmic antibody-associated vasculitis; EGF, epidermal growth factor; uEGF, urinary EGF.

uEGF/Cr in remission as compared with those in active stage (figure 2B). The proportion of patients with IFTA grade ≥2 in group B was significantly higher than that in group A (absolute frequencies and percentages: 14/18 (77.8%) vs 20/42 (47.6%), p=0.031, by χ^2 test). As expected, the eGFR slope in group B was significantly lower than that of patients in group A (−3.59±5.83 vs 0.94±6.47 mL/min/1.73 m^2 per year, p=0.014, by Student's t-test) during follow-up.

Association of uEGF/Cr levels with clinical–pathological parameters

Next, we assessed the association between the uEGF/Cr levels and clinical–pathological parameters using simple linear regression. For both active stage and remission stage of AAV, uEGF/Cr was significantly associated with eGFR(log_2) at sampling (β=0.60, p<0.001; β=0.74, p<0.001, respectively). The level of uEGF/Cr showed a negative association with IFTA grade (β=−0.37, p=0.005). However, there was no significant association between uEGF/Cr and CRP or BVAS. Note that analyses were based on log_2-transformed uEGF/Cr value, untransformed value was also used for data analysis, and results were similar.

Using the definition of renal response to treatment, treatment failure occurred in 32 out of 173 (18.5%) patients with active AAV. They experienced fast deterioration in renal function during the induction therapy, with a rapid rate of eGFR decline of −3.26±2.41 mL/min/1.73 m^2 per month. Eight out of 32 patients progressed to irreversible ESRD within the first month despite early institution of immunosuppressive therapy. Among these 32 patients, their extrarenal manifestations were ameliorated at various degrees after treatment. Patients who were 'treatment failure' had significantly lower level of uEGF/Cr (samples collected prior to treatment) than patients who had renal response to induction therapy (1.65±1.22 vs 2.16±1.26, p=0.04, by Student's t-test).

uEGF/Cr and renal outcomes of AAV

To determine whether uEGF/Cr could predict the loss of kidney function in patients with AAV after they achieved remission by the induction therapy, we analysed the association between uEGF/Cr level in remission stage and the slope of eGFR. Three or more eGFR values during follow-up were available for 133 out of 143 patients with AAV in remission. The level of uEGF/Cr was significantly associated with the eGFR slope (β=0.63, p<0.001, by linear regression).

Moreover, among all the 173 patients with active AAV, 143 patients had follow-up data. We investigated the association between uEGF/Cr level at baseline (acute stage of AAV) and the composite endpoint, defined as ESRD or 30% reduction of eGFR. During the follow-up (median 18.0 months, IQR 6.0–39.0 months), 38 patients developed ESRD, and 59 patients reached the composite endpoint. Univariable Cox regression analysis showed that urinary protein at renal biopsy, eGFR, protocols of induction therapy and uEGF/Cr were significantly associated with the composite endpoint. Multivariable Cox regression analysis showed that the level of uEGF/Cr remained an independent predictor for the composite endpoint (HR 0.61, 95% CI 0.45 to 0.83, p=0.001), even after adjusting for age, gender, eGFR, urinary protein at renal biopsy, type of ANCA and treatment protocols (table 3). Time-dependent ROC analysis showed that the area under the ROC curve for the reciprocal of uEGF/Cr at 72 months was 0.66. At the cut-off value of 0.46 for the reciprocal of uEGF/Cr, the sensitivity was 63% and the specificity was 63%.

In order to evaluate the additive effect of uEGF/Cr on traditional clinical markers for renal progression of AAV, we applied nested Cox proportional hazards models for this analysis. In comparison with the base model (M0, including age and gender), adding traditional clinical parameters to the base model improved the fitting capacity. Addition of uEGF/Cr to these models further improved the model fit significantly (table 4).

DISCUSSION

EGF is one of the typical peptide growth factors.[27 28] In humans, the kidney is the main organ for EGF production.[29] To be more specific, EGF expression can be found in the ascending portion of Henle's loop and the distal tubule in the kidney.[30] By activating the EGF receptors, EGF plays a variety of roles in controlling cell proliferation, differentiation, survival and motility.[31–33] However, whether the level of uEGF is associated

Table 3 Cox proportional hazard models for the composite endpoint in patients with AAV

	Univariable		Multivariable	
Factors	**HR (95% CI)**	**P values**	**HR (95% CI)**	**P values**
Age	0.99 (0.97 to 1.01)	0.416	0.98 (0.95 to 1.01)	0.126
Gender (male vs female)	0.99 (0.58 to 1.68)	0.966	1.41 (0.75 to 2.65)	0.281
uEGF/Cr(log_2)	0.56 (0.44 to 0.71)	<0.001	0.61 (0.45 to 0.83)	0.001
Urinary protein (per g/24 hours)*	1.19 (1.02 to 1.38)	0.024	1.17 (0.96 to 1.43)	0.121
eGFR (per 10 mL/min/1.73 m^2)	0.74 (0.64 to 0.87)	<0.001	0.82 (0.68 to 1.00)	0.044
Type of ANCA (MPO vs PR3)	1.42 (0.57 to 3.55)	0.458	2.64 (0.97 to 7.18)	0.057
Induction therapy		0.002		0.409
Corticosteroids+CTX	0.22 (0.10 to 0.48)	<0.001	0.47 (0.19 to 1.19)	0.111
Corticosteroids+CTX+MP	0.46 (0.23 to 0.93)	0.030	0.57 (0.26 to 1.22)	0.146
Corticosteroids+CTX+PE	0.44 (0.14 to 1.38)	0.157	0.70 (0.20 to 2.43)	0.570
Corticosteroids+CTX+MP+PE	Reference		Reference	
Maintenance therapy (AZA vs others)	1.12 (0.65 to 1.95)	0.679	1.83 (0.96 to 3.48)	0.067

*There were three patients with missing data for urinary protein, and these three patients were therefore not used in multivariable analysis.
ANCA, antineutrophil cytoplasmic antibody; AZA, azathioprine; CTX, cyclophosphamide; eGFR, estimated glomerular filtration rate; MP, methylprednisolone pulse therapy; MPO, myeloperoxidase; PE, plasma exchange; PR3, proteinase 3; uEGF, urinary epidermal growth factor.

with the disease severity and renal outcome of AAV is still far from clear.

In this study, similar to what has been reported in CKD cohorts, we observed a significant association between intrarenal *EGF* mRNA levels and eGFR in patients with AAV. Since uEGF is highly correlated with *EGF* mRNA levels,[12] it is not surprising that the level of uEGF/Cr was correlated with eGFR. The association between EGF and the degree of IFTA in the renal histology confirmed previous reports in patients with CKD and primary glomerulonephritis.[12 25] Meanwhile, we found that the uEGF/Cr level was associated with renal response to treatment. More importantly, the level of uEGF/Cr in remission stage was associated with the eGFR slope and the level of uEGF/Cr at baseline was an independent predictor for renal outcomes of AAV. While uEGF/Cr in the current study had moderately good sensitivity and specificity for predicting poor renal outcome, we found that with the addition of uEGF/Cr onto the traditional predictive model for AAV progression, the model's predictive performance improved. As urinary EGF is considered to reflect the degree of functional distal tubular cell mass, the reduced level of uEGF could be a reflection of the degree of tubular damage present in a patient with AAV. This could be the consequence of severity of ongoing autoimmune disease or a consequence of past immune damage to the tubular compartment. Both scenarios could explain the significant association between the lower level of uEGF/Cr and worse long-term outcome.

EGF is a main trophic factor for tubular cells and modulates tissue response to injury.[30] Moreover, exogenous EGF enhanced tubular cell repair processes, suggesting that EGF may regulate tubular recovery to damage.[34] Regarding the underlying protection mechanism of EGF in kidneys, studies have shown that high EGF levels reduced renal epithelial sodium channel activity, which could alleviate glomerular and renal tubular damage.[35 36] However, the protective mechanism of EGF in AAV progression needs to be further explored.

Strengths of the study include the assessment of association of uEGF/Cr level with active and remission AAV and the sequential longitudinal sampling from patients to evaluate the association of uEGF/Cr levels with renal progression of AAV. Limitations include the single-centre design with modest sample size, and relatively short and varied duration of follow-up, necessitating a validation in larger, independent multiethnic AAV study cohort. Important questions for future studies are whether uEGF level could predict treatment response and whether patients in remission with lower uEGF/Cr profiles should be monitored more closely for disease recurrence and potentially more aggressive therapy.

In conclusion, we show that lower uEGF/Cr level is associated with more severe renal disease, renal resistance to treatment and higher risk of progression to composite endpoint in patients with AAV. uEGF/Cr level may be a useful non-invasive biomarker to assess the degree of tubular damage and the potential for the transition into chronic progressive kidney disease in patients with AAV, which warrants validation in an independent, larger and multicentre cohort study.

Table 4 Comparison of nested models of survival analysis

Models*	R^2	AIC	Models compared	LR test (P values)
M0: age+gender	0.01	523.06	–	–
M1: age+gender+urinary protein	0.04	507.80	M1 vs M0	<0.001
M1′: M1+uEGF/Cr	0.19	487.53	M1′ vs M1	<0.001
M2: age+gender+eGFR	0.16	503.07	M2 vs M0	<0.001
M2′: M2+uEGF/Cr	0.23	485.05	M2′ vs M2	<0.001
M3: age+gender+urinary protein+eGFR+induction therapy+type of ANCA	0.19	491.42	M3 vs M0	<0.001
M3′: M3+uEGF/Cr	0.25	482.65	M3′ vs M3	0.001

*Three patients with missing data for urinary protein.
AIC, Akaike information criterion; ANCA, antineutrophil cytoplasmic antibody; eGFR, estimated glomerular filtration rate; LR, likelihood ratio; uEGF, urinary epidermal growth factor.

Contributors LW and X-QL participated in urine sample and clinical information collection. LW participated in the measurement of ELISA. LW, TG, SE and W-JJ participated in data analysis and interpretation. LW wrote the manuscript. MC, W-JJ, LW, TG and SE revised the manuscript. MC, W-JJ, MK and M-HZ participated in the study design and approved the final manuscript. All authors reviewed and approved the manuscript's content before submission.

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Competing interests None declared.

Patient consent Obtained.

Ethics approval The research was approved by the ethics committees of Peking University First Hospital and the ethics committees of the University of Michigan.

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EXTENDED REPORT

Number of individual ACPA reactivities in synovial fluid immune complexes, but not serum anti-CCP2 levels, associate with inflammation and joint destruction in rheumatoid arthritis

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ABSTRACT

Introduction Individual patients with rheumatoid arthritis (RA) show divergent specific anti-citrullinated protein/peptide antibodies (ACPA) patterns, but hitherto no individual ACPA specificity has consistently been linked to RA pathogenesis. ACPA are also implicated in immune complexes (IC)-associated joint pathology, but until now, there has been no method to investigate the role of individual ACPA in RA IC formation and IC-associated pathogenesis.

Methods We have developed a new technique based on IC binding to C1q-coated magnetic beads to purify and solubilise circulating IC in sera and synovial fluids (SF) from 77 patients with RA. This was combined with measurement of 19 individual ACPA in serum, SF and in the IC fractions from serum and SF. We investigated whether occurrence of individual ACPA as well as number of ACPA in these compartments was related to clinical and laboratory measures of disease activity and inflammation.

Results The majority of individual ACPA reactivities were enriched in SF as compared with in serum, and levels of ACPA in IC were regulated independently of levels in serum and SF. No individual ACPA reactivity in any compartment showed a dominating association to clinical and laboratory measures of disease activity and severity. Instead, the number of individual ACPA reactivities in the IC fraction from SF associated with a number of markers of joint destruction and inflammation.

Conclusions Our data highlight the polyclonality of ACPA in joint IC and the possibility that a broad ACPA repertoire in synovial fluid IC might drive the local inflammatory and matrix-degrading processes in joints, in analogy with antibody-induced rodent arthritis models.

INTRODUCTION

Antibodies directed against citrullinated peptides (anti-citrullinated protein/peptide antibodies, ACPA) are found in sera of 60%–80% of patients with rheumatoid arthritis (RA).[1 2] Numerous studies have confirmed that presence of ACPA in serum is associated with a more severe disease course.[3–5]

Total ACPA responses are commonly measured in serum with tests using proprietary cyclic citrullinated peptides (CCP) or other optimised mixtures of peptides.[6–8] Already the first publications showed a considerable ACPA epitope variation between patients with RA.[1 9] Commonly studied specificities include the originally described filaggrin molecule, as well as proteins found to be citrullinated in RA joints: α-enolase, vimentin, collagen type II, fibrinogen and histones.[10–13] A number of microarray techniques have been developed to study the pattern of ACPA responses in a simplified way, using proteome microarrays, surface plasmon resonance imaging or addressable laser bead immunoassay.[2 11 14] We have recently described a planar microarray where multiple ACPA are investigated in parallel to their native arginine-containing counterparts.[10] In an extended follow-up study using 2825 patients with RA, we found that individual subtraction of arginine reactivity was beneficial, as it both increased diagnostic specificity and association to HLA-DRB1* shared epitope, while diagnostic specificity was unchanged.[15]

No individual ACPA specificity or group of ACPA specificities has hitherto showed any unique and consistent association to clinical phenotype.[2 16] Such epitope studies have only been performed on serum samples. Two groups have reported divergent results concerning whether ACPA are enriched in synovial fluid after correction for IgG levels.[8 17 18] Enrichment would imply that ACPA might be preferably produced in the joints of patients with RA. Thus, if ACPA have any direct pathogenetic association to the inflammatory process in RA, determinations of total ACPA and individual ACPA reactivities in synovial fluid (SF) might show stronger associations to clinical phenotype than corresponding serum levels.

Already 50 years ago, immune complexes (IC) were found in RA, especially in SF but also in the circulation of patients with extra-articular disease.[19–23] However, results diverged considerably between studies and methods used.[24] More recent studies have focused on biological IC function in RA. We have shown that levels of polyethylene glycol-precipitated IC from RA SF relate to in vitro-induced tumour necrosis factor alpha (TNF-α) production and rheumatoid factor (RF) levels, supporting the hypothesis that IC are directly linked to cytokine-dependent inflammation in RA.[25] Using another in vitro model, we have produced surface-bound IC containing collagen

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type II (CII) and anti-CII, and related anti-CII IC-induced responses in vitro and anti-CII levels in vivo to an acute-onset RA phenotype.[26–29] This IC-dependent RA phenotype shows resemblance to collagen antibody-induced arthritis (CAIA), an antibody-mediated arthritis model dependent on neutrophil granulocytes.[28 30] Two groups have shown that ACPA-containing IC stimulate cytokine production via Fc receptors.[31 32] Notably, except for some early studies, none of these studies have actually determined autoantibody levels in RA IC obtained in vivo.[33 34] As previous studies have shown accumulation both of IC and of ACPA in SF as compared with in the circulation, ACPA levels in SF IC would then be an especially interesting target for such studies.

We have developed a bead-based assay for the quantification of multiple autoantibodies in soluble IC. Here, we have combined this assay with the ACPA multiplex array to investigate whether individual ACPA reactivities in sera and SF, and in IC from these compartments, can provide more or different prognostic information than conventional measurement of anti-CCP in serum.

Patients and methods

Subjects

The complete cohort study consisting of 121 patients with RA has been described previously, and of these, 77 patients had paired sera and knee SF available for the present study.[35] Patients fulfilling the 1987 American College of Rheumatology classification criteria for RA and with knee synovitis were included at the rheumatology departments in Gävle, Falun and Uppsala, Sweden.[36] Patients with function class 4 according to Steinbrocker, those receiving ≥10 mg oral prednisolone daily and those who had obtained glucocorticoid injection <3 months prior to the investigation were excluded.[37] SF were aspirated from the knees whereafter 20 mg triamcinolone hexacetonide was injected. Sera and SF were collected in parallel, centrifuged for 20 min at 1800×*g* within 1 hour and stored at −70°C until analysis. Levels of mononuclear (MN) and polymorphonuclear (PMN) cells in SF were recorded. Patients were followed for 6 months, or until relapse if clinical synovitis re-appeared earlier. Information on patient characteristics (age, sex, disease duration and smoking habits) was recorded at the study inclusion. Disability was evaluated using the Swedish version of the Health Assessment Questionnaire.[38] Disease Activity Score (DAS28)[39] was assessed from the number of tender and swollen joints, erythrocyte sedimentation rate and Visual Analogue Scale for global health. Radiographic examination of knees was performed and joint destruction recorded according to Larsen *et al*.[40] The study was approved by the regional ethical review board in Uppsala, and all patients had given informed consent in accordance with the Declaration of Helsinki.

Laboratory investigation of autoantibodies and measures of systemic and local inflammation was performed in parallel in serum and SF as described previously.[35 41 42] Measurements of C1q-binding IC and IgG levels in sera and SF are described in the online supplementary file .

Capturing and elution of circulating IC (CIC)

IC were purified from sera and SF by applying a technique we have established in our laboratory. Purified human C1q (Quidel, San Diego, California, USA; 1.2 mg/mL) was immobilised on magnetic tosylactivated microparticles (Dynabeads M-280; Life Technologies, Carlsbad, California, USA) according to the manufacturer's recommendations for activation of amine groups.

Ten-microlitre C1q beads were washed once with PBS–0.05% Tween–1% BSA (PBST-B) and incubated with 10 µL serum or SF and 30 µL PBST-B in a Bio-Plex flat-bottom 96-well plate for 1.5 hours on a microplate shaker (600 rpm) at 37°C. Antibodies from the C1q-bound IC were recovered in a two-step procedure. After washing with PBS–0.05% Tween, the beads were resuspended with a Bio-Plex hand-held magnetic washer in 50 µL 0.1 M glycine–HCl, pH 2.5 and incubated for 3 min on shaker at 37°C. IC eluates were collected and immediately neutralised with 4 µL 0.5 M NaOH on ice. In the second elution step, the beads were resuspended with 100 µL freshly prepared 25% methanol, pH 11.5, previously shown to dissociate antibodies without destroying antigen-binding capacity and incubated for additional 12 min on shaker at 37°C.[43 44] The latter fraction was mixed with the glycine eluates. IC eluates that were not assayed the same day were stored at −70°C until testing; freezing/thawing did not obviously change autoantibody results. The full procedure of IC purification is graphically illustrated in figure 1, and validation data are shown in online supplementary figure 1 for artificial IC and in online supplementary figures 2 and 3 for IC-rich SLE sera.

Microarray-based analysis for detection of ACPA fine specificities

ACPA in serum and SF and in solubilised IC from serum and SF were investigated on a custom-made microarray based on the ImmunoCAP ISAC system (Phadia AB, Uppsala, Sweden).[10 15] Each reaction site of the microarray slides contained 19 different citrullinated peptides and their native arginine-containing counterparts (table 1). Sera and SF were diluted 1:40 and IC eluates were, due to practical reasons, diluted twice with ImmunoCAP-specific IgA/IgG sample diluent (Phadia AB) corresponding

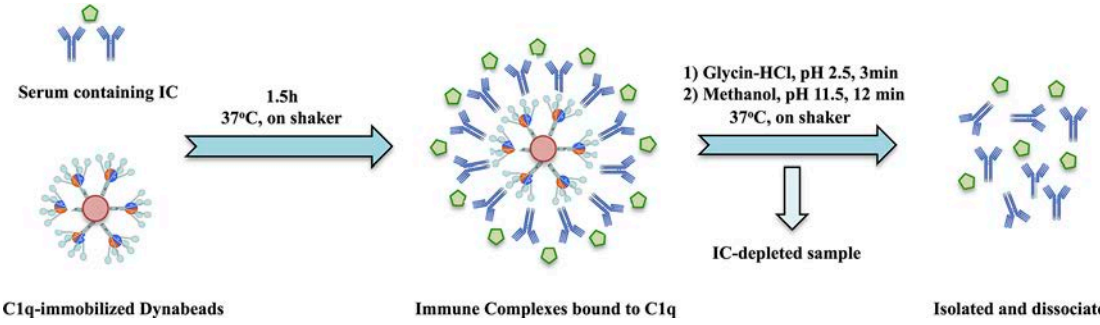

Figure 1 Schematic figure showing the process of immune complex purification with following solubilisation and measurement of individual anti-citrullinated protein/peptide antibody reactivities. Details of the procedure are described in the Patients and methods section and online supplementary file. IC, immune complexes.

Table 1 Relative concentration of specific ACPA, anti-CCP2 and circulating immune complexes (CIC) in sera and synovial fluids

	Sequence	Number of patients reacting in serum (%)	Serum median (mean)	SF median (mean)	P values (Wilcoxon)	Compartment with highest level	Serum/ IgG median (mean)	SF/ IgG median (mean)	P values (Wilcoxon)	Compartment with highest level
Filaggrin 307–324 (CCP1)	HQCHQEST(cit)GRSRGRCGRGS (cyclic)	55/77 (71)	38.89 (233.82)	17.07 (185.86)	**0.0005**	Serum	3.16 (22.69)	4.19 (36.59)	**0.0282**	SF
Vimentin 2–17	ST(cit)SVSSSSY(cit)(cit)MFGG	40/77 (52)	2.7 (14.78)	–0.43 (19.40)	0.9179		0.14 (1.27)	–0.11 (3.79)	0.561	
Vimentin 60–75	VYAT(cit)SSAV(cit)L(cit)SSVP	48/77 (62)	36.76 (195.93)	11.5 (168.09)	0.0575		2.63 (18.14)	4.00 (36.36)	**0.0009**	SF
Fibrinogen α36–50	GP(cit)VVE(cit)HQSACKDS	22/77 (29)	0 (61.38)	0 (53.56)	0.1822		0 (4.67)	0 (9.67)	**0.0355**	SF
Fibrinogen α563–583	HHPGIAEFPS(cit)GKSSSYSKQF	47/77 (61)	10.05 (107.30)	6.48 (82.61)	**0.0378**	Serum	1.02 (10.51)	1.08 (18.02)	**0.0216**	SF
Fibrinogen α580–600	SKQFTSSTSYN(cit)GDSTFESKS	25/77 (32)	0 (43.90)	0 (27.70)	0.1398		0 (4.65)	0 (7.61)	0.5338	
Fibrinogen α621–635	(cit)GHAKS(cit)PV(cit)GIHTS	36/77 (47)	0 (121.15)	0 (91.35)	0.3756		0 (13.33)	0 (20.48)	0.0987	
Fibrinogen β36–52	NEEGFFSA(cit)GHRPLDKK	47/77 (61)	14.38 (156.83)	7.44 (132.73)	**0.039**	Serum	1.33 (13.84)	2.32 (27.04)	**0.0087**	SF
Fibrinogen β60–74	(cit)PAPPPISGGGY(cit)A(cit)	52/77 (68)	17.7 (170.93)	7.49 (156.08)	**0.0292**	Serum	1.46 (14.62)	2.40 (30.47)	**0.0005**	SF
Fibrinogen β62-81(Fib72)	APPPISGGGY(cit)ARPAKAAAT	25/77 (32)	0 (11.19)	0 (7.09)	0.9311		0 (1.16)	0 (1.60)	0.936	
Fibrinogen β62-81(Fib74)	APPPISGGGYRA(cit)PAKAAAT	22/77 (29)	0.55 (35.02)	0.73 (32.12)	0.2692		0.06 (2.76)	0.11 (6.08)	**0.0305**	SF
α-Enolase 5-21(CEP-1)	CKIHA(cit)EIFDS(cit)GNPTVEC (cyclic)	49/77 (64)	72.82 (263.44)	32.4 (235.90)	**0.0183**	Serum	7.85 (22.65)	6.79 (55.09)	**<0.0001**	SF
hnRNP 1	(proprietary)*	28/77 (36)	9.79 (70.52)	6.44 (53.49)	**0.0003**	Serum	0.89 (6.06)	1.72 (10.64)	**<0.0001**	SF
hnRNP 5	(proprietary)*	49/77 (64)	19.8 (134.97)	9.14 (120.27)	0.1191		1.67 (12.52)	2.36 (26.61)	**0.0005**	SF
hnRNP Z1	(proprietary)*	35/77 (45)	0 (65.53)	0.83 (56.09)	0.4464		0 (6.47)	0.25 (13.54)	**0.0283**	SF
hnRNP Z2	(proprietary)*	46/77 (60)	4.96 (128.27)	6.21 (105.07)	0.1726		0.43 (13.44)	2.75 (24.81)	**0.0066**	SF
hnRNP Bla26	(proprietary)*	41/77 (53)	4.57 (45.95)	0.81 (45.32)	0.2491		0.43 (4.13)	0.46 (8.74)	**0.005**	SF
Histone4 14–34	GAK(cit)H(cit)KVL(cit)DNIQGITKPAI	32/77 (42)	18.77 (109.76)	11.64 (81.80)	**<0.0001**	Serum	1.66 (9.79)	2.14 (15.51)	**0.0053**	SF
Histone4 31–50	KPAI(cit)(cit)LA(cit)(cit)GGVK(cit)ISGLI	47/77 (61)	11.04 (101.92)	4.79 (67.97)	**0.029**	Serum	0.75 (10.42)	1.36 (11.51)	0.3402	
Anti-CCP2	–	55/77 (71)	132 (317.25)	55 (153.99)	**<0.0001**	Serum	11.60 (29.44)	10.48 (39.95)	**0.0053**	SF
CIC µg Eq/mL	–	–	0.53 (12.35)	0.27 (5.87)	**0.0252**	Serum	0.042 (1.112)	0.054 (0.914)	0.5018	

Values are shown both as arbitrary units in sera and SF, as well as after correction for total IgG levels in the corresponding serum or SF samples. Comparisons were performed with the Mann-Whitney U test; significant differences are depicted in bold. The second column details the sequences of the citrullinated peptides in the multiplex assay, using the single-letter amino acid code and '(cit)' for citrulline. The not listed arginine-containing control peptides have identical sequences except that they contain arginine residues instead of citrulline.

*Peptides are derived from hnRNP A3. Available on request from KS; see authors' details.

ACPA, anti-citrullinated protein/peptide antibodies; SF, synovial fluid.

to a 1:30 total dilution of the IC fraction as compared with serum and SF. For each individual ACPA reactivity, we subtracted the corresponding arginine peptide reactivity.[15] Cut-offs for individual ACPA in serum and SF were the 98th percentile among 944 healthy control serum samples. Measurement of ACPA in IC failed in one patient and in SF of one other patient for technical reasons. As there are no data on distribution of individual ACPA in IC, cut-offs were set as median for the n=76 group for serum and SF IC, respectively. In alternative parallel evaluations, cut-offs were estimated from mean +2 SD for levels in IC prepared from 20 healthy donor sera. Three SF IC were investigated on two occasions concerning occurrence of 17 individual ACPA reactivities, with total kappa coefficient 0.92. The strengths of individual ACPA reactivities with and without arginine correction are shown in online supplementary table 1.

Statistical analysis

Enrichment of individual ACPA reactivities in SF was defined as the ratio between ACPA levels in SF and serum, after normalisation for individual IgG levels. Groups were compared with the Mann-Whitney U test for unpaired comparisons, Wilcoxon signed-rank test for paired comparisons and χ^2 test for ratios. Correlation between variables was assessed by Spearman's rank correlation test. Multiple regression analyses were performed to determine which autoantibody measures predicted clinical and laboratory measures of disease activity and inflammation. Independent variables were in the first four regression analyses occurrence of 19 individual ACPA in serum, in SF and in the IC fractions obtained from serum and SF, respectively. Occurrence of ACPA was used as nominal variables, as ACPA distribution often is bimodal, grossly corresponding to anti-CCP2-positive and anti-CCP2-negative patients. In a second set of multiple regression analyses, the independent variables were anti-CCP2 levels in serum and in synovial fluid as well as the number of individual ACPA in serum, in SF and in the IC fractions obtained from serum and SF, respectively. Two independent ways to calculate cut-offs for individual ACPA reactivities in IC fractions were used, as described above.

The statistical calculations were performed with JMP V.11 (SAS Institute, Cary, North Carolina, USA). P values less than 0.05 were considered significant. No corrections for mass significance were performed.

RESULTS

Distribution of individual ACPA reactivities in serum, SF and corresponding IC fractions

Individual ACPA reactivities were found in serum from 29% to 71% of the patients (table 1). Levels in corresponding synovial fluids were significantly lower for 8/19 ACPA. This was, however, also the case for total IgG levels measured with identical technique in both body fluids (median (mean)) levels in serum and SF (11.0 (12.6) vs 4.3 (5.2) g/L; p<0.0001). When individual ACPA reactivities were corrected for total IgG, the majority (14/19) of ACPA reactivities instead showed an increase in the synovial compartment as compared with serum. A corresponding relation was also seen for anti-CCP2 levels. Levels of CIC were lower in SF than in serum, after IgG correction this difference disappeared (table 1). Total IgG levels in sera and SF did not show any correlation to levels of anti-CCP2, IgM RF or IgA RF in any compartment (data not shown).

The number of individual ACPA reactivities in serum showed a bimodal distribution corresponding to low numbers in anti-CCP2-negative patients and higher numbers in anti-CCP2-positive patients (figure 2A). This was not as obvious for the distribution in SF (figure 2B). Number of individual ACPA reactivities in the IC fractions in serum and SF instead showed only one modal value, especially prominent for serum IC (figure 2C, D for cut-offs based on medians). Corresponding figures using the alternate cut-offs are shown in online supplementary figure 4.

Relation between the appearance of individual ACPA reactivities in serum, SF and IC fractions

Levels of individual ACPA correlated strongly between sera and SF (Spearman's ρ between 0.56 and 0.93, mean 0.78) as was also seen for anti-CCP2 (ρ=0.82; table 2). A much lower degree of correlation was seen between levels in serum and in the corresponding IC fraction (Spearman's ρ 0.01–0.41, mean 0.20), whereas a somewhat larger degree of correlation was seen between levels in SF and in the corresponding IC fraction (ρ=0.06–0.61, mean 0.38; table 2).

Association between clinical and laboratory parameters and occurrence of individual ACPA reactivities in serum, SF and IC fractions

Analysis of the association between clinical and laboratory measures and the occurrence of individual ACPA in sera and SF yielded many parallel associations without any obvious dominance for certain ACPA (data not shown). When instead multiple regression with the occurrence of 19 individual ACPA reactivities in serum, SF and the corresponding IC fractions were used as independent variables, most of these associations disappeared, but again, no individual ACPA showed a general positive association to clinical and laboratory variables of disease activity and inflammation. For those ACPA showing many associations, there was often a mixture of positive and negative regression coefficients (online supplementary tables 2–5). Comparable data were found with the alternative approach of cut-off setting for ACPA in IC (data not shown). When the number of individual ACPA reactivities in serum, SF and in the corresponding IC fractions were employed as independent variables in parallel to anti-CCP2 levels in serum and SF, another picture emerged. The number of ACPA in the IC fraction in SF turned out to be the sole factor positively associating with Larsen-Dale score, and SF levels of PMN, IL-6, TNF-α, cathepsin S and VEGF, but without any association to the corresponding serum levels (table 3). This is graphically shown in figure 3, where Larsen-Dale index and SF levels of IL-6, cathepsin S and VEGF are shown dichotomised according to number of ACPA in the IC fraction in SF (optimal cut-offs for each variable were chosen according to calculations in online supplementary table 6). Also, when using the alternative cut-offs for the occurrence of individual ACPA in IC fractions from serum and SF, number of ACPA reactivities in SF IC was the determining factor for swollen joint count, and SF levels of PMN, IL-6, cathepsin S and VEGF, but also for serum IL-6 (data not shown).

DISCUSSION

In this study, we have shown that the majority of individual ACPA reactivities are enriched in SF as compared with in serum, and that levels of ACPA in IC are regulated independently of levels in serum and SF. No individual ACPA reactivity in serum or SF shows a dominating association with clinical and laboratory measures of disease activity and severity. Instead, the number of individual ACPA reactivities in the IC fraction from SF associates to a number of markers of joint destruction and inflammation.

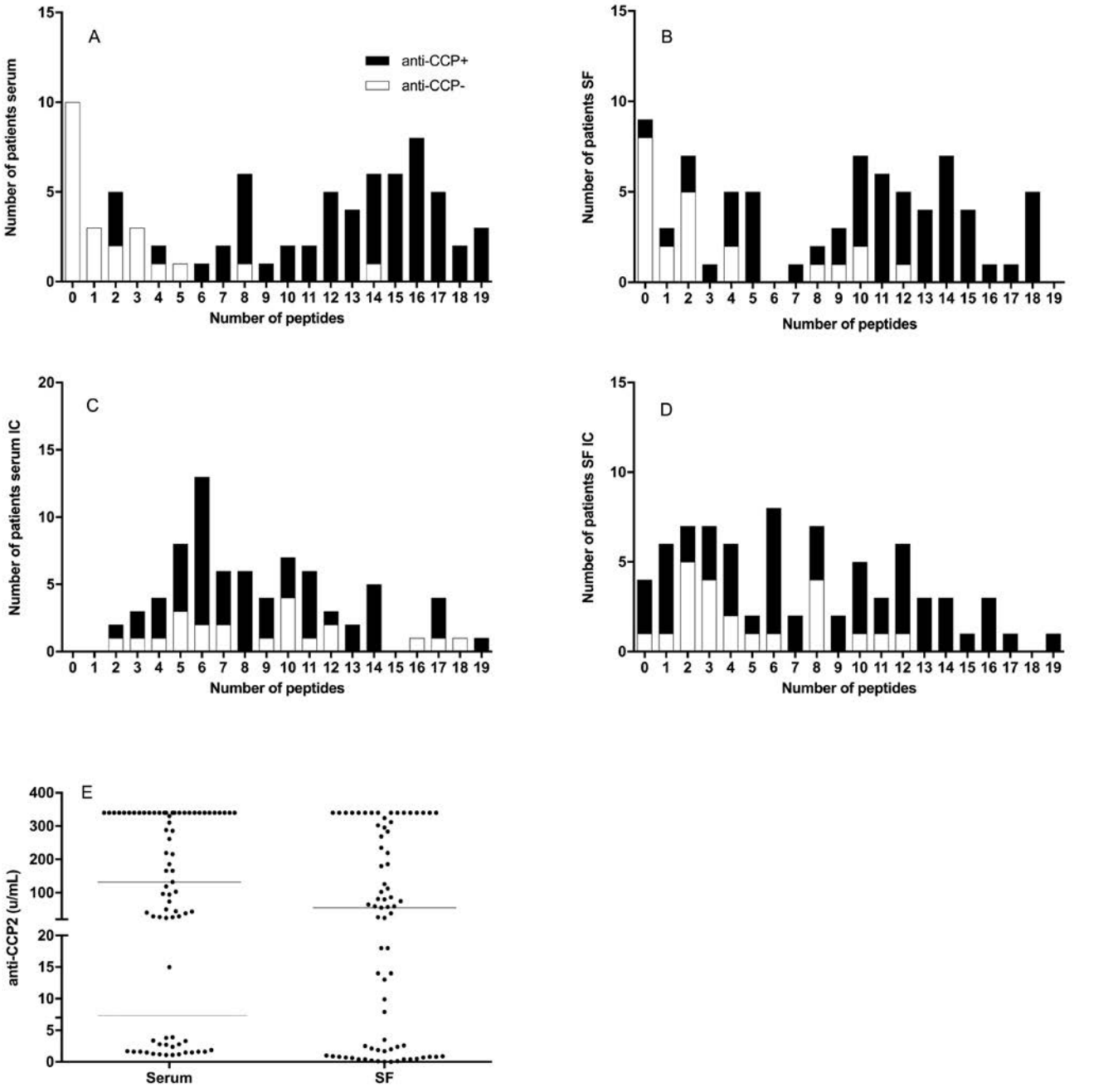

Figure 2 Distribution of number of antibodies against 19 citrullinated peptides in sera and synovial fluid (SF) as well as in immune complexes (IC) from sera and SF. Cut-offs for sera and SF (A, B) were determined as the 98th percentile for net anti-citrullinated protein/peptide antibodies (ACPA) reactivity among 944 healthy control samples, whereas cut-offs in the IC fractions from sera and SF (C, D) were determined as the median values for the respective compartment. For each compartment, empty bars represent the anti-CCP2-negative and filled bars represent the anti-CCP2-positive patients. In (E), the corresponding levels of anti-CCP2 in the investigated sera and SF are shown; horizontal bars representing medians. Corresponding figures using the alternate cut-offs for IC fractions from sera and SF are shown in online supplementary figure 4.

We focused on determination of individual ACPA reactivities in sera and synovial fluid, and in IC fractions purified from sera and SF, in order to investigate if measurement of individual ACPA may provide more prognostic information than conventional measurement of CCP2 in serum and SF. We could not determine that any individual ACPA reactivity was dominant in the association with clinical disease activity or inflammation.

Anti-CCP2 and the majority (14/19) of individual ACPA reactivities were enriched in SF compared with in serum. When measuring crude ACPA levels, the majority of ACPA reactivities were lower in SF than in serum; enrichment in SF only appeared when antibody concentrations were corrected for total IgG, and also then, median values differed only modestly. No individual ACPA was considerably more enriched in SF than other ACPA. These data argue that most ACPA are locally produced in the joints. Snir and colleagues demonstrated that ACPA levels were increased in synovial fluid compared with in serum using two different techniques for IgG measurement.[8 18] We used identical ELISA tests for serum and SF with γ-specific $F(ab')_2$ fragments of both the capture and detection antibodies to reduce the risk

Table 2 Correlation between levels of specific ACPA in serum, synovial fluid and the immune complex fractions in serum and synovial fluid (correlation values are Spearman's ρ values)

ACPA specificity	Serum vs SF	Serum vs serum IC	SF vs SF IC
Filaggrin 307-324 (CCP1)	0.85	0.31	0.61
Vimentin 2–17	0.87	0.13	0.19
Vimentin 60–75	0.93	0.15	0.38
Fibrinogen α36–50	0.73	0.10	0.34
Fibrinogen α563–583	0.85	0.24	0.49
Fibrinogen α580–600	0.70	0.18	0.22
Fibrinogen α621–635	0.87	0.33	0.55
Fibrinogen β36–52	0.83	0.27	0.42
Fibrinogen β60–74	0.82	0.28	0.53
Fibrinogen β62–81(Fib72)	0.56	0.01	0.17
Fibrinogen β62–81(Fib74)	0.63	0.04	0.43
α-Enolase 5–21(CEP-1)	0.88	0.41	0.58
hnRNP 1	0.76	0.10	0.31
hnRNP 5	0.78	0.36	0.34
hnRNP Z1	0.67	0.14	0.22
hnRNP Z2	0.82	0.32	0.55
hnRNP Bla26	0.58	0.11	0.06
Histone4 14–34	0.88	0.14	0.43
Histone4 31–50	0.78	0.21	0.45
Mean for 19 ACPA	0.78	0.20	0.38
Median for 19 ACPA	0.82	0.18	0.42
Anti-CCP2	0.82		

ACPA, anti-citrullinated protein/peptide antibodies; IC, immune complexes; SF, synovial fluid.

of non-specific binding of RF to Fc parts of assay antibodies. Consequently, we found no correlation between total IgG levels and anti-CCP2 or RF in serum or SF.

We found a strong correlation between serum and SF levels of individual ACPA, while associations were much lower between sera/SF and the corresponding IC fractions (table 2). This suggests that antibody levels in serum and SF are regulated by the same mechanisms, but that levels in IC are regulated differently. However, the correlations differed between peptides: anti-filaggrin 307–324 and anti-α-enolase 3–17 displayed strong correlation between serum and SF and the corresponding IC fractions, whereas the opposite was found for anti-fibrinogen β62–81 and anti-hnRNP Bla26 (table 2). This might implicate that certain individual ACPA reactivities have higher propensity to enrich in IC as compared with other ACPA, irrespective of body compartment. This first study using our new technique is however small, and such hypotheses should be confirmed in larger cohorts. We are aware that our results imply that some anti-CCP2-negative patients have ACPA in their IC, not found in the corresponding body fluids. Tentatively, occurrence of ACPA in IC might be a new measure of ACPA positivity with its own individual clinical associations, but there are also methodological concerns. Degree of ACPA positivity in IC varies with how cut-offs for ACPA in IC are determined (compare figure 2C and 2D with online supplementary figure 4) and might also be affected by method robustness. This will be subject to future studies.

The main finding of this study was that polyclonality in SF IC was the dominating factor that appeared in a number of independent estimates (8/27), always in positive association to clinical and laboratory measures of inflammation and joint destruction. Apart from serum IL-6 that associated with many

Table 3 Multiple regression with levels of anti-CCP2 in serum and synovial fluid together with number of ACPA reactivities in serum, synovial fluid, immune complex fraction in serum and in synovial fluid were used as independent variables and compared with clinical and laboratory measures (only significant p values (<0.05) are shown, and underlined if the regression coefficient and T statistics were negative)

	Age	Disease duration	DAS28	Swollen joint count	Tender joint count	Global VAS	ESR	CRP	HAQ	Larsen-Dale	Time to relapse	SF PMN	SF MNC	Serum IL-6	SF IL-6	Serum TNF	SF TNF	Serum MMP3	SF MMP3	Serum PTX3	SF PTX3	Serum cathepsin L	SF cathepsin L	Serum cathepsin S	SF cathepsin S	Serum VEGF	SF VEGF
Serum anti-CCP2																0.0182								0.0034			
SF anti-CCP2									0.0157	0.0438						0.0026								0.0109			
No of ACPA in serum																0.0385						0.0373					
No of ACPA in SF																											
No of ACPA in s-IC																0.0149											
No of ACPA in SF IC										0.0166		0.0459			0.0007	0.048	0.0441								<0.0001		0.0377

ACPA, anti-citrullinated protein/peptide antibodies; CRP, C reactive protein; DAS, Disease Activity Score; ESR, erythrocyte sedimentation rate; HAQ, Health Assessment Questionnaire; MNC, mononuclear cell; MMP3, matrix metalloproteinase 3; PMN, polymorphonuclear; PTX3, pentraxin 3; SF, synovial fluid; TNF, tumour necrosis factor; VAS, visual analogue scale; VEGF, vascular endothelial growth factor.

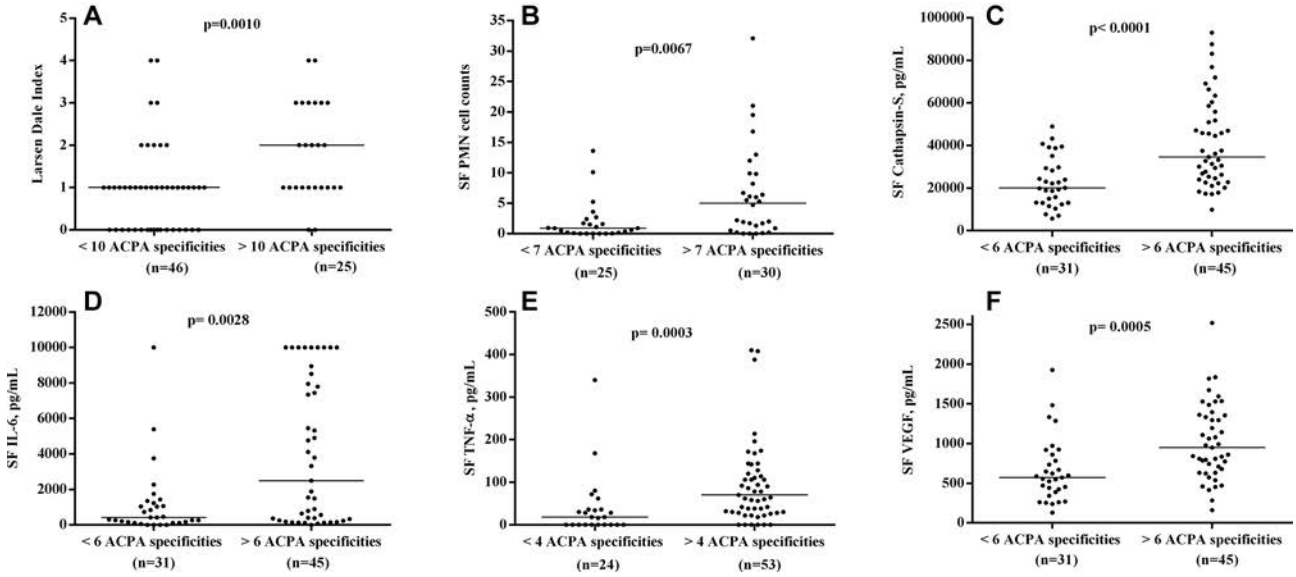

Figure 3 Difference between patients with high and low number of individual ACPA reactivities in SF IC concerning (A) Larsen-Dale index, (B) polymorphonuclear (PMN) cell counts, synovial fluid (SF) levels of (C) cathepsin S, (D) IL-6, (E) tumour necrosis factor alpha (TNF-α) and (F) vasclular endothelial growth factor (VEGF). For each measure, the optimal cut-off was chosen according to online supplementary table 6. Horizontal solid line represents the median levels in each group. For TNF-α, two measurements are outside of the axis limit but have been included in the statistics.

ACPA measures, the only other ACPA measure that appeared frequently in the regression analyses was anti-CCP2 levels in SF, however usually with negative association. Regardless of which cut-off setting algorithm was used to determine occurrence of individual ACPA in IC fractions, the number of individual ACPA reactivities in SF IC appeared to be the most strongly associated factor.

Our finding indicates a key role for ACPA polyclonality in IC in the target organ. Thus, the breadth of the ACPA response, but no particular individual ACPA specificity, may be the determining factor. Our findings also put focus on the tentative role of ACPA in IC-driven joint pathology in RA. Our technique for the determination and quantification of individual autoantibodies in IC obtained in vivo was developed to target this kind of scientific questions, and we aim to pursue such studies in larger RA cohorts, including whether ACPA in general or with predilection for certain individual ACPA specificities are enriched in IC. We also plan to investigate IC heterogeneity concerning other antibody specificities, like RF and anti-type II collagen.

The tentative role for autoantibody polyclonality in RA IC makes it tempting to compare the present findings with two autoantibody-driven arthritis models in rodents. Both the CAIA as well as the K/BxN models depend solely on antibodies against type II collagen and glucose 6-phosphate isomerase, respectively. Injection of individual monoclonal IgG antibodies specific against major epitopes of the antigens into healthy recipients will only induce mild arthritis. However, when multiple antibodies directed against nearby epitopes on the same protein are injected simultaneously as a cocktail, they will induce severe arthritis, suggesting that pathogenicity is associated not to recognition of a specific epitope on the antigen but the ability to form polyclonal IC that may activate FcγR on the target cell.[45 46]

Although our results hitherto have not implicated any individual ACPA, it is of course tempting to speculate that our method might define a core group of individual ACPA reactivities in SF IC, which in vivo might target multiple epitopes on a pathogenetically central citrullinated antigen, and thereafter activate and perpetuate arthritis in RA as we have described for anti-type II collagen antibodies.[26–29 47–49] It is possible that individual ACPA reactivities and also individual citrullinated antigen(s) can be recovered in isolated SF IC obtained with our new technique, and we intend to investigate this possibility.

The present version of our technique is a prototype method. If further studies will prove our findings to be of tentative interest in patient care by adding clinically useful information not provided by conventional autoantibody measurement, the laboratory procedures have to be further validated, for example, concerning robustness and variability.

Our data highlight the polyclonality of the ACPA in joint IC, and the possibility that a broad ACPA repertoire might drive the local inflammatory and matrix degrading process in the joint. Thus, our findings argue that ACPA are locally produced and participate in RA pathogenesis via formation of IC locally at the site of inflammation.

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Contributors AS and JR conceived the study. AS developed the IC purification technique described in this paper and performed most of the laboratory work. MH and LM-A developed the multiplex microarray. MC, KS and GS provided peptides for the analyses, including validation of their performance. AK, JL and TW included and performed clinical characterisation of the investigated patients. AL investigated inflammatory markers. AS and JR drafted the manuscript. All authors read, commented on and approved the final manuscript.

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Competing interests LM-A is employed by Thermo Fisher Scientific. KS is co-inventor of the patents US 13/141,960, EP 09799354.7 describing the diagnostic use of the hnRNP-A3 peptide epitopes. GS is co-inventor of several international patents about ACPA antigens held by BioMérieux Cy and licensed to Eurodiagnostica Cy, and Axis-Shield Cy for commercialisation of the CCP2 assays; according to French laws, he receives a part of the royalties paid to the Toulouse III University and the University Hospital of Toulouse.

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Ethics approval The ethics board in Uppsala.

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Data sharing statement Most data are shown in the manuscript and supplementary files. However, calculations using the alternative cut-offs (see Results section) are not included but can be obtained from JR.

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EXTENDED REPORT

Expansion of T follicular helper-T helper 1 like cells through epigenetic regulation by signal transducer and activator of transcription factors

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ABSTRACT

Objectives T follicular helper (Tfh) cells are critical in the development and progression of systemic lupus erythematosus (SLE). To assess the characteristics and mechanisms of differentiation of Tfh cells, we investigated the phenotype of T helper cells in patients with SLE and underlying epigenetic modifications by cytokine-induced signal transducer and activators of transcription (STAT) family factors.

Methods Peripheral blood mononuclear cells from patients and healthy donors were analysed by flow cytometry. $CD4^+$ T cells were isolated and cultured under various stimulations. Expression of characteristic markers and phosphorylation of STATs were analysed by flow cytometry and quantitative PCR. Histone modifications were analysed by chromatin immunoprecipitation (ChIP)-PCR.

Results Differentiation of $CD4^+CXCR5^+CXCR3^+$Bcl-6^+T-bet$^+$IL-21^+IFN-γ^+Tfh-Th1-like cells was induced by interleukin (IL)-12-induced activation of STAT1 and STAT4 simultaneously. The loci of Bcl-6 and T-bet at STAT binding sites were marked by bivalent histone modifications. After IL-12 stimulation, both STAT1 and STAT4 directly bound on *BCL6* and *TBX21* gene loci accompanied by suppression of repressive histone mark trimethylated histone 3 lysine 27. Levels of serum IL-12 and interferon (IFN)-γ, expression of IL-12 receptors and proportion of $CXCR5^+CXCR3^+$ activated Tfh-Th1-like cells were increased in patients with SLE. Furthermore, the level of pSTAT1, pSTAT4 and T-bet were higher in activated Tfh-Th1-like cells than non-Tfh-Th1 cells.

Conclusion Our findings suggest that IL-12-mediated co-activation of STAT1 and STAT4 alters histone modification, resulting in differentiation of Tfh-Th1-like cells that are characteristically expanded in patients with SLE. This could be one of the underlying mechanisms responsible for expansion of Tfh-Th1-like cells and potentially helpful towards development of cell-specific treatment for SLE.

INTRODUCTION

Several types of immune cells are thought to be involved in the pathogenesis of SLE. Among them, $CD4^+$ T helper (Th) cells serve important roles in the development and progression of SLE, by amplifying inflammation, helping B cells to generate autoantibodies and accumulation of autoreactive memory B cells.[1] It is reported that loss of the Th1/Th2 balance is related to the development of SLE.[2–4] Treg cells are implicated in suppressing autoreactive effector cells and maintenance of peripheral immune tolerance.[5] The role of Th17 cells in autoantibody production is contentious[6 7]; however, interleukin (IL)−17 was proved to promote local inflammation in the lupus kidney.[8] In addition to these findings, recent studies have also demonstrated increased numbers of circulating T follicular helper (Tfh)-like cells bearing markers of Tfh cells in patients with SLE.[9 10] Moreover, our study revealed the importance of Tfh and plasmablast axis in patients with lupus.[11]

Tfh cells express the chemokine receptor 5 (CXCR5) and migrate into B cell follicles. The inducible co-stimulator (ICOS), a cell surface co-stimulatory molecule, is highly expressed on Tfh cells and crucial for Tfh cell interactions with B cells. Tfh cells support the differentiation and survival of germinal centre B cells through the secretion of IL-21.[12] In addition, the master regulator transcription factor required for Tfh cell formation is the transcriptional repressor B cell lymphoma 6 (Bcl-6); the absence of Bcl-6 results in failure of Tfh cell formation with consequently failure of germinal centre formation.[13]

Accumulating evidences point to differences between human and murine Tfh cell differentiation mechanisms. In murine model, IL-6 and IL-21 activated signal transducer and activators of transcription (STAT)1/3 and promoted the differentiation of Tfh cells; where Tfh cells secreted IL-21, which in turn provided a positive feedback loop to enhance its secretion.[14 15] In addition, type I/II interferon also contributed to some functional features of murine Tfh cells.[16 17] Whether human $CD4^+$ T cells share the same pathways remains controversial.

IL-12 is the most efficient cytokine known to promote murine and human Tfh cell differentiation.[18–20] IL-12 is produced by activated dendritic cells and can induce naive $CD4^+$ T cells to express Tfh cell characteristic molecules and upregulates transcriptional factors essential for Tfh cell differentiation, such as *BCL6*, *BATF* and *CMAF* in human.[21] However, IL-12 also drives the differentiation programme towards Th1 cell phenotype by increasing T-bet expression, followed by IFN-γ secretion.[22] We and other investigators have identified IFN-γ^+IL-21^+CD4^+ T cells, which are with

co-expression of Bcl-6 and T-bet and thus we defined them as 'Tfh-Th1-like cells'.[23–26]

The differentiation of T helper cells from naive cells to distinct subsets is regulated by the coordinated functions of various cytokines and transcription factors. In addition, epigenetic modification also controls the regulation and function of T helper cells along with transcription factors.[27] The present study was designed to assess the characteristics and mechanisms of differentiation of human Tfh-Th1-like cells by examining the regulation of epigenetic modifications and to elucidate its association in the pathogenesis of SLE.

METHODS

Patients

The study subjects were Asian patients with SLE diagnosed according to the American College of Rheumatology revised criteria for SLE[28] and age-matched and sex-matched healthy subjects free of autoimmune and infectious diseases. The online Supplementary figure S1 shows the gating strategy. The clinical features of patients are listed in the online Supplementary tables S1 and S2.

Cell isolation

Peripheral blood mononuclear cells (PBMCs) were isolated from blood samples using lymphocyte separation medium (ICN/Cappel Pharmaceuticals). Naive $CD4^+$ T cells from healthy donors were purified by naive $CD4^+$ T Cell Isolation Kit II (Miltenyi Biotec). Memory $CD4^+$ T cells were purified by $CD4^+$ T Cell Isolation Kit II, then naive $CD4^+$ T cells were removed using the aforementioned method. Cell purity was always >85%.

Cell stimulation

Cells were cultured with complete RPMI1640 medium (Wako) supplemented with 10% fetal calf serum (FCS) in flat-bottomed plates coated with T cell receptor (TCR) stimulation, cytokines and antibodies (see online Supplementary table S3). Cytokine production was detected by Cytokine Bead Array system.

Flow cytometry

Cells were stained for 20–50 min with antibodies (see online Supplementary table S4). For intracellular staining, cells were fixed for 50 min at 4°C with Transcription Factor Buffer (BD Biosciences) and then incubated for 50 min at 4°C in Perm/Wash Buffer I. For intracytoplasmic staining, cells were stimulated for 2 hours with phorbol 12-myristate 13-acetate (50 ng/mL), ionomycin (1 µg/mL) and brefeldin A (2.5 µg/mL) for 3 hours. For phosflow, cells were hatched for 10 min with Phosflow Fix Buffer I and treated for 30 min at 4°C with Perm Buffer III. Isotype-matched mouse IgG controls were used to evaluate the background. Flow cytometric analysis was performed on a BD FACSVerse, and data were analysed using FlowJo software.

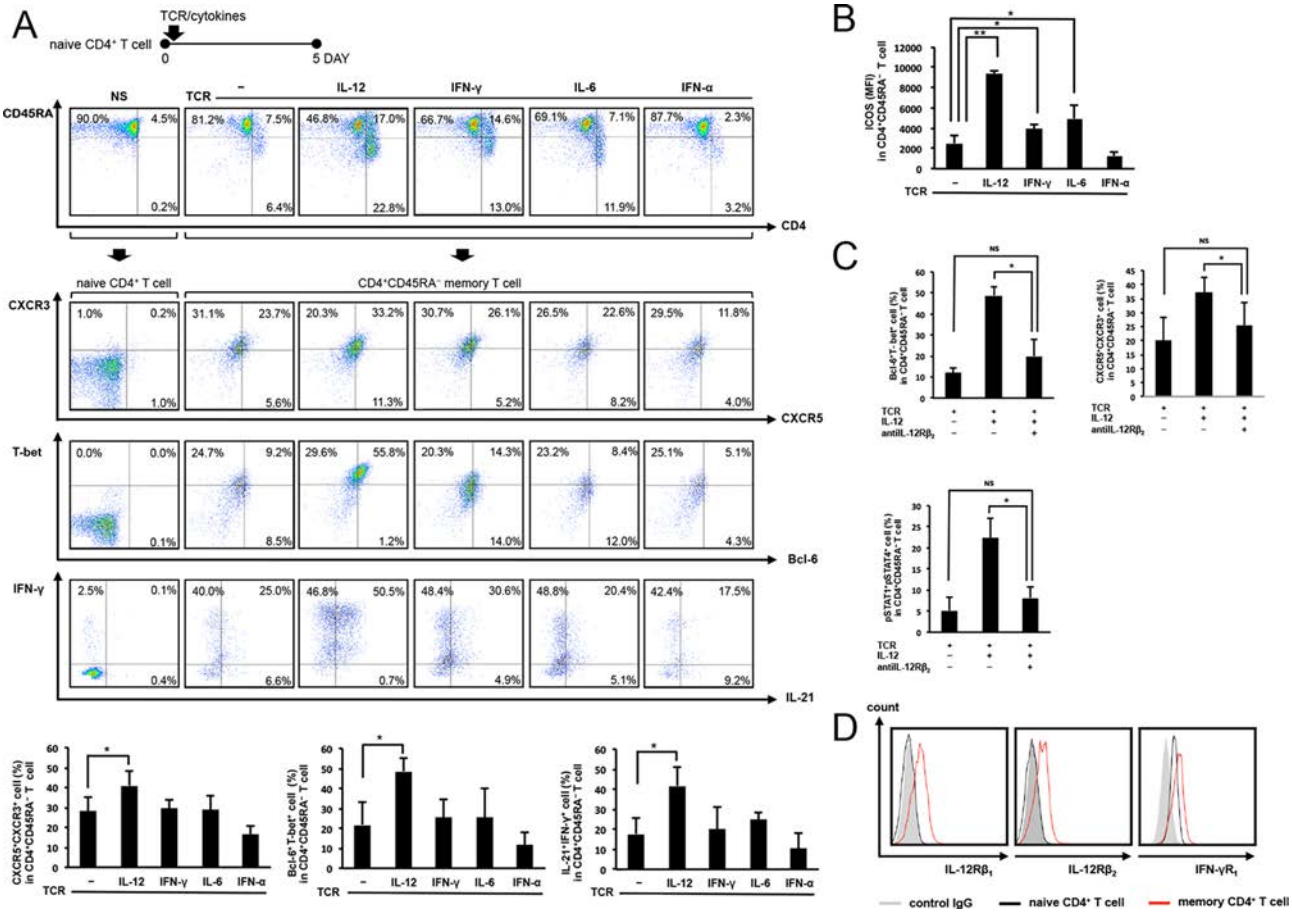


Figure 1 Interleukin (IL)-12 induced the differentiation of T follicular helper (Tfh)-Th1-like cells. Naive $CD4^+$ T cells of healthy donors were cultured for 5 days with T cell receptor (TCR) in the presence of the indicated cytokines or antibodies. (A) Representative flow cytometry plots showing expression of chemokine receptor (CXCR)5/CXCR3, Bcl-6/T-bet and IL-21/interferon (IFN)-γ in $CD4^+CD45^-$ memory T cells; percentages of $CXCR5^+CXCR3^+$ cells, $Bcl\text{-}6^+T\text{-}bet^+$ cells and $IL\text{-}21^+IFN\text{-}\gamma^+$ cells expressed in bar graphs. (B) Inducible co-stimulator (ICOS) levels expressed in mean fluorescence intensity (MFI). (C) Percentages of $Bcl\text{-}6^+T\text{-}bet^+$ cells, $CXCR5^+CXCR3^+$ cells and $pSTAT1^+pSTAT4^+$ cells in the presence or absence of anti-IL-12Rβ_2 antibody. (D) Expression of receptors of IL-12 and IFN-γ in naive $CD4^+$ T cells (black line) and in memory $CD4^+$ T cells (red line). Data are mean±SEM of three independent experiments using different donors. NS, not significant, *p<0.05, **p<0.01.

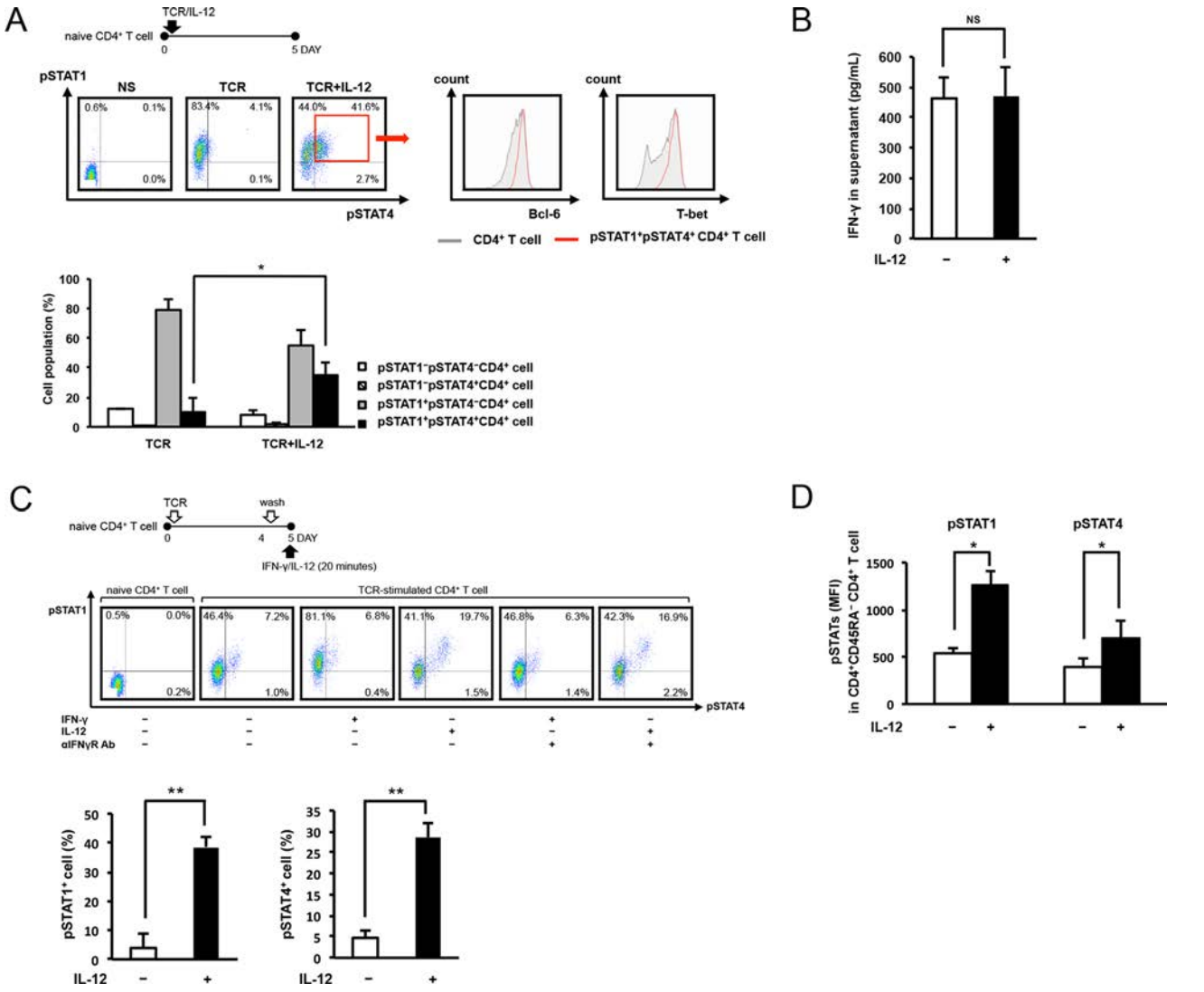


Figure 2 Interleukin (IL)-12 directly phosphorylated both signal transducer and activators of transcription (STAT)1 and STAT4 in CD4$^+$ T cells. (A) Naive CD4$^+$ T cells of healthy donors were cultured for 5 days with T cell receptor (TCR) in the presence or absence of IL-12. Representative flow cytometry plots showing phosphorylated STAT (pSTAT)1 and pSTAT4 in CD4$^+$ T cells; expression of Bcl-6 and T-bet in CD4$^+$ T cells expressed in grey line, in pSTAT1$^+$pSTAT4$^+$ cells expressed in red line; expansion of the different cell populations expressed in bar graphs. (B–D) Naive CD4$^+$ T cells of healthy donors were cultured for 4 days with TCR, then cells were washed three times with complete medium and recultured in new cytokine-free medium for 24 hours, then restimulated with or without IL-12 or interferon (IFN)-γ for 20 min. Pretreatment of anti-IFN-γR antibody was carried out 1 hour before restimulation. (B) IFN-γ level in the supernatant was analysed by Cytokine Bead Array. (C) Representative flow cytometry plots showing pSTAT1 and pSTAT4 in TCR-stimulated CD4$^+$ T cells; percentages of pSTAT1$^+$ cells and pSTAT4$^+$ cells expressed in bar graphs. (D) The levels of pSTAT1 and pSTAT4 in the presence or absence of IL-12 expressed in mean fluorescence intensity (MFI). Data are mean±SEM of three independent experiments using different donors. NS, not significant, *p<0.05, **p<0.01.

Quantitative real-time PCR

Total RNA was extracted from cells and purified using RNeasy Mini Kit (Qiagen), and cDNA was prepared using the high capacity RNA-to-cDNA kit (Applied BioSystems). Quantitative PCR was performed with sequence detection system with site-specific primers and probes. The comparative threshold cycle method and an internal control were used to normalise the expression of the target genes. The primers and probes used are shown in the online Supplementary table S5.

STAT knockdown

Memory CD4$^+$ T cells were incubated in Accell siRNA delivery media and 1 µM STAT1, STAT4 or non-targeting control siRNA Accell SMART pool from Dharmacon (ABgene) for 48 hours, then cells were stimulated with TCR for another 24 hours. Knockdown efficiency was assessed by quantitative real-time PCR.

Chromatin immunoprecipitation (ChIP)-PCR

Cells were cross-linked with formaldehyde and chromatin was fragmented to 200–300 bp by sonication after 20 min stimulation with or without IL-12. DNA was extracted from the cells and purified using the EZ ChIP Kit (Millipore). DNA was immunoprecipitated with antibodies; PCR was performed with designed primers (online Supplementary tables S3 and S5).

Statistical analysis

Comparison between the disease groups was performed with the Mann-Whitney's U test. In the in vitro experiments, data

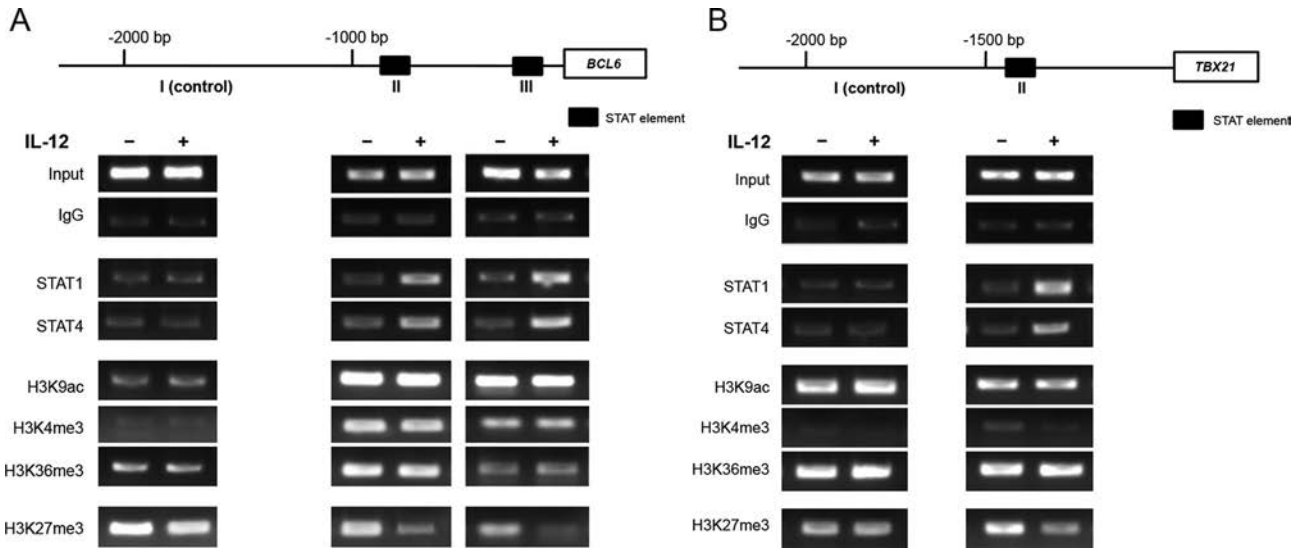


Figure 3 Direct effects of signal transducer and activator of transcription (STAT)1 and STAT4 on T follicular helper (Tfh)-Th1-like cell gene loci. Naive $CD4^+$ T cells of healthy donors were cultured for 4 days with T cell receptor (TCR), then cells were washed three times with complete medium and subsequently recultured in new cytokine-free medium for 24 hours, then restimulated with or without interleukin (IL)-12 for 20 min before proceeding with chromatin immunoprecipitation (ChIP). PCR was used to detect protein association at the indicated sequences. Immunoglobulin (Ig) G was used as the control in each experiment. Input: the chromatin solution used to perform the ChIP. PCR reactions from each input chromatin solution at representative chromosome loci are as follows: STAT1, STAT4, H3K9ac, H3K4me3, H3K36me3 and H3K27me3. (A) ChIP analysis of *BCL6* at the indicated loci (region I: negative control, regions II and III: promoter regions) in the presence or absence of IL-12. (B) ChIP analysis of *TBX21* at the indicated loci (region I: negative control, region II: promoter region) in the presence or absence of IL-12. Data are representative one from three independent experiments using different donors.

were expressed as mean±SEM of three independent experiments using different donors. Differences between groups were examined by the Student's t-test. A p value less than 0.05 was considered statistically significant. All statistical analyses were conducted using the IBM SPSS software V.22.0.

RESULTS

IL-12 promoted differentiation of Tfh-Th1-like cells

To determine the type of cytokine involved in the development of Tfh-Th1-like cells in human, we conducted a series of cell differentiation experiments. IL-12, but not other cytokines, such as IFN-γ, IL-6 and IFN-α, increased the proportions of $CXCR5^+CXCR3^+$, Bcl-6^+T-bet^+, IL-21^+IFN-γ^+ Tfh-Th1-like cells and expression of ICOS among $CD4^+CD45^-$ memory cells (figure 1A,B, online Supplementary figure S2). In addition, the treatment with anti-IL-12 receptor antibody inhibited the induction of Tfh-Th1 associated molecules by IL-12 (figure 1C).

We noted that TCR stimulation produced abundant amount of IFN-γ (online Supplementary figure S3) and promoted differentiation of Tfh-Th1-like cells, and it was IL-12 but not other cytokines had the capacity to enhance the production of IFN-γ on the basis of TCR. Moreover, neutralisation of IFN-γ signal by anti-IFN-γR antibody diminished IL-12-induced differentiation of Tfh-Th1-like cells at the early phase (online Supplementary figure S4). We then tested whether higher concentration of exogenous IFN-γ had an impact on the differentiation of Tfh-Th1-like cells in the presence of IL-12. Since stimulation with IFN-γ (10 ng/mL) induced marginal upregulation of Tfh-Th1-like cells, we investigated the dose-dependent effect of IFN-γ. The result proved that even high concentrations of IFN-γ did not influence the proportion of differentiated Tfh-Th1-like cells (data not shown).

However, the effect of IFN-γ was observed when the experiments of IL-12 receptor expression were carried out. Although IL-12 receptorβ_1 (IL-12Rβ_1) and IL-12Rβ_2 were not expressed on naive $CD4^+$ T cells, they were robustly induced after priming with IFN-γ derived from TCR stimulation (figure 1D, online Supplementary figure S5), indicating that IFN-γ is required for induction of IL-12 receptors on naive $CD4^+$ T cells.

Next, we evaluated the expression levels of the markers of Th17, Treg and Th2 cells. Compared with the upregulation of *IFNG* and *IL21*, the expression levels of *IL17A* and *FOXP3* induced by IL-12 were unstable and insignificant (online Supplementary figure S6). IL-17 and IL-4 were not detected in the cell culture supernatants (online Supplementary figure S3). In addition, IL-12 did not promote expression of CCR4 or CCR6 (online Supplementary figure S7).

We also detected differentiation of Tfh-Th1-like cells in memory $CD4^+$ T cells subsets. The results showed that IL-12 could upregulate Tfh-Th1-like cells in total memory $CD4^+$ T cells, as well as quiescent subsets of PD-$1^-$$ICOS^-$ and PD-$1^+$$ICOS^-$ (online Supplementary figures S8 and S9).

Above all, our findings suggest that IL-12 is the most important cytokine for the differentiation of Tfh-Th1-like cells.

IL-12 phosphorylated both STAT1 and STAT4

Next, we investigated how IL-12 promotes the differentiation of Tfh-Th1 like cells. The STAT family mediates cytokine-induced gene expression. Our study confirmed that TCR stimulation induced production of IFN-γ and activated phosphorylation of STAT1 (pSTAT1); stimulation with TCR and IL-12 induced pSTAT1 and pSTAT4. Furthermore, Bcl-6 and T-bet highly expressed in pSTAT1^+pSTAT4^+ cells (figure 2A). IL-12R blockade suppressed IL-12-induced pSTAT1^+pSTAT4^+ cells (figure 1C).

To elucidate the direct effect of IL-12 on STAT phosphorylation, cells were washed to remove TCR and endogenous IFN-γ, thereafter stimulated with IL-12 for 20 min. Transitory

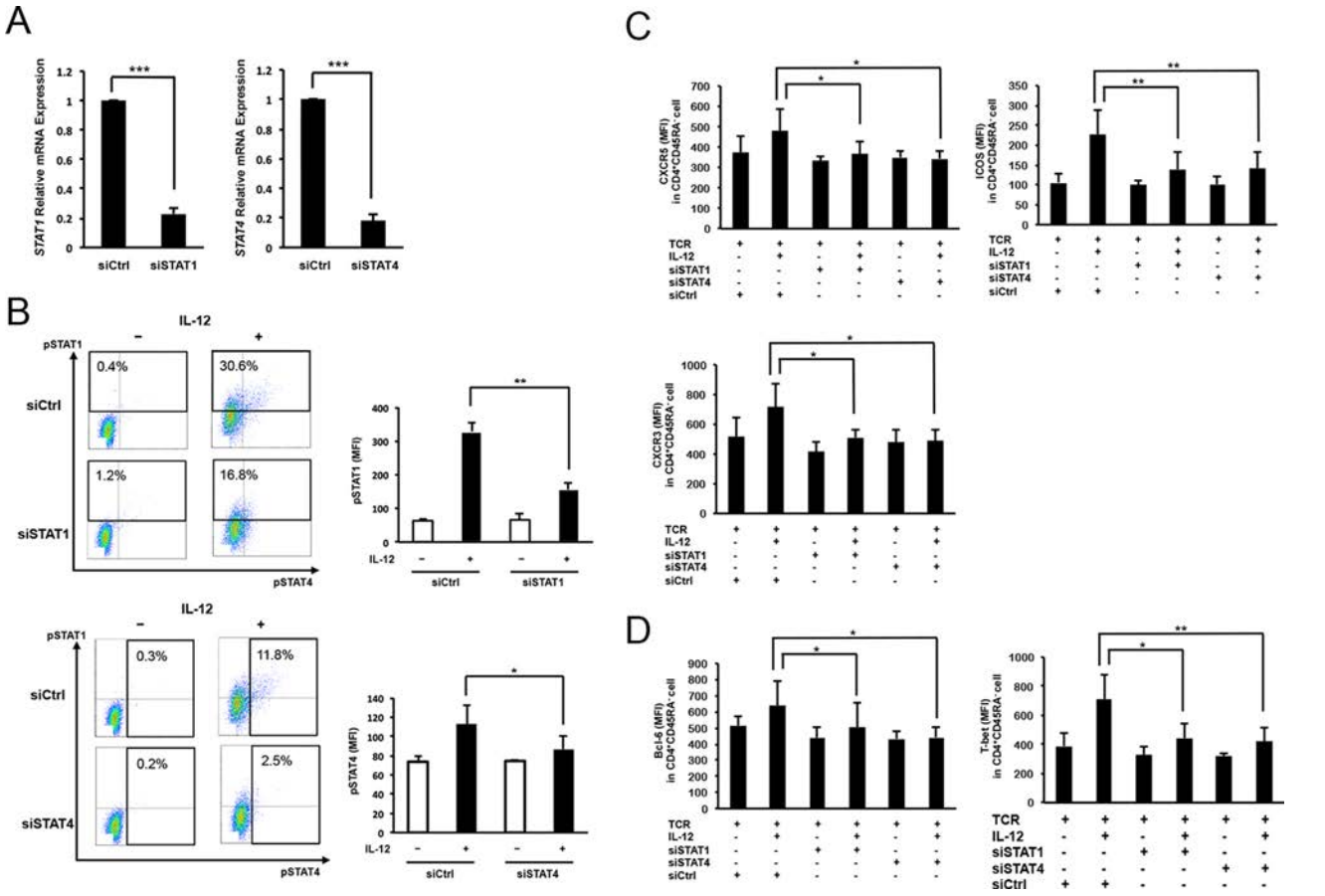


Figure 4 Effects of signal transducer and activator of transcription (STAT)1 and STAT4 knockdown on interleukin (IL)-12-induced T follicular helper (Tfh)-Th1-like cell differentiation. (A) Relative messenger RNA expression of *STAT1* and *STAT4* in transfected CD4$^+$ T cells was evaluated by quantitative PCR. (B–D) Transfected memory CD4$^+$ T cells of healthy donors were cultured for further 24 hours with T cell receptor (TCR) in the presence or absence of IL-12. (B) Levels of phosphorylated STAT (pSTAT)1 and pSTAT4 expressed in representative flow cytometry plots and mean fluorescence intensity (MFI). (C) Levels of chemokine receptor (CXCR)5, inducible co-stimulator (ICOS) and CXCR3 expressed in MFI. (D) Levels of Bcl-6 and T-bet expressed in MFI. Data are mean±SEM of three independent experiments using different donors; *p<0.05, **p<0.01, ***p<0.001.

stimulation with IL-12 had no effect on IFN-γ secretion level (figure 2B). We found that IL-12 alone promoted not only phosphorylation of both STAT4 but also that of STAT1 (figure 2C,D, online Supplementary figure S10). The addition of anti-IFN-γR antibody failed to inhibit IL-12-induced phosphorylation of both STAT1 and STAT4 (figure 2C). In contrast, *IRF4* was an important transcription factor in signalling pathways of Tfh cells; however, it was not increased by IL-12 (online Supplementary figure S11).

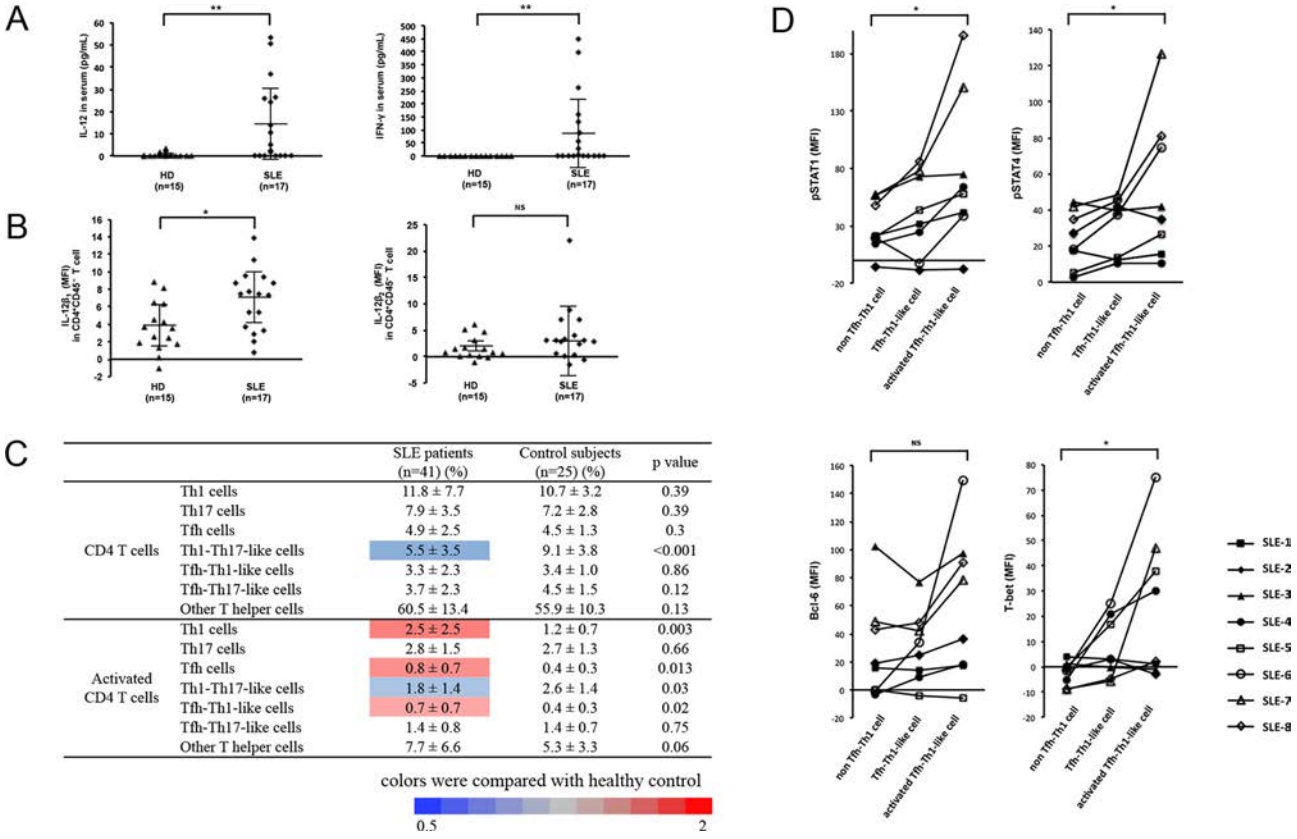
Collectively, the above results indicate that IL-12 directly phosphorylated both STAT1 and STAT4, resulting in the induction of Tfh-Th1-like cells.

STAT1 and STAT4 directly bind to *BCL6* and *TBX21* gene loci and alter histone modification

Next, ChIP-PCR was performed to investigate whether IL-12-induced pSTAT1 and pSTAT4 directly regulate *BCL6* and *TBX21* promoter regions. Both STAT1 and STAT4 directly bound to *BCL6* and *TBX21* loci around STAT binding sites following stimulation with IL-12 (figure 3A,B). We also evaluated the effect of IL-12 on epigenetic modification of *BCL6* and *TBX21* loci. Both *BCL6* and *TBX21* loci in TCR-stimulated CD4$^+$ T cells exhibited bivalent histone modifications, such as permissive marks H3K4me3, H3K9Ac and H3K36me3 and repressive mark H3K27me3. After IL-12 stimulation, no changes were observed in permissive marks H3K4me3, H3K9Ac and H3K36me3 on *BCL6* and *TBX21* loci. Conversely, repressive mark H3K27me3 on *BCL6* and *TBX21* loci was strongly suppressed after IL-12 stimulation (figure 3A,B, online Supplementary figure S12). These results suggest that IL-12 promotes the differentiation of Tfh-Th1-like cells by binding STAT1 and STAT4 to *BCL6* and *TBX21* gene, with simultaneous suppression of H3K27me3.

Silencing of *STAT1* or *STAT4* represses IL-12-mediated differentiation of Tfh-Th1-like cells

We evaluated whether combined effect of STAT1 and STAT4 was indispensable in the differentiation of Tfh-Th1-like cells by knocking down *STAT1* and *STAT4*, respectively. We selected memory CD4$^+$ T cells which already expressed IL-12 receptors and could receive IL-12 signal to promote phosphorylation of STAT1 and STAT4 (figure 2D). mRNA expression of *STAT1* and *STAT4* were effectively silenced by siRNA, respectively (figure 4A). Moreover, IL-12-mediated pSTAT1 and pSTAT4 was also suppressed by siRNA (figure 4B). Ether knocking down of *STAT1* or *STAT4* abolished the expression of ICOS, Bcl-6 and CXCR5. Besides, CXCR3 and T-bet were also clearly suppressed by knockdown of either *STAT1* or *STAT4* in CD4$^+$ T cells (figure 4C, D). These results indicated that IL-12 and its subsequent signals of coexisting pSTAT1 and pSTAT4 were essential for the generation of Tfh-Th1 like cells.



		SLE patients (n=41) (%)	Control subjects (n=25) (%)	p value
CD4 T cells	Th1 cells	11.8 ± 7.7	10.7 ± 3.2	0.39
	Th17 cells	7.9 ± 3.5	7.2 ± 2.8	0.39
	Tfh cells	4.9 ± 2.5	4.5 ± 1.3	0.3
	Th1-Th17-like cells	5.5 ± 3.5	9.1 ± 3.8	<0.001
	Tfh-Th1-like cells	3.3 ± 2.3	3.4 ± 1.0	0.86
	Tfh-Th17-like cells	3.7 ± 2.3	4.5 ± 1.5	0.12
	Other T helper cells	60.5 ± 13.4	55.9 ± 10.3	0.13
Activated CD4 T cells	Th1 cells	2.5 ± 2.5	1.2 ± 0.7	0.003
	Th17 cells	2.8 ± 1.5	2.7 ± 1.3	0.66
	Tfh cells	0.8 ± 0.7	0.4 ± 0.3	0.013
	Th1-Th17-like cells	1.8 ± 1.4	2.6 ± 1.4	0.03
	Tfh-Th1-like cells	0.7 ± 0.7	0.4 ± 0.3	0.02
	Tfh-Th17-like cells	1.4 ± 0.8	1.4 ± 0.7	0.75
	Other T helper cells	7.7 ± 6.6	5.3 ± 3.3	0.06

Figure 5 Expansion of T follicular helper (Tfh)-Th1-like cells and high levels of pSTAT1 and pSTAT4 in activated Tfh-Th1 cells in patients with systemic lupus erythematosus (SLE). (A–C) Serum and peripheral blood mononuclear cells (PBMCs) were, respectively, obtained from patients with SLE and healthy donors. Results shown in the scatter plots. (A) Interleukin (IL)-12 and interferon (IFN)-γ levels in serum were analysed by Cytokine Bead Array. (B and C) PBMCs were isolated and analysed by flow cytometry without incubation. (D) Whole blood of patients with SLE was collected, stained directly and analysed by flow cytometry. Levels of pSTAT1, pSTAT4, T-bet and Bcl-6 in different subsets of cells from different patients expressed in scatter plots. Data are mean±SD. NS, not significant, *p<0.05, **p<0.01.

Expansion of circulating activated Tfh-Th1-like cells in SLE

Finally, we investigated how IL-12/STAT1/STAT4/Tfh-Th1-like cell axis contributed to the pathogenesis in SLE. The serum IL-12 and IFN-γ and the expression of IL-12 receptors on $CD4^+$ T cells were increased in patients with lupus compared with healthy subjects (figure 5A,B). In response to these increased levels, activated Th1 cells and activated Tfh cells among helper T cell subsets were increased in patients with SLE (figure 5C). In addition, there was a significant increase in the proportion of circulating activated Tfh-Th1-like cells in SLE (figure 5C). In contrast, the proportions of Th1-Th17-like cells and activated Th1-Th17-like cells were lower in patients with SLE relative to the control. Finally, to assess the association between pSTAT1/4 and Bcl-6/T-bet in peripheral blood of SLE subjects, we measured the basal level of pSTAT1/4, Bcl-6 and T-bet expression in Tfh-Th1-like cells, In patients with SLE, pSTAT1, pSTAT4 and T-bet were higher in activated Tfh-Th1-like cells than in non-Tfh-Th1 cells, though there was no difference in expression of Bcl-6 (figure 5D). These results suggest that IL-12-mediated phosphorylation of STAT1 and STAT4 promotes the expansion of Tfh-Th1-like cells in patients with SLE.

DISCUSSION

Tfh-Th1-like cells possess characteristics and functions of both Tfh and Th1 cells, co-express Bcl-6, T-bet, CXCR5 and CXCR3 and produce IL-21 and IFN-γ. Although many previous studies have confirmed that Th1 or Tfh cells play an important role in SLE,[9 10 29] no reports have ever paid close attention to crucial role of Tfh-Th1-like cells in SLE. In our study, we believe IL-12-induced bi-characteristics of Tfh and Th1 cells could not be observed separately. Due to the co-activation of STAT1 and STAT4 mediated by IL-12, circulating activated Tfh-Th1-like cells significantly expanded in patients with SLE.

High serum IL-12 level is commonly seen in patients with SLE and treatment with glucocorticoid is known to result in reduction of these levels.[30–32] Our study added support to those findings by demonstrating that IL-12 is indispensable for differentiation of Tfh-Th1-like cells in SLE. However, IL-12 alone did not mediate the differentiation of Tfh-Th1-like cells from naive cells in the early phase, since naive $CD4^+$ T cells do not express IL-12 receptors. We confirmed that endogenous IFN-γ produced by TCR stimulation was required for the expression of IL-12 receptors on naive $CD4^+$ T cells, as reported in murine.[33] These results provide explanation for why neutralisation of IFN-γ or IFN-γR diminishes IL-12-induced expression of Bcl-6 and T-bet at the early phase but not the late phase.[24]

There is a general agreement that IL-12/STAT4 signal plays an important role in the development of Th1 cells,[22 34 35] Tfh cells[18 20] and Tfh-Th1-like cells.[21 24] Although IL-12 was reported to activate STAT1 and STAT5,[36] the importance towards differentiation of T cells in human is still unclear. In the inflammatory process, interferons and IL-6 all involve phosphorylation of STAT1,[15 16 37] but direct action from IL-12 is overlooked. Our results show that IL-12 also induced phosphorylation of STAT1 and that coexisting of pSTAT1 and pSTAT4 is requisite for the human Tfh cell differentiation.

We consider that IL-12-induced pSTAT1$^+$pSTAT4$^+$ cells are critical for the differentiation of Tfh-Th1-like cells based on the following evidence. First, high expression of Bcl-6 and T-bet was detected only in pSTAT1$^+$pSTAT4$^+$ cells. Second, IL-12 induced pSTAT1 and pSTAT4 even in the absence of IFN-γ; neutralisation of IFN-γR on cells had no impact IL-12-mediated pSTAT1. Third, our ChIP data indicated that both STAT1 and STAT4 directly bound to *BCL6* and *TBX21* gene loci after IL-12 stimulation. Fourth, either *STAT1*-silenced or *STAT4*-silenced memory CD4$^+$ T cells displayed non-responsiveness to IL-12 stimulation and failed to differentiate into Tfh-Th1-like cells. Finally, evidence from peripheral blood of patients with SLE showed basal level of pSTAT1, pSTAT4 and T-bet upregulated in activated Tfh-Th1-like cells, which indicated the correlative association between pSTATs and master transcription factors for Tfh-Th1-like cells. Although we could not detect upregulation of Bcl-6 in unstimulated T cells, this may not be surprising because expression of Bcl-6 in circulating peripheral Tfh cells could be rapidly decreased outside lymphoid tissues, as previously reported.[38] Thus, co-activation of STAT1 and STAT4 by IL-12 is required for the differentiation of Tfh-Th1-like cells.

The term epigenetic modifications refer to changes in gene expression that are not caused by modification in DNA sequence. Among the various epigenetic regulatory mechanisms, histone methylation is reported to influence the differential expression of key genes.[39] Breaking the balance by suppressing either permissive or repressive modification can change bivalent to open or close chromatin state, followed by changes in gene transcription.[40] Recent studies have demonstrated that STATs promote the deposition of permissive or repressive epigenetic marks at loci that exhibit both STAT binding and STAT-dependent transcriptional regulation.[41] Our results showed that the loci of *BCL6* and *TBX21* at STAT binding sites in TCR-stimulated CD4$^+$ T cells were marked by bivalent histone modifications. Following IL-12 stimulation, both STAT1 and STAT4 directly bound on *BCL6* and *TBX21* gene loci accompanied by suppression of repressive histone mark H3K27me3. We consider that permissive modifications on *BCL6* locus are already initiated by TCR and maintained after IL-12 stimulation; since previous studies indicated that TCR stimulation induced early activation of T helper cell gene expression, including *BCL6*.[23 42] However, our data showed that H3K4me3 modification on *TBX21* locus was always at low level regardless of the presence or absence of IL-12, a finding different from previous studies.[43] Thus, we consider that IL-12 simulation removed H3K27me3 modification at *BCL6* and *TBX21* regions, leading to enhanced Tfh-Th1-like cell gene expression in CD4$^+$ T cells.

The mechanisms we found respond the expansion of activated Tfh-Th1-like cells in patients with SLE, especially in active SLE and serum IL-12^{high} SLE (online Supplementary figures S13 and S14). By contrast, different from pervious view,[44 45] Th1-Th17-like cells decreased in patients with SLE in our research. Although the plasticity and diversity of T helper subset are still open issue, Tfh cells appear to be more plastic. We consider the balance of Tfh and Th17 subphenotype of Th1 cell could be tipped towards Tfh polarity, due to the high level of IL-12 in SLE.

Taken together, our study demonstrated that IL-12 directly activated both STAT1 and STAT4 and regulated *BCL6* and *TBX21* gene by alteration of histone modifications, resulting in differentiation of Tfh-Th1-like cells. This could be one of the underlying mechanisms responsible for the expansion of circulating activated Tfh-Th1-like cells in patients with SLE. The findings of epigenetic modifications that result in the induction of pathogenic Tfh cells could be potentially helpful towards the development of cell specific and effective treatment for SLE.

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Contributors XM and SN designed the study. XM conducted the experiments, analysed the data and wrote the manuscript. SK, KS, KY, YM, MY, YK and MZ helped to conduct the experiments and YT created the research concept and supervised the research and writing of the manuscript.

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Competing interests YT has received consulting fees, speaking fees and/or honoraria from Daiichi-Sankyo, Astellas, Pfizer, Mitsubishi-Tanabe, Bristol-Myers, Chugai, YL Biologics, Eli Lilly, Sanofi, Janssen, UCB and has received research grants from Mitsubishi-Tanabe, Takeda, Bristol-Myers, Chugai, Astellas, Abbvie, MSD, Daiichi-Sankyo, Pfizer, Kyowa-Kirin, Eisai, Ono. SN has received speaking fees from Bristol-Myers, UCB, Astellas, Abbvie, Eisai, Pfizer, Takeda and has received research grants from Mitsubishi-Tanabe, Novartis and MSD. All other authors declare no conflict of interest. None of the material presented in our manuscript has been previously submitted or published.

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Ethics approval The study was approved by Institutional Review Board of the University of Occupational and Environmental Health. Informed consent was obtained from each subject.

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EXTENDED REPORT

Therapeutic interleukin-6 blockade reverses transforming growth factor-beta pathway activation in dermal fibroblasts: insights from the faSScinate clinical trial in systemic sclerosis

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ABSTRACT

Objectives Skin fibrosis mediated by activated dermal fibroblasts is a hallmark of systemic sclerosis (SSc), especially in the subset of patients with diffuse disease. Transforming growth factor-beta (TGFβ) and interleukin-6 (IL-6) are key candidate mediators in SSc. Our aim was to elucidate the specific effect of IL-6 pathway blockade on the biology of SSc fibroblasts in vivo by using samples from a unique clinical experiment—the faSScinate study—in which patients with SSc were treated for 24 weeks with tocilizumab (TCZ), an IL-6 receptor-α inhibitor.

Methods We analysed the molecular, functional and genomic characteristics of explant fibroblasts cultured from matched skin biopsy samples collected at baseline and at week 24 from 12 patients receiving placebo (n=6) or TCZ (n=6) and compared these with matched healthy control fibroblast strains.

Results The hallmark functional and molecular-activated phenotype was defined in SSc samples and was stable over 24 weeks in placebo-treated cases. RNA sequencing analysis robustly defined key dysregulated pathways likely to drive SSc fibroblast activation in vivo. Treatment with TCZ for 24 weeks profoundly altered the biological characteristics of explant dermal fibroblasts by normalising functional properties and reversing gene expression profiles dominated by TGFβ-regulated genes and molecular pathways.

Conclusions We demonstrated the exceptional value of using explant dermal fibroblast cultures from a well-designed trial in SSc to provide a molecular framework linking IL-6 to key profibrotic pathways. The profound impact of IL-6R blockade on the activated fibroblast phenotype highlights the potential of IL-6 as a therapeutic target in SSc and other fibrotic diseases.

Trial registration number NCT01532869; Post-results.

INTRODUCTION

Systemic sclerosis (SSc) is characterised by fibrosis of the skin and internal organs, autoimmunity and vasculopathy.[1] Although the biological mechanisms underlying SSc are not fully understood, interplay between vasculopathy, autoimmunity and fibrosis may perpetuate a state of aberrant wound repair.[2] Disease modification remains elusive, and treatment focuses on complications such as interstitial lung disease, pulmonary artery hypertension and peripheral vasculopathy.[3] Clinical heterogeneity is a hallmark of SSc, and patients with severe progressive diffuse skin involvement have poor outcomes; more effective treatment for this subset of patients is an important unmet medical need.[4]

Fibroblasts play a central role in the biology of the disease[5] by interacting with endothelial cells[6] and leucocytes[7] in a complex biological network involving cytokines and adhesion molecules,[8] resulting in excess deposition of extracellular matrix proteins and in tissue stiffening. Transforming growth factor-beta (TGFβ) plays a central role in fibrosis[9] by promoting the differentiation of fibroblasts into myofibroblasts and by inducing profibrotic molecules and proliferation.[10] Interleukin-6 (IL-6) also plays an important role in SSc[11] by regulating the function of immune and non-immune cells. Dermal fibroblasts from patients with SSc express increased levels of IL-6[12]; elevated levels of IL-6 are associated with early disease,[13] and increased serum IL-6 level predicts higher mortality risk, worse skin involvement and increased pulmonary decline.[14 15] IL-6 is known to induce TGFβ production by cardiac fibroblasts[16] and to enhance TGFβ-signalling in dermal fibroblasts[17] and cardiac fibroblasts.[18] Conversely, TGFβ regulates the expression of IL-6 by lung fibroblasts[19] and airway smooth muscle cells.[20]

To elucidate the specific effect of IL-6 on the biology of fibroblasts in vivo, we took advantage of a unique clinical experiment—the faSScinate study—in which SSc patients were treated for 24 weeks with tocilizumab (TCZ), an IL-6 receptor-α blocking antibody. The faSScinate study was a randomised, double-blind, placebo (PBO)-controlled phase 2 study of TCZ in adult SSc patients with <5 years' disease duration, a modified Rodnan skin score (mRSS) between 15 and 40 units and active progressive disease according to specified clinical or laboratory features. No concomitant immunosuppressive medication was permitted at study entry or during the first 24 weeks of the trial.[21] In this study, 43 patients with SSc received weekly TCZ 162 mg subcutaneously and 44 patients with SSc received weekly PBO subcutaneously. As previously reported, primary clinical data

from the faSScinate trial showed a trend of benefit in favour of TCZ for the primary endpoint or mRSS and a strong trend at 48 weeks together with congruent benefit in exploratory endpoints including lung function. Based on these results, a phase 3 trial of TCZ is under way (ClinicalTrials.gov, NCT02453256).

Here we define the molecular and functional phenotype of explant dermal fibroblasts from a representative subset of patients with SSc enrolled into faSScinate and demonstrate a profound reversal of the hallmark profibrotic properties of these cells after treatment with TCZ for 24 weeks compared with controls from the PBO arm of the trial.

METHODS

Sample collection and analysis

Culture of dermal explant fibroblasts from patients with SSc enrolled in the faSScinate study (ClinicalTrials.gov, NCT01532869) and matched controls was performed as described, as was protein quantification using Western blot analysis.[15] Cell migration was assessed using scratch wound assay,[22] and contractility assays were performed on 3D collagen gel lattices.[23] Gene expression patterns were analysed using Ingenuity Pathway Analysis (IPA; Qiagen). Additional methodology is described in online supplementary appendix S1.

Statistical analysis

For functional assays, means were compared using Tukey's multiple comparison test. Correlations were calculated using the Spearman ρ. The Benjamini and Hochberg False Discovery Rate method was used to correct for multiple comparisons. All statistical calculations were conducted in JMP V.11.1 (SAS Institute).

RESULTS

SSc explant dermal fibroblasts present hallmark functional properties of activated fibrotic cells

Dermal fibroblasts were cultured from fresh skin biopsy samples collected at baseline and at week 24 from consecutive PBO-treated and TCZ-treated patients enrolled in the faSScinate study (online supplementary table S1).[21] Confirming earlier reports,[22] dermal fibroblasts isolated from patients with SSc at baseline produced significantly higher levels of connective tissue growth factor (CTGF), collagen alpha 1 (Col1) and alpha-smooth muscle actin (αSMA) than normal fibroblasts (NFs) (NF vs SSc; $p<0.0001$ for all three factors) (figure 1A–C). Migratory capacity and contractility activity were also enhanced compared with NF (NF vs PBO baseline and NF vs TCZ baseline, $p<0.0001$ for both) (figure 1D,E). These results indicate that the SSc explant dermal fibroblasts obtained in this study have the hallmark functional characteristics of activated fibrotic fibroblasts.

SSc dermal fibroblasts collected at baseline: typical profibrotic signature dominated by genes regulated by TGFβ

Using RNA sequencing (RNA-Seq), we compared the expression profiles of SSc fibroblasts collected at baseline (SSc) with those of NF. Figure 2 depicts the expression values of NF and SSc baseline fibroblasts for 578 protein-coding genes significantly differentially expressed (criteria: fold difference ≥1.5; median reads per kilobase of transcript per million mapped reads ≥1, adjusted $p\leq0.05$). Of the 578 selected genes, 145 are known to be regulated by TGFβ in human dermal or lung fibroblasts,[20 24–26] indicating that this pathway is dominant in these cells (figure 2). Of note, baseline erythrocyte sedimentation rate (ESR) and C reactive protein and IL-6 levels were not associated with distinct baseline gene expression profiles (online supplementary table S2).

TCZ treatment for 24 weeks attenuates the functional properties of SSc explant fibroblasts

Comparison of functional measures between fibroblasts collected at baseline and week 24 from patients treated with TCZ showed a significant decrease in protein production, in migration (increase in gap size) and in contractility (increase in gel weight) (figure 3), indicating that therapeutic treatment with TCZ results in a significant attenuation of the hallmark functional properties of SSc fibroblasts. In contrast, the phenotype of SSc fibroblasts was stable in PBO-treated patients.

Profibrotic expression profile of SSc dermal fibroblasts: normalised in TCZ-treated patients and stable in PBO-treated patients

Figure 4 depicts expression levels for the PBO and TCZ groups at week 24 compared with the matching sample at baseline. In the PBO-treated group (figure 4), no significant difference was detected (see also online supplementary figure S1), indicating that baseline expression profile is sustained over ≥24 weeks in the absence of immunosuppressants. In contrast, in the TCZ group (figure 4), we identified 2136 significantly differentially expressed genes; of those, 411 were also differentially expressed in the SSc/NF comparison. Remarkably, the expression pattern of the 411 shared genes in the TCZ group was opposite to that observed in the SSc versus NF comparison (figure 4). Of note, 167 and 1725 genes were significantly differentially expressed in SSc/NF and TCZ week 24/baseline comparisons, respectively. For most genes, differential expression trends were similar to those observed in the 411 shared genes (online supplementary figure S2). Thus, gene expression data confirm that therapeutic treatment with TCZ for 24 weeks profoundly alters the SSc-associated molecular phenotype of explanted dermal fibroblasts. Considering this striking biological effect of TCZ, we explored the correlation between gene expression and mRSS across all patients and time points. Of the 411 genes analysed, 35 had a nominal correlation $p\leq0.05$ (online supplementary table S3), and only one, PRKCE, remained significant after correction for multiple testing. Treatment with TCZ resulted in a significant upregulation of PRKCE expression that was underexpressed in SSc dermal fibroblasts at baseline, as previously reported in SSc lung fibroblasts.[27]

Treatment with TCZ for 24 weeks: reversal of activation status of key pathological pathways associated with SSc

We used the IPA (Qiagen) to assign biological functions to networks of all significantly differentially expressed genes (2303 genes) from the SSc versus NF and the TCZ week 24 versus baseline comparisons (online supplementary figure S1). Of the 609 significantly enriched pathways, we selected the top 50 pathways with the highest combined absolute Z scores (figure 5) and grouped them into 10 major biological nodes; annotations for the selected pathways are provided in (online supplementary table S4). Most pathways defined by genes that were only differentially expressed in one comparison overlapped with the 50 pathways.

The fibrosis node is dominated by TGFβ-related pathways but also includes a pathway related to the activity of lysophosphatidic acid and to the antifibrotic transcription factor KLF2. The contraction node regroups 11 pathways related to cellular contractility machinery. The migration node includes four pathways positively

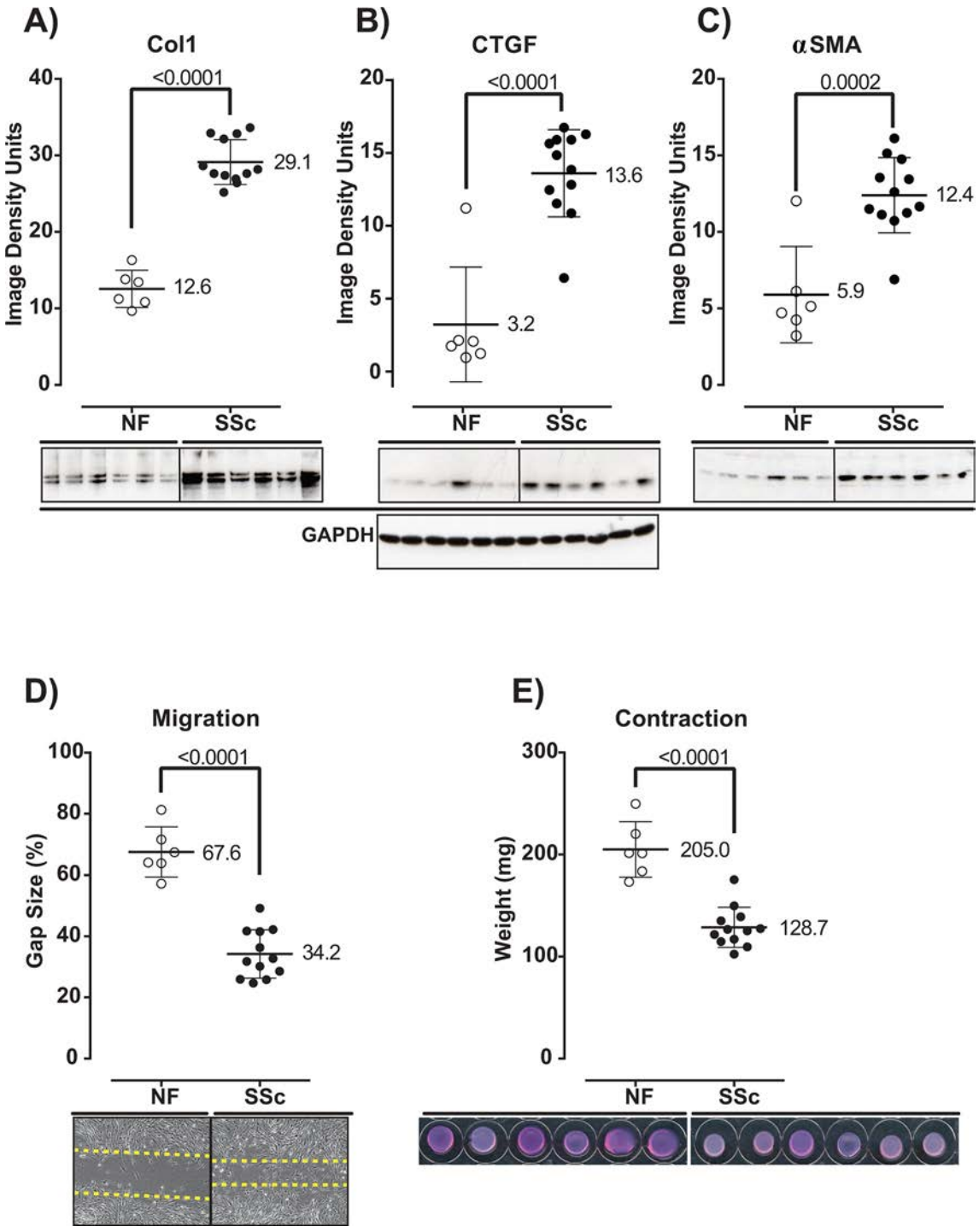

Figure 1 Explant dermal fibroblasts present hallmark functional properties of activated fibrotic fibroblasts. Production of Col1 (A), CTGF (B) and αSMA (C); migration activity (D); and contractility (E) by normal and SSc dermal fibroblasts collected at baseline were analysed by western blot densitometry of cell lysates (A–C), scratch assay: percentage of remaining gap size (D) and 3D gel contraction assay: weight of the lattice plug (E), respectively. Representative western blot images are presented for each protein analysed (A–C, lower panel); western blot of the reference protein glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is shown for the corresponding samples. Representative images of scratch assay performed over an incubation period of 48 hours are shown (D, lower panel). Representative images of gel contraction assay performed over an incubation period of 24 hours are shown (E, lower panel). BL, baseline; Col1, collagen alpha 1; CTGF, connective tissue growth factor; NFs, normal fibroblasts; αSMA, alpha-smooth muscle actin; SSc, systemic sclerosis.

related to cell movement. The growth node contains three pathways positively related to proliferation and cell survival. The five pathways grouped under the cardiovascular node are generally associated with endothelial and vascular smooth muscle cell biology but are also known to be positively related to fibrosis. Although the main function of the two pathways grouped under the coagulation node is blood clotting, these pathways play an important role in linking vascular events to fibrotic events in SSc. The activation node regroups eight pathways representing major signalling molecules and transcription factors positively

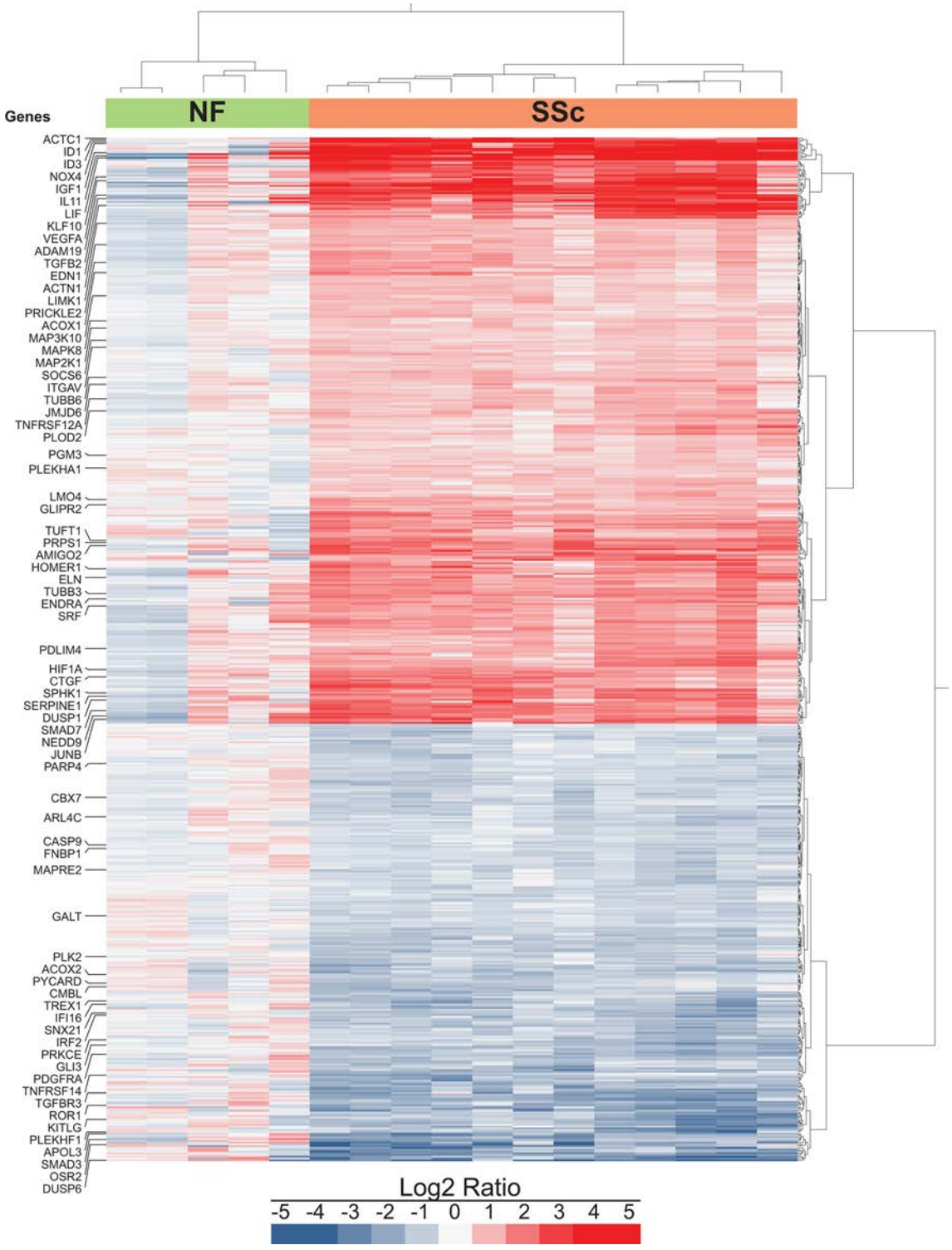

Figure 2 Genes differentially expressed by SSc dermal fibroblasts collected at baseline compared with normal dermal fibroblasts represent a typical profibrotic signature dominated by genes regulated by TGFβ. Gene expression of normal, SSc PBO and SSc TCZ dermal fibroblasts collected at baseline and normalised to the average NF expression. Protein coding RNA showing a median RPKM ≥1, 1.5-fold overexpressed or underexpressed compared with NF, with an adjusted p≤0.05, was selected as significantly differentially expressed (n=578). Genes and samples were clustered using the Ward method. BL, baseline; NFs, normal fibroblasts; PBO, placebo; RPKM, reads per kilobase of transcript per million mapped reads; SSc, systemic sclerosis; TCZ, tocilizumab; TGFβ, transforming growth factor beta.

associated with cellular activation. The two pathways grouped in the hormones node are thought to contribute to the pathobiology of SSc, but the exact mechanisms by which this occurs remain ill defined. The inflammation node includes two pathways related to the innate immune system. Three of the four pathways included in the morphogenesis node belong to the bone morphogenetic protein family, and the fourth pathway in this node is related to neuregulin biology. Overall, for the baseline SSc explant dermal fibroblasts (SSc vs NF comparison), the functions and activation status of the top 50 selected pathways depict a highly activated

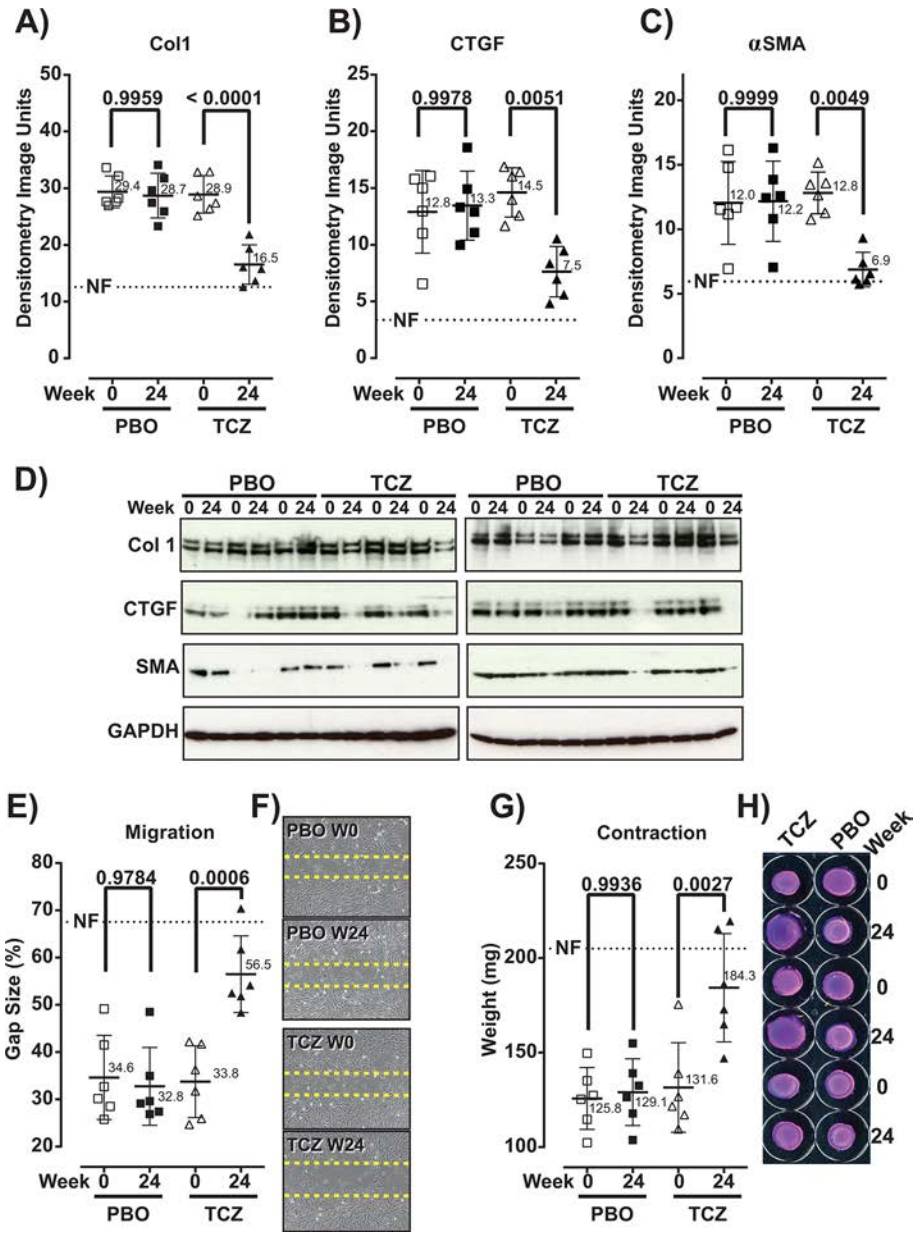

Figure 3 Hallmark functional properties of SSc explant dermal fibroblasts are attenuated after treatment of patients with TCZ for 24 weeks. Comparison of functional properties of explant dermal fibroblasts from PBO-treated and TCZ-treated patients collected at baseline (week 0) and at week 24. Production of Col1 (A), CTGF (B) and αSMA (C); migration activity (E); and contractility (G) were analysed by western blot densitometry of cell lysates (A–C), scratch assay: percentage of remaining gap size (E) and 3D gel contraction assay: weight of the lattice plug (G), respectively. Representative western blot images are presented for each protein analysed (D, lower panel). Western blot of the reference protein GAPDH is shown for the corresponding samples. Representative images of scratch assay performed over an incubation period of 48 hours are shown (F). Representative images of gel contraction assay performed over an incubation period of 24 hours are shown (H). For each graph, the average measure of NFs is depicted as a dotted line. BL, baseline; Col1, collagen alpha 1; CTGF, connective tissue growth factor; NFs, normal fibroblasts; PBO, placebo; αSMA, alpha-smooth muscle actin; SSc, systemic sclerosis; TCZ, tocilizumab.

fibrotic, motile and contractile phenotype dominated by TGFβ superfamily member-regulated biology, where positive regulators (n=47) are activated and negative regulators (n=3) are predicted to be inhibited. In contrast, for the dermal fibroblasts collected from patients treated with TCZ for 24 weeks (TCZ week 24 vs baseline comparison), the same 50 pathways are predicted to have the opposite activation status. Notably, the level of inhibition observed with TCZ generally matches the levels of activation observed at baseline. Thus, in keeping with the functional assay and the gene expression data, the pathway analysis results indicate that the 24-week treatment of patients with TCZ almost completely reverses the SSc-associated fibrotic phenotype of explant dermal fibroblasts.

DISCUSSION

This study provides exceptional insight into the molecular pathology of dermal fibroblasts in severe SSc and unique insight into the likely key pathways and mediators involved in SSc pathogenesis, building on the original observations of LeRoy.[28] By linking the ex vivo data to promising clinical responses in a PBO-controlled trial of TCZ, our results also elucidate new and

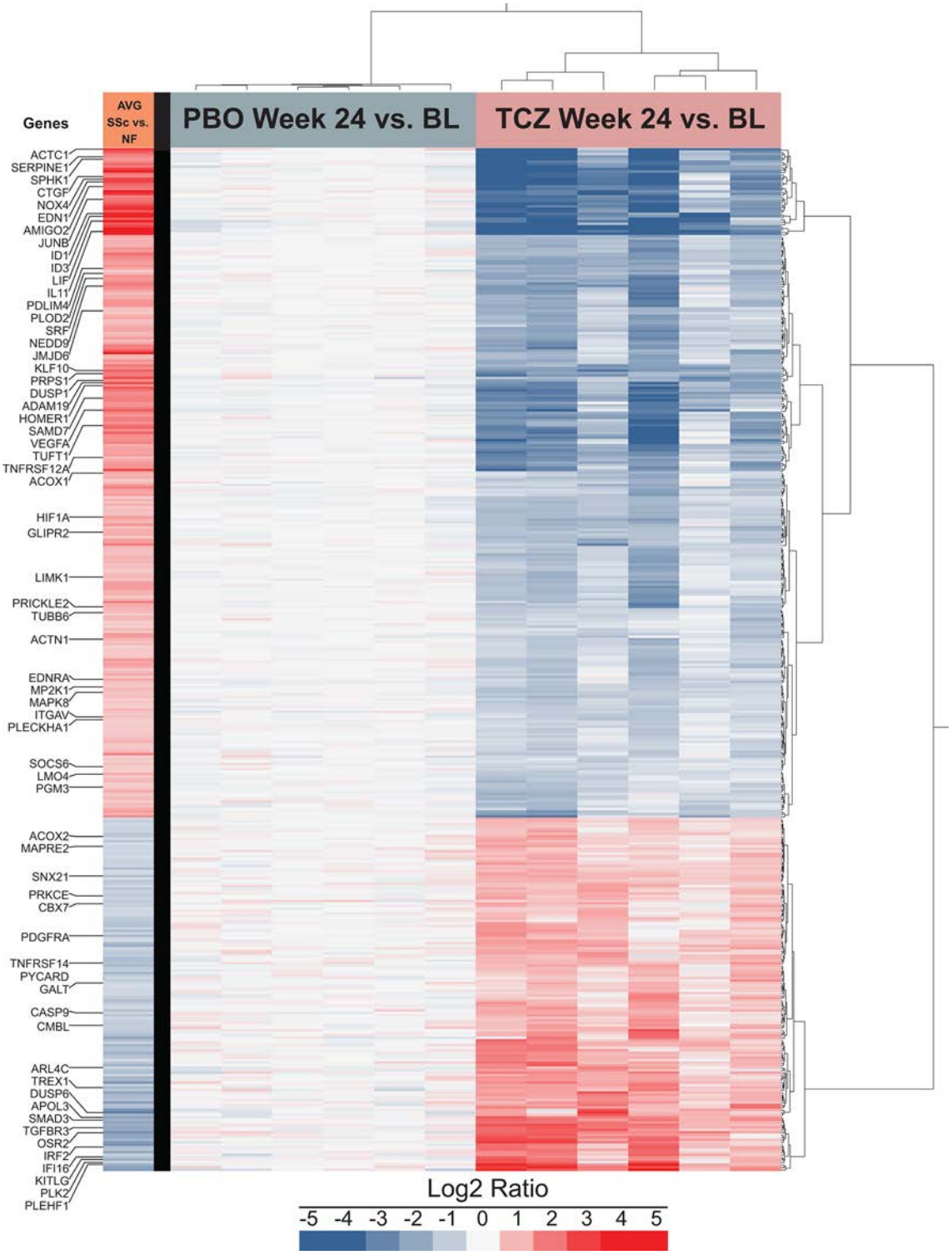

Figure 4 The profibrotic expression profile of SSc dermal fibroblasts tends to be normalised by 24-week treatment of patients with TCZ and tends to be stable in PBO-treated patients. Gene expression levels of SSc PBO and SSc TCZ dermal fibroblasts were measured at week 24 and normalised to matching baseline expression levels. Protein coding RNA showing a median RPKM ≥1, 1.5-fold overexpressed or underexpressed at week 24 compared with baseline with an adjusted p≤0.05 was marked as significantly differentially expressed (n=2136). For clarity, only the subset of 411 genes differentially expressed in both SSc versus NF and in TCZ week 24 versus baseline is shown. Genes and samples were clustered using the Ward method. The first column represents the average differential expression of SSc baseline samples compared with NF samples. AVG, average; BL, baseline; NF, normal fibroblast; PBO, placebo; RPKM, reads per kilobase of transcript per million mapped reads; SSc, systemic sclerosis; TCZ, tocilizumab.

important mechanisms by which TCZ may exert an antifibrotic effect and build on data from whole-skin gene expression analysis from the faSScinate phase 2 study.[21] Although it was demonstrated that treatment with TCZ for 24 weeks resulted in a specific down-regulation of genes associated with M2 macrophages and with the IL-6 pathway, little specific effect of TCZ treatment could be

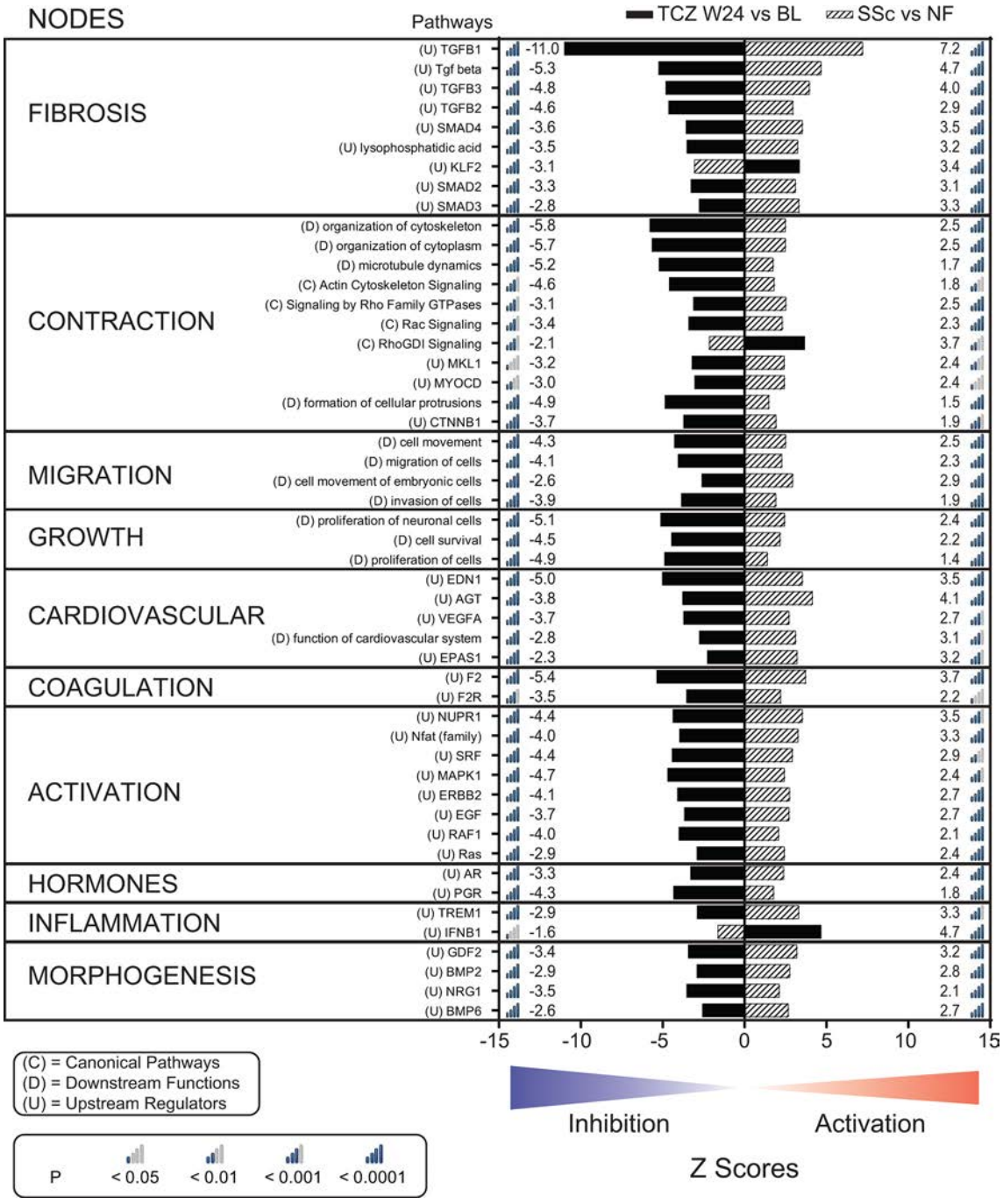

Figure 5 Treatment with TCZ for 24 weeks normalises the activation status of molecular and functional pathways associated with the biology of SSc fibroblasts. Activation status Z scores for the top 50 associated pathways grouped according to functional nodes. Genes significantly differentially expressed in the SSc versus NF (hatched bars) and the TCZ W24 versus baseline (solid black bars) comparisons (online supplementary figure S1) were analysed with IPA (Qiagen). Differential gene expression data for all differentially expressed genes are available in online supplementary appendix S2. BL, baseline; EGF, epidermal growth factor; IPA, Ingenuity Pathway Analysis; MAPK1, mitogen-activated protein kinase 1; NF, normal fibroblast; SSc, systemic sclerosis; TCZ, tocilizumab; TGFβ1, tumour necrosis factor beta 1; VEGFA, vascular endothelial growth factor A; W24, week 24.

detected on genes associated with fibrosis at the whole-skin biopsy level. Considering the multicellular complexity and relative heterogeneity of whole-skin biopsy samples used in this study, we hypothesised that the effect of TCZ on fibroblasts might be masked by the gene expression of other cellular compartments. The clinical homogeneity of the faSScinate patients and the access to samples collected from the same patients before and after 24 weeks of treatment with TCZ or PBO allowed us to use relatively small numbers of samples to generate robust and reliable results. As a result, we are able to confirm and expand on the description of the profibrotic properties of SSc dermal fibroblasts at the functional and genomic levels, establish that the profibrotic phenotype of SSc dermal fibroblasts is stable over ≥24 weeks in patients treated with PBO in the absence of immunosuppressant (cyclophosphamide, mycophenolate mofetil or methotrexate) and demonstrate that treatment of SSc patients with TCZ for 24 weeks profoundly alters the biological characteristics of dermal fibroblasts.

The baseline functional and genomic properties of the dermal explant fibroblasts analysed in this study closely match those described in previous publications.[23 24 29] Specifically, the baseline

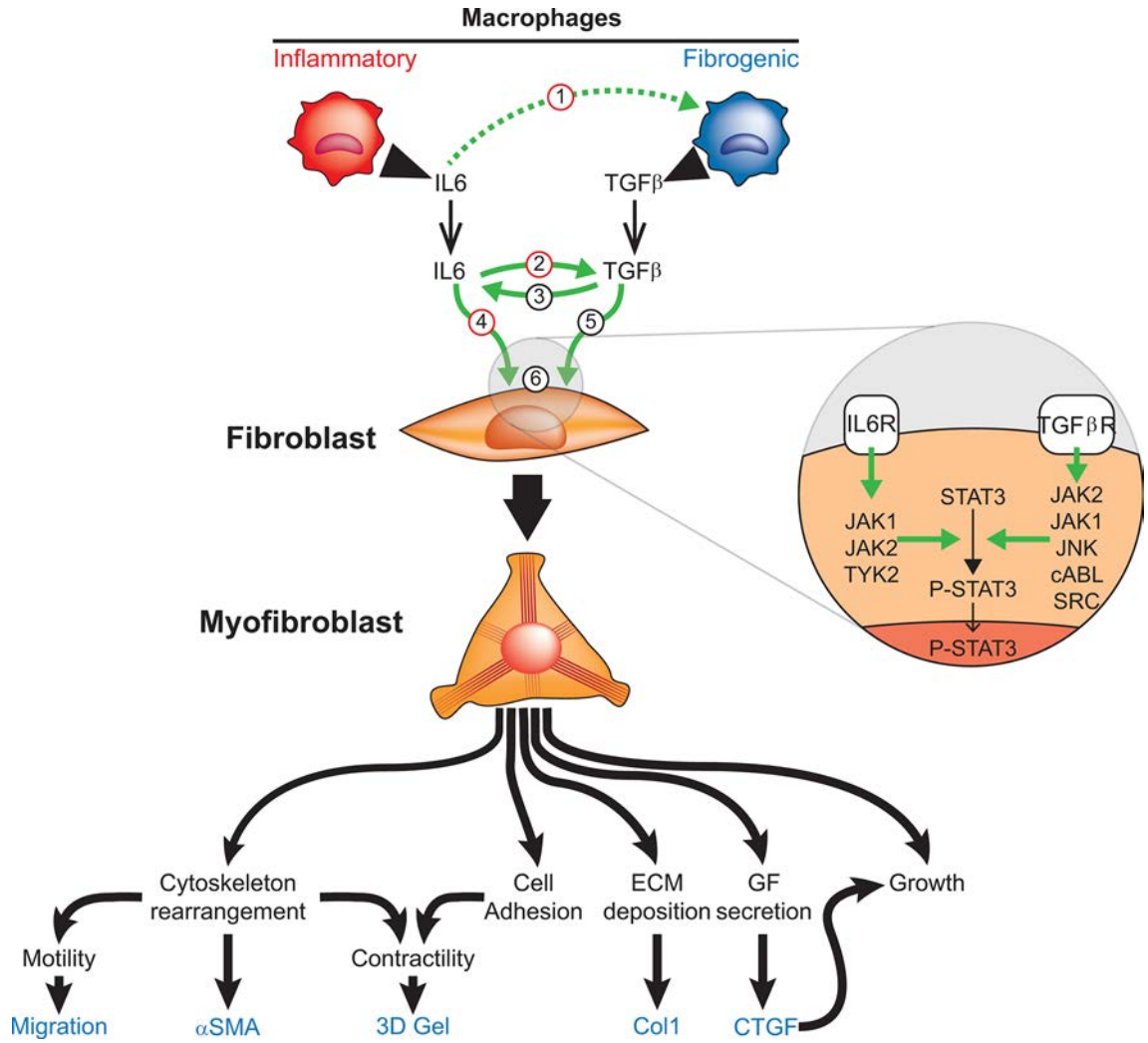

Figure 6 Potential mechanisms of interaction between IL-6 and TGFβ leading to fibrosis in SSc. (1) Inflammatory macrophage-produced IL-6 enhances fibrogenic macrophage function (potential TCZ target). (2) IL-6 induces TGFβ production and enhances its signalling in fibroblasts (potential TCZ target). (3) TGFβ enhances IL-6 production by fibroblasts. (4) IL-6 directly activates fibroblasts to produce profibrotic proteins such as Col1 (potential TCZ target). (5) TGFβ directly induces fibroblast differentiation into myofibroblasts. (6) Intracellularly, IL-6 classic signalling and TGFβ alternative signalling converge towards the activation of STAT3 (STAT3 → P-STAT3) resulting in aberrant expression of STAT3-dependent genes. αSMA, alpha-smooth muscle actin; Col1, collagen alpha 1; CTGF, connective tissue growth factor; ECM, extracellular matrix; GF, growth factor; IL-6, interleukin-6; JAK, Janus-activated kinase; JNK, Jun N-terminal kinase; P-STAT, phosphorylated STAT; STAT, signal transducer and activator of transcription; TGFβ, transforming growth factor beta; TGFβR, transforming growth factor beta receptor; TYK, tyrosine kinase.

SSc dermal fibroblasts produced elevated levels of profibrotic proteins and of migration and contractility activities and differentially expressed a distinct set of fibrosis-related genes with a high prevalence of TGFβ-regulated genes. Considering that the faSScinate patients were experiencing active progressive disease at study entry,[21] our observation is consistent with the work of Sargent *et al*,[24] who reported that the differential expression of genes regulated by TGFβ is associated with more severe disease when detected at the whole-biopsy level. Over the years, the study of explant dermal fibroblasts in vitro has provided valuable insight into the pathobiology of SSc.[6 7 22 28 30 31] The fact that the phenotypic abnormalities of SSc dermal fibroblasts persist in vitro over multiple passages has been critical to this line of investigation. However, it is important to note that dermal fibroblasts are heterogeneous and that they manifest different properties as a function of their microenvironment.[32] The potential selection pressure imposed by isolation, plating and passage procedures makes it likely that only a subset of fibroblasts is accessible to in vitro studies. These may represent fibroblasts that are more motile, better able to acclimate to the culture conditions or have higher growth potential, thus perhaps representing stem cell population(s). For any of these reasons, we might have selected a fibroblast subpopulation in the explant culture conditions more sensitive to the effects of IL-6 inhibition.

We used IPA (Qiagen) to associate gene expression with biological pathways, focusing on the molecular and cellular biology of relevance to fibroblasts. The top 50 pathways associated with SSc differentially expressed genes could be grouped into 10 core biological nodes that covered two levels of biology: biology intrinsic to fibroblasts (fibrosis, contraction, migration, growth and activation) and biology extrinsic to fibroblasts (cardiovascular, coagulation, hormones, inflammation and morphogenesis). The pathways forming the intrinsic group reflect canonical features of fibrotic fibroblasts[5] with a dominant contribution by TGFβ. This dominance of TGFβ-related biology is apparent in the fibrosis node and in the contraction node, where activation of Rho GTPases can be triggered by TGFβ in a SMAD-independent process,[33] and in the activation node, where Ras, RAF1 and

ERK are part of one branch of SMAD-independent TGFβ signalling.[34] Conversely, the pathways forming the extrinsic group represent biological processes that link inflammation and vasculopathy to fibrosis. For example, not only are the cardiovascular pathways EDN1 and AGT potent mediators of vasoconstriction,[35 36] they are profibrotic mediators relevant to the pathobiology of SSc.[37 38] This is notable considering the prominent vasculopathy of SSc and the putative benefit for patients with SSc of drugs that target these pathways. Similarly, in addition to playing a key role in response to angiogenesis, vascular endothelial growth factor A (VEGFA) and the VEGFA-inducing transcription factor endothelial PAS domain-containing protein 1 amplify fibrosis,[39] creating a link between response to hypoxia and fibrosis. Although excessive inhibition of angiogenesis could be detrimental, our data support normalisation of the activation status of cardiovascular-related pathways rather than complete blockade of these pathways, which might be detrimental for wound healing or tissue repair.

Patient treatment with TCZ for 24 weeks profoundly affected the properties of explant dermal fibroblasts. It is not possible to determine whether treatment with TCZ modified the phenotype of resident cells or the cellular composition of the dermis; both these mechanisms are non-exclusive. Independent of the exact mechanism, the effect of IL-6Rα blockade on biological pathways dominated by TGFβ-related biology is striking and highlights the interaction between IL-6 and TGFβ. Although TGFβ is the quintessential profibrotic factor,[9] IL-6 has also been shown to induce the production of fibrotic markers (CTGF, αSMA and collagen 1) by human fibroblasts in vitro.[15] Hence, IL-6 and TGFβ may form a self-sustaining loop leading to fibrosis that could be interrupted by blockade of either or both cytokines. This is supported by recent work demonstrating that STAT3, the main downstream signalling element for IL-6, is an important TGFβ-dependent molecular checkpoint of fibrosis in SSc.[40] Altogether, these results and our observations at the whole-biopsy level[21] suggest a potential interaction network between IL-6 and TGFβ involving fibroblasts and macrophages that leads to fibrosis in SSc (figure 6). Thus, blocking IL-6 signalling with TCZ may represent a novel approach to control both the TGFβ pathway and the inflammatory pathways, supporting further exploration of IL-6 and related mediators as potential therapeutic targets in SSc and other inflammation-driven fibrotic diseases.

A unique strength of this study is longitudinal sampling of well-characterised and otherwise untreated SSc patients with clinically active disease receiving TCZ or PBO treatment. The combination of clinical homogeneity of patients, repeat sampling at two time points and quality of characterisation assays allowed us to use relatively small numbers of samples to generate robust and reliable results. In addition, the combination of well-characterised functional assays with gene expression analysis using RNA-Seq provides a rare window into the biology of active SSc. However, this study does not address the apparent discrepancy between biological and clinical effects of TCZ on skin fibrosis. Although additional studies are needed to fill this gap, we hypothesise that TCZ primarily affects de novo fibrosis and has little impact on the reduction of established fibrosis. Hence, in established skin fibrosis, the clinical effect of TCZ would be substantially delayed after its biological effect.

In conclusion, this study in the field of SSc translational research sheds new light on SSc biology by leveraging the conduct of a well-designed clinical trial in SSc and rigorous functional and molecular analysis of skin biopsy samples to elucidate the links between IL-6 biology and other key pathways and mediators in determining the hallmark profibrotic phenotype of SSc dermal fibroblasts. Moreover, our findings strongly support further exploration of IL-6 and related mediators as potential therapeutic targets in SSc and other fibrotic diseases.

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Contributors All authors were involved in drafting the manuscript or revising it critically for important intellectual content, made substantial contributions to the conception or design of the study or the acquisition, analysis or interpretation of data, approved the final version of the manuscript to be published and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of the work are appropriately investigated and resolved.

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Competing interests CPD reports personal fees from Roche/Genentech, Actelion, GlaxoSmithKline, Sanofi-Aventis, Inventiva and Boehringer-Ingelheim; grants and personal fees from Bayer; and grants from CSL Behring during the conduct of the study. VHO and SX have nothing to disclose. HC-H is an employee of Genentech. ZM, JS and TS are employees of and own stock in Genentech. RL reports personal fees from Biocon and Merck, grants from Elpidera and grants and personal fees from PRISM Biolab outside of the submitted work. DK reports personal fees from Actelion, Cytori, EMD Serono, Gilead, GlaxoSmithKline, Sanofi-Aventis, Corbus, ChemomAb, Eicos and UCB Pharma; grants from Bristol-Myers Squibb, the National Institutes of Health and Pfizer; grants and personal fees from Bayer, Roche/Genentech and Boehringer-Ingelheim during the conduct of the study; and personal fees from AstraZeneca outside the submitted work. AJ is an employee of Genentech, owns stock and stock options in Roche and has a patent issued for tocilizumab.

Patient consent Obtained.

Ethics approval The study protocol was approved by the institutional review boards or ethics committees before the study commenced. All subjects provided written consent. The study was conducted in accordance with the Declaration of Helsinki and with Good Clinical Practice. 'Control' skin biopsy samples were obtained with informed consent from healthy volunteers (London-Hampstead NRES Committee, MREC Reference 6398).

Provenance and peer review Not commissioned; externally peer reviewed.

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EXTENDED REPORT

Connective tissue growth factor contributes to joint homeostasis and osteoarthritis severity by controlling the matrix sequestration and activation of latent TGFβ

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ABSTRACT

Objectives One mechanism by which cartilage responds to mechanical load is by releasing heparin-bound growth factors from the pericellular matrix (PCM). By proteomic analysis of the PCM, we identified connective tissue growth factor (CTGF) and here investigate its function and mechanism of action.

Methods Recombinant CTGF (rCTGF) was used to stimulate human chondrocytes for microarray analysis. Endogenous CTGF was investigated by in vitro binding assays and confocal microscopy. Its release from cut cartilage (injury CM) was analysed by Western blot under reducing and non-reducing conditions. A postnatal, conditional $Ctgf^{cKO}$ mouse was generated for cartilage injury experiments and to explore the course of osteoarthritis (OA) by destabilisation of the medial meniscus. siRNA knockdown was performed on isolated human chondrocytes.

Results The biological responses of rCTGF were TGFβ dependent. CTGF displaced latent TGFβ from cartilage and both were released on cartilage injury. CTGF and latent TGFβ migrated as a single high molecular weight band under non-reducing conditions, suggesting that they were in a covalent (disulfide) complex. This was confirmed by immunoprecipitation. Using $Ctgf^{cKO}$ mice, CTGF was required for sequestration of latent TGFβ in the matrix and activation of the latent complex at the cell surface through TGFβR3. In vivo deletion of CTGF increased the thickness of the articular cartilage and protected mice from OA.

Conclusions CTGF is a latent TGFβ binding protein that controls the matrix sequestration and activation of TGFβ in cartilage. Deletion of CTGF in vivo caused a paradoxical increase in Smad2 phosphorylation resulting in thicker cartilage that was protected from OA.

INTRODUCTION

Articular cartilage is an avascular, non-elastic connective tissue in which chondrocytes, the only cells in the tissue, are embedded in a type II collagen and proteoglycan dense matrix. This part of the matrix is designed to withstand mechanical stress and inability to do so can lead to joint failure and osteoarthritis (OA).[1] Individual chondrocytes are also surrounded by a discrete pericellular matrix (PCM) that is structurally distinct from the adjacent type II collagen-rich matrix.[2] The PCM is rich in the heparan sulfate proteoglycan, perlecan and type VI collagen.[3 4] Reduced stiffness of the PCM compared with the adjacent type II collagen rich matrix suggests that this region will compress preferentially on mechanical load.[5] One mechanism by which cells of cartilage respond to mechanical stress is by release of sequestered heparin-bound molecules, such as FGF2, from the PCM.[6 7] The mechanism for this release may be due to a rapid flux in sodium that is displaced from the highly sulfated aggrecan-rich matrix on tissue compression. Release of FGF2 drives an immediate injury response in chondrocytes and protects animals from development of OA.[8 9]

TGFβ is another important cartilage growth factor that controls chondrogenesis and contributes to OA pathology.[10–12] TGFβ is secreted from cells in a latent complex in which a covalent dimer of active TGFβ is non-covalently associated with two latency-associated peptides (LAPs) to form a small latent complex (SLC).[13] In most cell types, the SLC covalently associates with one of four described latent TGFβ binding proteins (LTBPs) to form a large latent complex (LLC).[14] They exhibit a range of functions including facilitating folding and secretion and sequestration of the LLC, and activation of latent TGFβ. A number of mechanisms for latent TGFβ activation have been proposed, including integrin-dependent activation in response to mechanical stress,[15–17] protease-dependent mechanisms[18–20] and those mediated by thrombospondin.[21] Genetic manipulation in mice and identification of human mutations in TGFβ ligands, receptors and the LTBPs demonstrate the collective importance of these molecules in many aspects of tissue biology. The modest overlap in the phenotypes suggests that there are temporal and tissue-specific roles for these molecules, and raises the possibility that alternative mechanisms of TGFβ activation exist.[22–26]

In this study, we describe the search for other sequestered molecules of the PCM that are released on cartilage injury. Using a proteomic analysis, we identify connective tissue growth factor (CTGF, also known as CCN2) and determine its function and role in OA development.

Materials and methods

Reagents

See online supplementary file.

Mice

The *Ctgf*$^{fl/fl}$ line was developed by AL.[27] The *Ubi*-Cre/ER^{T2} line was purchased from Jackson Laboratories (strain no. 007001). Gene deletion was induced at week 4 of age (for avulsion hip injury) (men and women) and 8 weeks (for in vivo knee joint studies) (men only) with three intraperitoneal injections of tamoxifen on three consecutive days (50 mg/kg).

Cartilage and isolation of chondrocytes, confocal microscopy, siRNA transfection, microarray and RT-PCR, co-immunoprecipitation, ELISA-based binding assay for CTGF and perlecan, TGFβ1 ELISA: See online supplementary methods.

Statistical analysis

Paired Student's t-tests were performed when comparing the same cell population with two different treatments. Unpaired t-tests were performed when comparing groups of mice. Not significant (ns), p≤0.05 (*), p≤0.01 (**), p≤0.001 (***).

RESULTS

CTGF is a cartilage PCM protein

Four known heparin-binding growth factors were identified by proteomic analysis of purified PCM from human articular cartilage. These included FGF2, CTGF, hepatoma-derived growth factor and CCN1, also known as Cyr61 (data not shown). CTGF was of particular interest because *Ctgf*$^{-/-}$ mice have a severe musculoskeletal and vascular phenotype resulting in perinatal lethality[28–30] and the mechanism for this is unexplained.

Confocal microscopy confirmed pericellular localisation of CTGF in normal human articular cartilage (figure 1A). Binding of CTGF to perlecan was detected in vitro, in a heparan sulfate-dependent manner (figure 1B). Like FGF2,[6] CTGF was rapidly released into the medium of injured cartilage (injury CM) (figure 1C). Sequential collection of injury CM after cutting demonstrated that most protein was released in a single burst (figure 1D, none). Subsequent slow accumulation was from an actively translated and secreted pool as it could be inhibited by cycloheximide (figure 1D, +CHX).

CTGF activates chondrocytes in a TGFβ-dependent manner

To investigate the role of CTGF in articular chondrocytes, we performed a microarray analysis of isolated human articular chondrocytes stimulated with recombinant CTGF. To take into account endogenous production of CTGF in chondrocytes, we silenced endogenous CTGF by siRNA. Four CTGF-induced genes were identified: BMP receptor 2 (*BMPR2*), Prostate Transmembrane Protein, Androgen Induced 1 (*PMEPA1*), latent TGFβ binding protein 2 (*LTBP2*) and CTGF itself (*CTGF*) (figure 2A). Apart from *BMPR2*, regulation of each of these was robust in CTGF-stimulated human dermal fibroblasts and human articular chondrocytes by RT-PCR (figure 2B) even though the responses

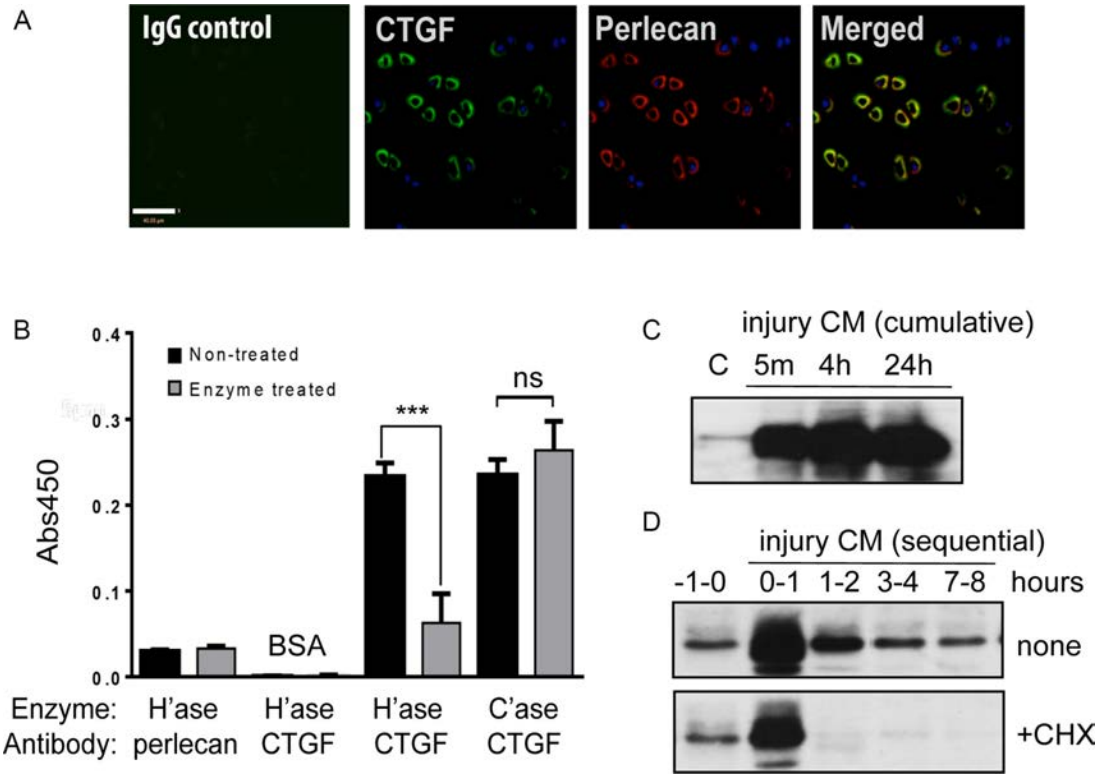

Figure 1 Connective tissue growth factor (CTGF) binds to perlecan in the pericellular matrix of articular cartilage and is released rapidly on injury. (A) Confocal microscopy of normal human articular cartilage, showing pericellular colocalisation of CTGF (green) and perlecan (red). Scale bar, 40 µm. (B) bovine serum albumin (BSA) or perlecan was precoated onto ELISA plates and wells were treated with or without 10 mU/mL of heparitinase (H'ase) or chondroitinase (C'ase) prior to incubation with 0.05 µg recombinant CTGF for 3 hours. CTGF was detected with anti-CTGF antibodies using a standard ELISA plate reader. Levels of bound perlecan pre-enzyme and post-enzyme treatment were checked with an anti-perlecan antibody. (C, D) Porcine articular cartilage explants (5×4 mm discs) were rested in serum-free (SF) medium for 48 hours and re-cut in fresh SF medium in the presence or absence of 10 µg/mL cycloheximide (+CHX). Injury conditioned medium (CM) was collected cumulatively (C) or sequentially (D) after specific time points and immunoblotted for CTGF. ***P<0.001; ns, not significant by a two-sided Student's t-test.

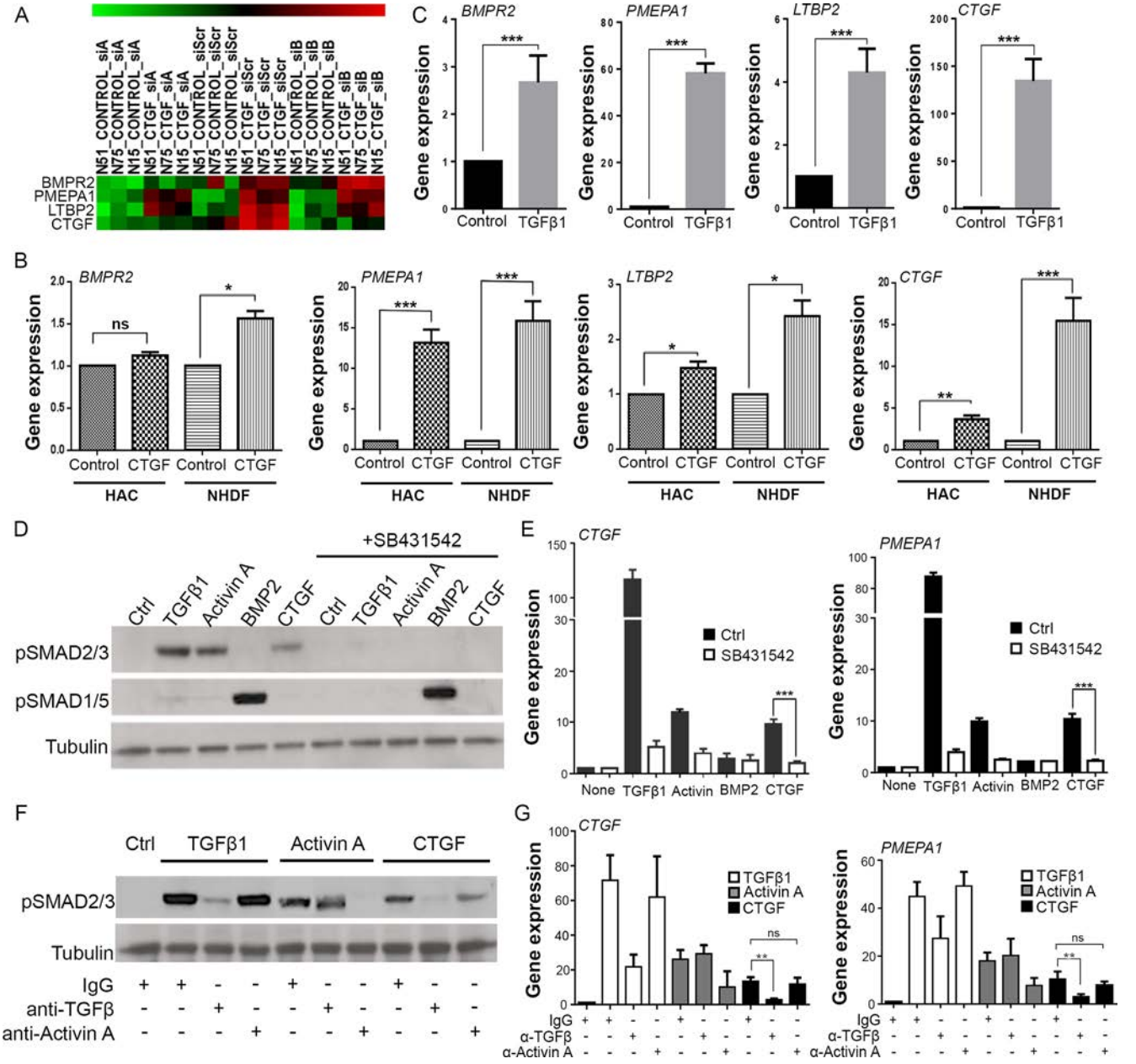

Figure 2 Connective tissue growth factor (CTGF) induces TGFβ-dependent SMAD2 phosphorylation and gene regulation. (A) Heat map of microarray *Z*-scores for the genes upregulated in human chondrocytes 8 hours after stimulation with 100 ng/mL CTGF (in triplicate). siRNAs targeting CTGF (siA, siB) were used to silence endogenous Ctgf prior to stimulation with recombinant ligand. The scrambled siRNA (siScr) is also shown. (B) RT-PCR validation of CTGF-induced genes in human articular chondrocytes (HAC) and normal human dermal fibroblasts (NHDF). (C) RT-PCR of NHDF treated with 10 ng/mL TGFβ1. All gene expressions expressed relative to GAPDH. Porcine chondrocytes were stimulated for either 45 min (D) or 8 hours (E) with 10 ng/mL TGFβ1, 100 ng/mL activin A, 100 ng/mL BMP2 or 100 ng/mL CTGF in the presence or absence of 5 µM SB431542. Lysates were immunoblotted for pSMAD2 and pSMAD1/5 (D), or RT-PCR performed for expression of *CTGF* and *PMEPA1* (E). (F, G) Porcine chondrocytes treated with TGFβ1, activin A or CTGF as above in the presence of 1 µg/mL anti-TGFβ or activin A neutralising antibodies. 45 min lysates were immunoblotted for pSMAD2 (F), or 8-hour RT-PCR performed for expression of CTGF and PMEPA1 (G). Western blots are representative of three independent experiments. All error bars represent SE. *p<0.05, **p<0.01, ***p<0.001 by a two-sided Student's t-test. ns, not significant. n=3.

were slightly less strong in chondrocytes. The four CTGF-induced genes were known to be TGFβ-responsive genes, which we confirmed by RT-PCR in human dermal fibroblasts (figure 2C). We checked to see whether recombinant CTGF was able to activate the canonical pathway of TGFβ involving phosphorylation of SMAD2. Phosphorylation of SMAD2, but not SMAD1/5/8, occurred following TGFβ, activin A (a family member of TGFβ that signals through the ALK4 receptor, also known as ACVR1B) or CTGF stimulation (figure 2D). Phosphorylation by all three ligands was abrogated in the presence of the ALK4/5/7 receptor inhibitor (figure 2D, SB431542), as was the gene regulation of *CTGF* and *PMEPA1* by these ligands (figure 2E). CTGF did not stimulate the TGFβ receptor directly, as phosphorylation of SMAD2 by CTGF was inhibited by a neutralising antibody to TGFβ (figure 2F), as was CTGF-induced gene regulation (figure 2G). SMAD2 phosphorylation and gene regulation by CTGF was not abrogated by an activin A neutralising antibody (figure 2F, G).

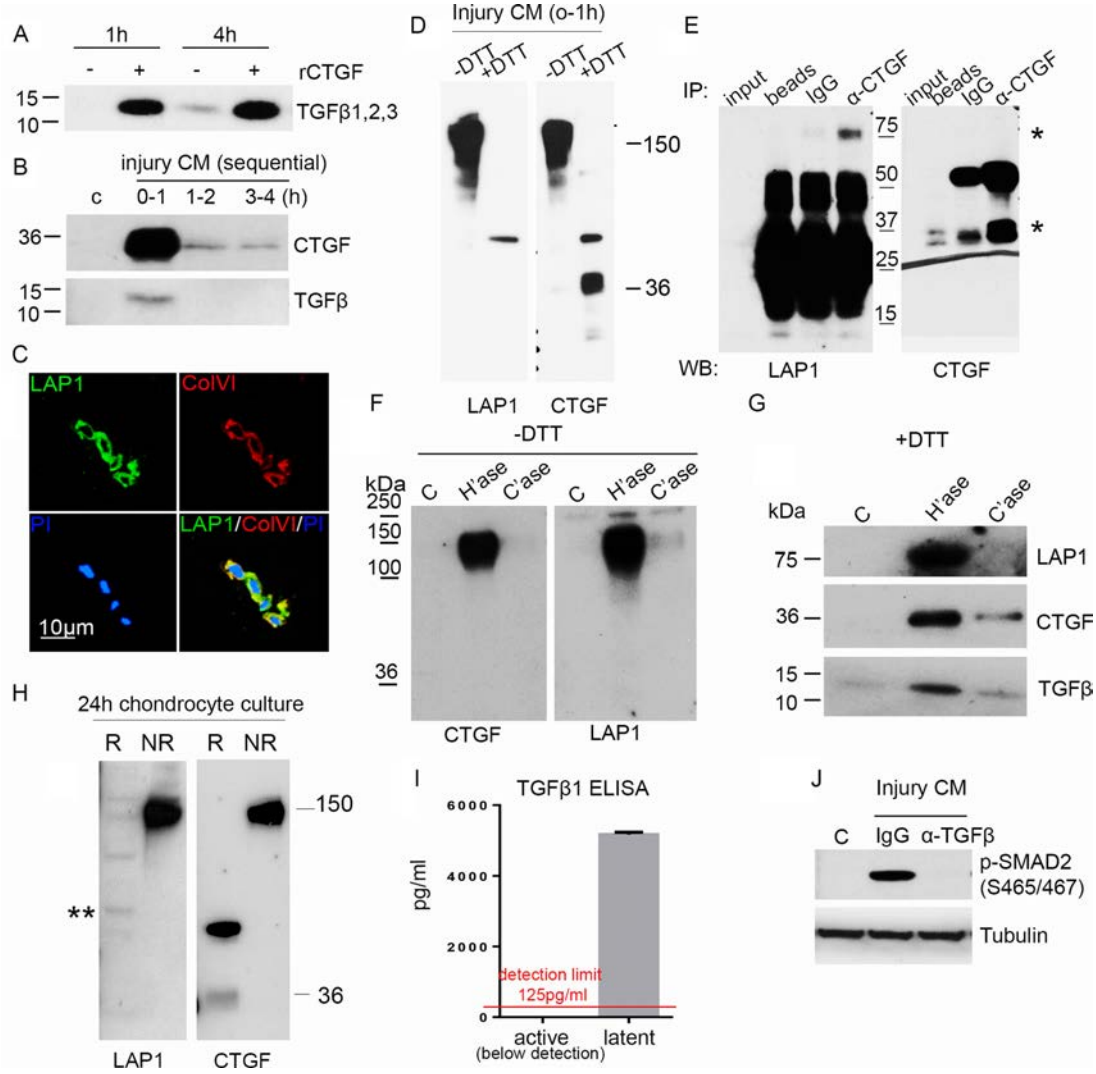

Figure 3 Connective tissue growth factor (CTGF) is covalently bound to latent TGFβ and is sequestered in the pericellular matrix (PCM) on heparan sulfate. (A) Rested porcine articular cartilage was treated with 100 ng/mL CTGF for 1 hour or 4 hours and the medium immunoblotted (under reducing conditions) for TGFβ. (B) Rested porcine cartilage was re-cut in fresh medium and the injury CM, at times specified, immunoblotted for CTGF and TGFβ (under reducing conditions). (C) Confocal microscopic images showing LAP1 and type VI collagen (ColVI) colocalising in the PCM of normal human articular cartilage, propidium iodide (PI). Scale bar, 10 µm. (D) Cartilage injury CM (1 hour) was electrophoresed under both reducing (+DTT) or non-reducing (–DTT) conditions and immunoblotted for LAP1 and CTGF. (E) CTGF was immunoprecipitated from injury CM using goat anti-CTGF antibody and immunoblotted for LAP1 (first panel) or CTGF (second panel) (under reducing conditions). * indicating bands for LAP1 and CTGF. (F) Cartilage explants were treated with or without 10 mU/mL heparitinase or chondroitinase for 4 hours. Medium was run under either non-reducing (–DTT) (F) or reducing (+DTT) (G) conditions and immunoblotted for LAP1, CTGF and TGFβ. (H) Culture medium was collected from isolated monolayer porcine chondrocytes over 24 hours and immunoblotted for CTGF or LAP under reducing or non-reducing conditions. ** shows weak 75 kDa band of LAP1. (I) Injury CM was treated with or without hydrochloric acid to calculate active and latent TGFβ1 protein levels by ELISA. Lower limit of detection, 125 pg/mL (n=3). (J) Porcine chondrocytes were treated (45 min) with or without injury CM pre-incubated with 1 µg/mL anti-TGFβ neutralising antibody or isotype control for 1 hour. Lysates were immunoblotted for pSMAD2 and tubulin.

We checked that there was no biologically significant contamination of TGFβ in our purified CTGF preparation (<0.1 ng TGFβ/100 ng CTGF) (data not shown), and we were unable to demonstrate synergy between suboptimal doses of recombinant TGFβ and CTGF in isolated cells (data not shown).

CTGF is secreted and sequestered in the PCM in a covalent complex with latent TGFβ

CTGF did not induce mRNA for TGFβ (data not shown), but as TGFβ-dependent activity was increased by CTGF, we next investigated whether CTGF controlled TGFβ protein levels. Stimulation of articular cartilage explants with recombinant CTGF led to strong accumulation of TGFβ protein in the medium within 1 hour of stimulation (figure 3A). Moreover, endogenous TGFβ and CTGF were detected in the medium following simple cutting injury within 1 hour (figure 3B). The rapid release of TGFβ from injured cartilage suggested that it was also in a pre-formed sequestered store. We determined TGFβ was stored in an extracellular pool by demonstrating staining for the latency-associated protein (LAP1) of latent TGFβ in the PCM (colocalising with type VI collagen) (figure 3C).

As latent TGFβ usually exists as a complex in which there are several disulfide bonds, injury CM was separated by SDS-PAGE under non-reducing and reducing conditions and immunoblotted

for LAP1 and CTGF. Surprisingly, LAP1 and CTGF co-migrated at 150 kDa (figure 3D), and immunoprecipitation of CTGF pulled down LAP (figure 3E, band at 75 kDa) indicating that CTGF and latent TGFβ were in a covalent (disulfide) complex. These results were strengthened further by showing that treatment of cartilage with heparitinase led to release of the 150 kDa CTGF/LAP complex (figure 3F), which, when run under reduced conditions, contained both CTGF (at 37 kDa) and components of the small latent complex (LAP1 and TGFβ) (figure 3G). Having established that CTGF was covalently bound to latent TGFβ in the extracellular matrix, we determined what fraction of secreted CTGF was bound to latent TGFβ. Examining the non-reduced 24-hour culture medium from isolated chondrocytes, all detectable CTGF co-migrated with LAP as a single high molecular weight band at 150 kDa, suggesting that CTGF's principal role in chondrocytes is as a latent TGFβ binding protein (figure 3H, PACs). In this experiment, both CTGF and LAP1 failed to be reduced fully; CTGF was seen at 36 kDa (monomeric form) as well as migrating at 70 kDa. LAP1 migrated at several molecular weights including its predicted fully reduced form, 75 kDa (asterisk).

CTGF is required for activation of latent TGFβ

We next investigated whether TGFβ released from the PCM on injury was all in its latent form or whether injury also caused activation of the latent complex. Free TGFβ was not detected in the injury CM by ELISA (figure 3I). However, chondrocytes stimulated with injury CM showed strong TGFβ-dependent SMAD2 activity (figure 3J), suggesting that the complex is stored and released in its latent form then activated on contact with the cell. We were never able to detect free LAP or CTGF in the medium of these stimulated cells indicating that these are rapidly cleared (most likely through an endocytic pathway).

To determine whether CTGF was required for controlling release of the latent complex on injury and its activation at the cell surface, we generated mice in which CTGF had been deleted ubiquitously in an inducible (postnatal) manner (*Ctgf*$^{fl/fl}$/*UbiCreERT*^{2}). Successful deletion was confirmed by showing reduced release of CTGF from knockout hip cartilage in the first hour following injury compared with wild-type hips (figure 4A). TGFβ release within the first hour of hip injury was also significantly reduced and correlated with the level of CTGF in the injury medium suggesting that CTGF is required for the release of TGFβ on cartilage injury (figure 4A,B). In the absence of both CTGF and TGFβ, the 1 hour injury CM from *Ctgf*^{cKO} hips was unable to activate SMAD2 in isolated chondrocytes (figure 4C). When *Ctgf*^{cKO} and wild-type cartilage was cultured for 24 hours following injury, CTGF levels remained suppressed in *Ctgf*^{cKO} 24-hour CM (figure 4D,E), but TGFβ accumulated in the medium (due to constitutive secretion of the SLC by the chondrocytes over this time) (figure 4D,F). Despite the presence of TGFβ, the 24-hour injury CM from *Ctgf*^{cKO} hips was unable to phosphorylate SMAD2 in isolated porcine chondrocytes (figure 4D lower panel, 4G), and the ability of the 24-hour injury CM to phosphorylate SMAD2 strongly correlated with levels of CTGF ($r=0.80$, $p=0.0085$) but not with TGFβ ($r=0.42$, $p=0.151$).

Activation of the latent CTGF–TGFβ complex requires CTGF binding to cell surface TGFβR3 in a heparan sulfate-dependent manner

We speculated that the CTGF-bound TGFβ complex was binding to a cell surface receptor to allow activation of latent TGFβ. Published mechanisms for activation of latent TGFβ in other tissues point towards a role for integrin binding or metalloproteinase activity.[31 32] To establish whether cell surface integrins were involved in CTGF-dependent SMAD2 activation by the injury CM, we stimulated human chondrocytes after pretreatment with the soluble arginylglycylaspartic acid (RGD) peptide (to block integrin binding) or with neutralising antibodies to αv, β1 or β3 integrins. None of these approaches affected SMAD2 activation by the injury CM (online supplementary figure 1a). Nor was activity affected by preincubation with a pan-metalloproteinase inhibitor, GM6001 (online supplementary figure 1a). As CTGF is known to bind and be cleared from the extracellular space by the scavenger receptor low density lipoprotein receptor-related protein 1 (LRP1), we treated cells with receptor-associated protein (RAP), an inhibitor of LRP1 re-uptake, or knocked down LRP1 by siRNA. Neither of these approaches affected activity of the injury CM (online supplementary figure 1b,c).

Finally, we addressed whether activation of the injury CM was dependent on cell surface heparan sulfate. Treatment of isolated chondrocytes with heparitinase, but not chondroitinase, prior to stimulation with the injury CM significantly blunted activation of SMAD2 (figure 4H). One transmembrane heparan sulfate proteoglycan that has been described as a regulator of TGFβ signalling (but not latent TGFβ activation) is betaglycan, also known as TGFβR3.[33 34] Soluble TGFβR3 was able to abrogate injury CM-induced activation of SMAD2 (figure 4I) and activity was also suppressed following knockdown of TGFβR3 using two separate siRNA oligonucleotides (figure 4J).

CTGF deletion causes a paradoxical hyper-Smad2 phosphorylation and protects cartilage from OA

To assess the role of CTGF in vivo, *Ctgf*$^{fl/fl}$/*UbiCreERT*^{2} male mice were treated with tamoxifen at 8 weeks of age to induce deletion of CTGF and the joints examined 10 weeks later. No overt ill health was observed in these mice. Surprisingly, deletion of Ctgf was associated with markedly increased phosphorylation of SMAD2 in the chondrocytes across all compartments of the joint (figure 5A–C) and the articular cartilage was significantly thicker in the Ctgf^{cKO} control mice (figure 5D–F). Joint destabilisation was performed at 10 weeks of age and histomorphometry of the operated and control (contralateral) joints was performed. The thicker cartilage of the *Ctgf*^{cKO} mice was more resistant to degradation induced by surgical joint destabilisation (figure 5G–L). Osteophyte size and maturity were not affected by genotype (online supplementary figure 2c).

SMAD2 phosphorylation of the articular cartilage following CTGF deletion may be due to compensatory regulation of TGFβ ligands from other tissues of the joint

In order to explore the paradoxical increase in SMAD2 phosphorylation in the cartilage of Ctgf^{cKO} mice, we extracted mRNA either from cartilage (auricular) or the whole joint of mice 2 weeks following tamoxifen treatment. A total of 38 genes relating to TGFβ, including ligands, receptors and target responses, were investigated. Gene regulation was expressed relative to wild-type tissue (online supplementary table 1). The results confirmed knockdown of CTGF in both cartilage and whole joints (97% and 87%, respectively). When the cartilage was considered separately, a small number of genes were regulated; these included a statistically significant reduction in follistatin, BMP6, aggrecan and LTBP2, and a striking increase in type II collagen (approaching threefold). This was quite different

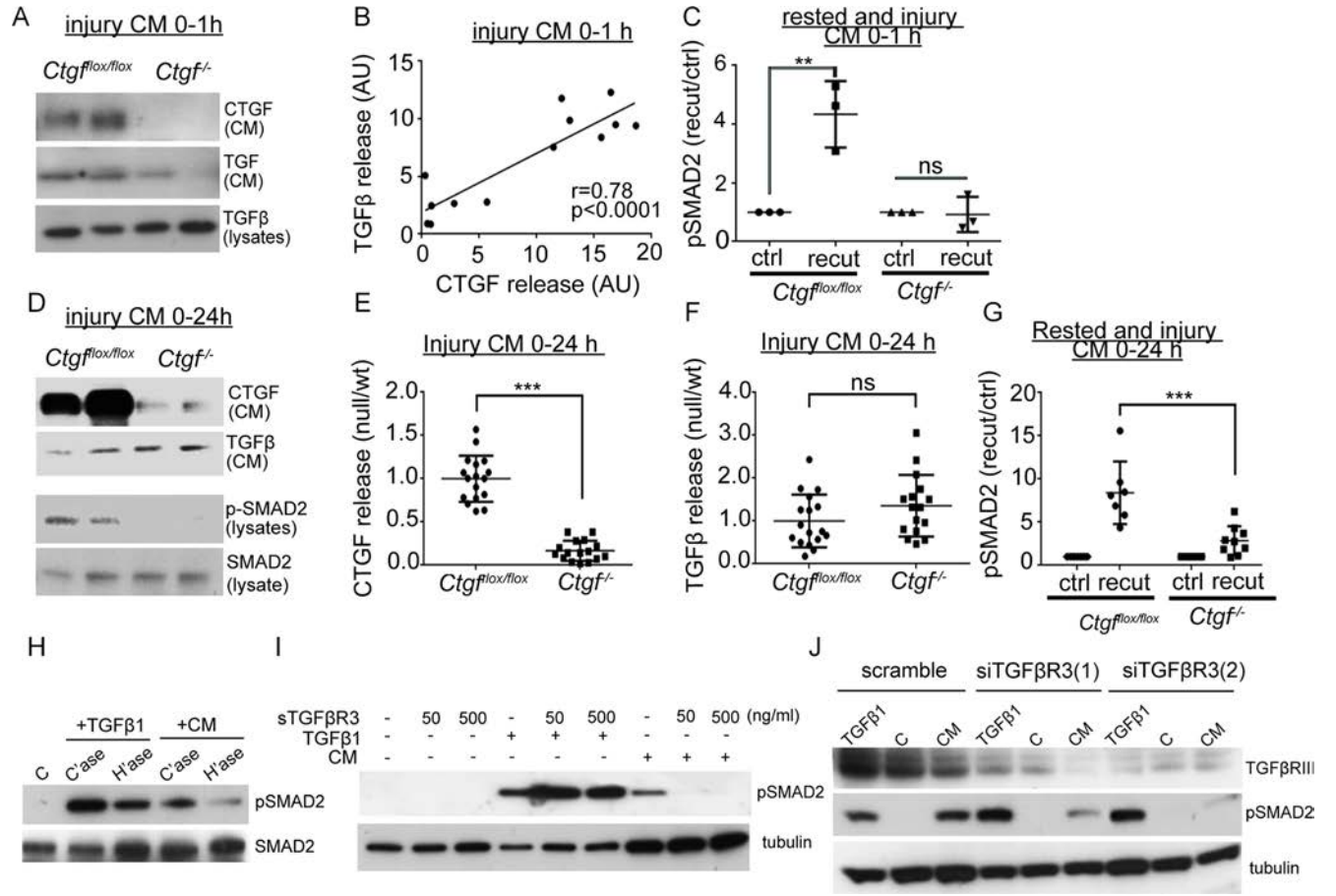

Figure 4 Connective tissue growth factor (CTGF) is required for release and activation of latent TGFβ in a TGFβR3-dependent manner. Conditional, inducible deletion of CTGF ($Ctgf^{cKO}$) was achieved by crossing $Ctgf^{fl/fl}$ (wt) mice with an inducible Cre recombinase driven by the ubiquitin promoter (*UbiCreER*^{T2}). Mice were treated with tamoxifen at 4 weeks to induce deletion. Hip avulsion, a model of murine cartilage injury, was performed in 6-week-old $Ctgf^{cKO}$ or wt mice. Injury medium (serum free) was conditioned for either 1 hour (A–C) or 24 hours (D–G). Some medium was conditioned from hips that had been rested for 48 hours then re-cut to obtain control (ctrl) and re-cut injury medium. (A) Injury CM or explant lysates were immunoblotted for CTGF and TGFβ (run under reducing conditions). (B) Protein levels of injury CM CTGF and TGFβ were quantified (wt, n=7; null, n=6) and their correlation examined. (C) Control and injury CM was used to stimulate porcine chondrocytes (45 min) and lysates immunoblotted for pSMAD2. (D) Injury CM (24 hours) was generated from $Ctgf^{cKO}$ and wt hips and were immunoblotted for CTGF and TGFβ (run under reducing conditions). Injury CM was also used to stimulate monolayer chondrocytes for 45 min and the lysates were immunoblotted for pSMAD2 (lower panels). Released CTGF (E) and TGFβ (F) from the injury CM were quantified (wt, n=17; null, n=16) and expressed relative to wt levels. (G) Injury CM was used to stimulate porcine chondrocytes (45 min) and pSMAD2 was quantified from Western blots. (H–J) To determine the mechanism of activation of latent injury CM, porcine chondrocytes were stimulated with either injury CM or TGFβ with the following pre-treatments: (H) 10 mU/mL heparitinase or chondroitinase for 4 hours prior to stimulation. (**I**) 50 ng/mL or 500 ng/mL soluble TGFβR3, 1 hour prior to stimulation. (J) TGFβR3 was knocked down by siRNA for 72 hours and the cells were rested in serum-free dulbecco modified eagles medium (DMEM) for 18 hours prior to stimulation. Error bars represent SE. **p<0.01, ***p<0.001 by a two-sided Student's t-test; ns, not significant. Pearson's coefficients of linear correlation and p values are shown.

to that observed in the whole joint where a number of TGFβ family members were increased (inhibin βA, TGFβ2, BMP7) and regulators of these pathways (TGFβR1, Acvrl1, Bmpr2, Bmpr1a, Smurf1).

DISCUSSION

Here, we describe CTGF as a novel latent TGFβ binding protein, binding covalently to the small latent complex of TGFβ prior to secretion, sequestering latent TGFβ in the matrix of cartilage in a heparan sulfate-dependent manner and controlling its release on cartilage injury. Activation of the complex in chondrocytes occurs exclusively in a CTGF-dependent and TGFβR3-dependent manner. It may also explain how cells that do not express $\alpha_v\beta_6$, an important latent TGFβ-activating integrin,[15 16] are able to activate the latent growth factor.

There is a strong existing literature to support a link between CTGF and TGFβ. Although some publications point towards a synergistic relationship between the two cytokines, the mechanism by which CTGF influences TGFβ has remained controversial.[35–37] In vitro, fragments of CTGF have been shown to bind directly to recombinant (active) TGFβ to activate the TGFβ receptor synergistically.[38] However, other diverse mechanisms of cellular activation by CTGF have been described including through extracellular integrin engagement,[30] and binding to cell surface receptors, TRK-A[39] and LRP1.[40] We found no evidence for activation of CTGF by these mechanisms. Most studies presented in our study were performed on endogenously secreted and released CTGF, which likely explains why we uncovered this novel mechanism of action. Our ability to demonstrate a cellular response with recombinant protein in chondrocytes (figure 2)

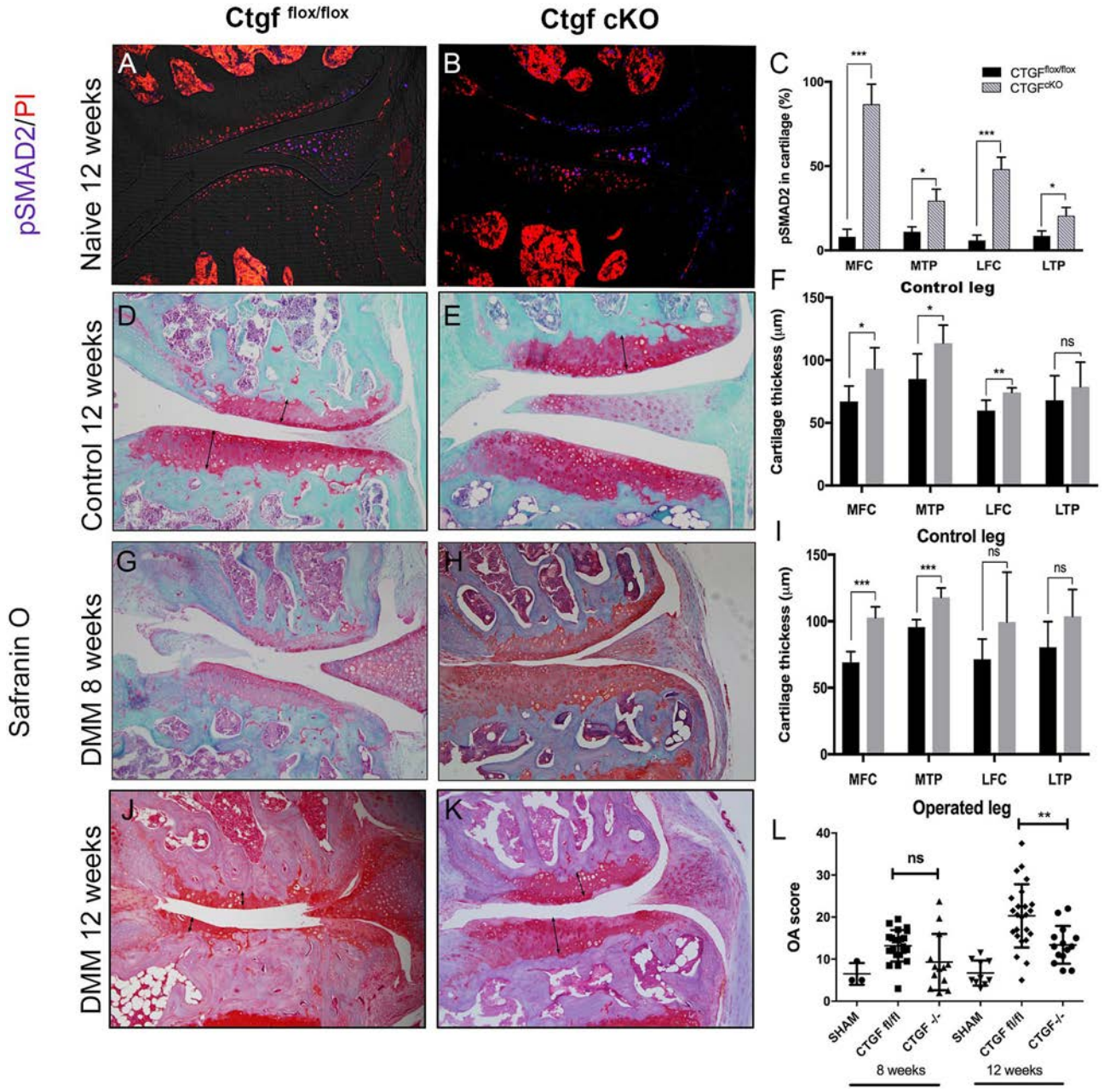

Figure 5 Paradoxical increase in SMAD2 phosphorylation in cartilage of CTGF$^{-/-}$ mice is associated with thicker cartilage and protection against osteoarthritis (OA). Male Ctgf$^{fl/fl}$;UbiCreER^{T2} and Ctgf$^{fl/fl}$ (control) mice were treated with tamoxifen at 8 weeks of age to induce postnatal, pan-tissue deletion of connective tissue growth factor (CTGF^{cKO}). At 10 weeks, surgical destabilisation of the joint, by cutting the meniscotibial ligament (DMM), or sham surgery, was performed on the right knee joint. The contralateral limb was used as a control. Joints were examined histologically 8 and 12 weeks postsurgery. Cartilage degradation (OA score) and cartilage thickness measures were performed on Safranin O-stained sections. Immunohistochemistry was performed for phospho-SMAD2. Magnification ×20.

was most likely due to displacement of endogenous extracellular CTGF–TGFβ from the chondrocyte cultures.

The role of TGFβR3 in the activation of latent TGFβ has not been described before. Active TGFβ is known to bind to the core protein of TGFβR3 in a glycosaminoglycan-independent fashion where it enhances TGFβ signalling.[33] TGFβR3 does not have the ability to signal directly as it has only a short cytoplasmic tail, but the N-terminal region is thought to interact with the TGFβ type II receptor and thereby facilitate recruitment of the receptor complex and TGFβ-induced SMAD2 phosphorylation.[41] In renal mesangial cells, active ligand binds to TGFβR3 to antagonise signalling.[42] In chondrocytes, we observed that TGFβR3-dependent activation of latent TGFβ required heparan sulfate (figure 4h). As we demonstrated that CTGF binds to heparan sulfate (on perlecan) in vitro and within the PCM, we hypothesise that it is a CTGF–heparan sulfate interaction that mediates initial binding of the latent complex to TGFβR3 (figure 6). Thereafter, we propose that this facilitates activation of latent TGFβ, through a mechanism not yet understood, but not involving integrin ligation or metalloproteinase activity, to allow it to activate the adjacent TGFβR1/2 receptor complex.

TGFβ is regarded as a chondroprotective agent in articular cartilage, promoting chondrogenesis in mesenchymal stem cells and inhibiting terminal differentiation.[43 44] Similar biological

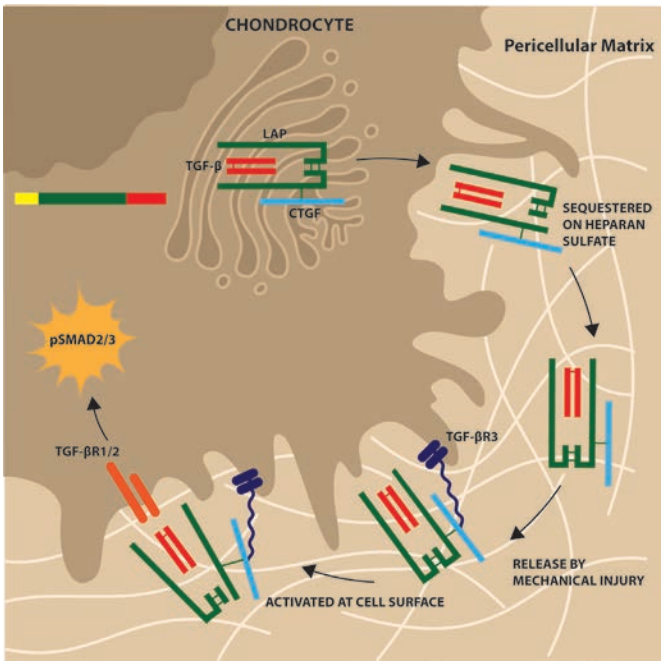

Figure 6 Schematic of role of connective tissue growth factor (CTGF) in cartilage. CTGF is covalently bound (disulfide bond) to latent TGFβ in the endoplasmic reticulum of the chondrocyte. It is secreted as a large latent complex and sequestered in the pericellular matrix attached to the heparan sulfate chains of perlecan. Mechanical compression causes release of heparan sulfate bound factors (mechanism likely involving sodium flux) and latent complex engages with TGFβR3 on cell surface (in heparan sulfate/CTGF-dependent manner). This allows activation of latent complex with autocrine activation of canonical pathway of TGFβ involving SMAD2/3 phosphorylation.

effects have been described for CTGF.[45 46] Canonical TGFβ signalling is through phosphorylation of SMAD2/3, leading to the activation and nuclear translocation of SMAD4. Human mutations in SMAD3 or deletion of SMAD3 in mice is associated with increased risk of osteoarthritis.[43 47 48] Although we did not examine SMAD3 phosphorylation directly, SMAD2 was phosphorylated both in vitro after stimulation with TGFβ or CTGF and, somewhat paradoxically, in vivo after CTGF deletion. This was associated with thicker cartilage, which was more resistant to degradation. SMAD2/3 phosphorylation also occurs after stimulation with other members of the TGFβ family such as nodal and activin βA. In our RNA analysis of CTGF cKO joints, we found no detectable nodal expression, but we did find an increase in mRNA expression of inhibin βA (the dimer of which forms activin βA), as well as an increase in TGFβ2. Although it could be that these effects are due to CTGF deletion in the chondrocytes, when the cartilage was considered separately, these ligands did not appear increased raising the possibility that pan-deletion of CTGF is leading to increased activin and TGFβ synthesis from other tissues of the joint. Interestingly, a similar paradox is documented in patients with Loeys-Dietz syndrome where loss of function of TGFβ (through mutations in TGFβ receptors or ligands) is associated with unexplained high SMAD2 phosphorylation in patient tissues.[49] Another possible explanation for the increased SMAD2 phosphorylation in the chondrocytes of $CTGF^{cKO}$ mice is that there is increased soluble latent TGFβ either because it is not being sequestered in the matrix of cartilage or because it is derived from other cells of the joint. The chondrocytes may, under these circumstances, be able to compensate for the loss of CTGF by activating latent TGFβ in a CTGF-independent and TGFβR3-independent manner. Whether this is through an LTBP-dependent mechanism remains unclear.

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Contributors TLV and JS devised the project. XT, HM, CM, JM-Z, JF, AD and PÖ generated experimental data. AL developed the CTGF floxed mouse. TLV and XT wrote the manuscript. All authors critically reviewed the manuscript and approved the final version.

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Competing interests AL is a shareholder of FibroGen.

Patient consent Not required.

Ethics approval All animal experiments were carried out with full ethical approval in accordance with local and national regulations.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement Mainly full data sets are presented. Further information is available on request to corresponding author.

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European families reveal MHC class I and II associations with autoimmune-mediated congenital heart block

Congenital heart block (CHB) may develop in the fetus of Ro/SSA autoantibody-positive women. A population-based recurrence rate of only 12% however suggests that factors other than maternal autoantibodies influence the risk of developing CHB.[1] In the present study, we aimed to identify genetic contribution to the disease by analysing HLA associations in European families with a Ro/SSA antibody-positive mother and at least one child affected by CHB.

HLA allele genotypes were imputed for 173 families comprising 635 individuals (291 parents, 173 cases and 167 unaffected siblings) from Sweden, Norway, Finland and Italy (online supplementary table 1) based on 5636 tag SNPs. Parental HLA allele transmission frequencies to patients with CHB were assessed and OR and significance of the association with CHB calculated. We considered nominal P values <0.05 suggestive of significance, and P values below the threshold of Bonferroni correction for multiple testing, or if the association had been previously reported at P<0.05, significant. Detailed information on patients and methods are available online (online supplementary methods).

In the MHC class I gene locus, we replicated the previously identified[2] protective association of HLA-Cw*06 (OR 0.22 (0.07–0.67), P=0.003) (figure 1). None of the HLA-A, HLA-B or other HLA-C alleles were associated with CHB. In the MHC class II locus we observed a novel protective association of the HLA-DQB1*06 allele with CHB (OR=0.48 (0.28 to 0.81), P=0.004), and replicated that previously reported[2] for HLA-DRB1*13 (OR 0.47 (0.24–0.91), P=0.007) (figure 1). Suggestive associations were observed for HLA-DQA1*01 (OR 0.52 (0.28–0.95), P=0.016), -DQA1*04 (OR 1.25 (0.56–2.79), P=0.025) and DRB1*07 (OR 0.55 (0.27–1.14), P=0.037) alleles (online supplementary table 2). Suballele and haplotype analysis led to the identification of two novel suggestive haplotype associations with CHB, DRB1-DQA1-DQB1 13-01:03-06:03 (OR 0.38 (0.11–1.3), P=0.025) and DRB1-DQA1-DQB1 08-04:01-04:02 (OR 1.55 (0.58–4.2), P=0.022) (online supplementary tables 3 and 4). No associations were observed for the HLA-DP locus.

One of the most robust associations with CHB appears to be a protective effect of the MHC class I allele Cw*06. Potent cytotoxic responses can be mediated by HLA-C both in the context of NK and $CD8^+$ T cells recognition, but with low level cell surface expression and a more restricted peptide binding, this HLA distinguishes itself among the class I molecules.[3] Interestingly, immunohistology of heart tissue of fetuses deceased from CHB shows $CD8^+$ T cells in the mononuclear cell infiltrates,[4] indicating a direct role for HLA class I peptide presentation to $CD8^+$ T cells in the disease pathogenesis.

The HLA-C alleles can be divided into the C1 and C2 groups, based on the presence of an asparagine or lysine at position 80 (Asn80 and Lys80), respectively. The C2 group was recently reported as more frequent in siblings affected by CHB than those not.[5] HLA-Cw*06, for which we observed a highly significant protective association in this study, as in a previous report,[2] carries a lysine at position 80 and thus belongs to the C2 group. A detailed analysis of HLA-C alleles was not included in the paper by Ainsworth and colleagues[5] making comparisons difficult. Notably though, neither any of the other C2 group alleles nor the C2 group reached a nominal level of significance in our analysis, indicating that the protective effect is mediated specifically by HLA-Cw*06, and not the C2 group in general.

We also observed HLA class II alleles associated with CHB, of which both significantly associated alleles, HLA-DQB1*06 and DRB1*13, had protective effects. There are many reports of negative DRB1*13 associations in European populations with autoimmune diseases.[6] We therefore hypothesise that the protective DRB1*13 association with CHB depends on a general mechanism of protection from inflammation shared among autoimmune diseases, and interpret the observed lack of significantly associated HLA alleles that increase susceptibility to CHB as consistent with the fact that the pathogenic CHB-initiating autoantibodies are generated in the mother.

In summary, we identify and validate several protective MHC class I and II associations with CHB across different European populations. Defining genes contributing to the risk of CHB is important for understanding the disease pathogenesis, and building knowledge to develop better diagnostic and therapeutic options.

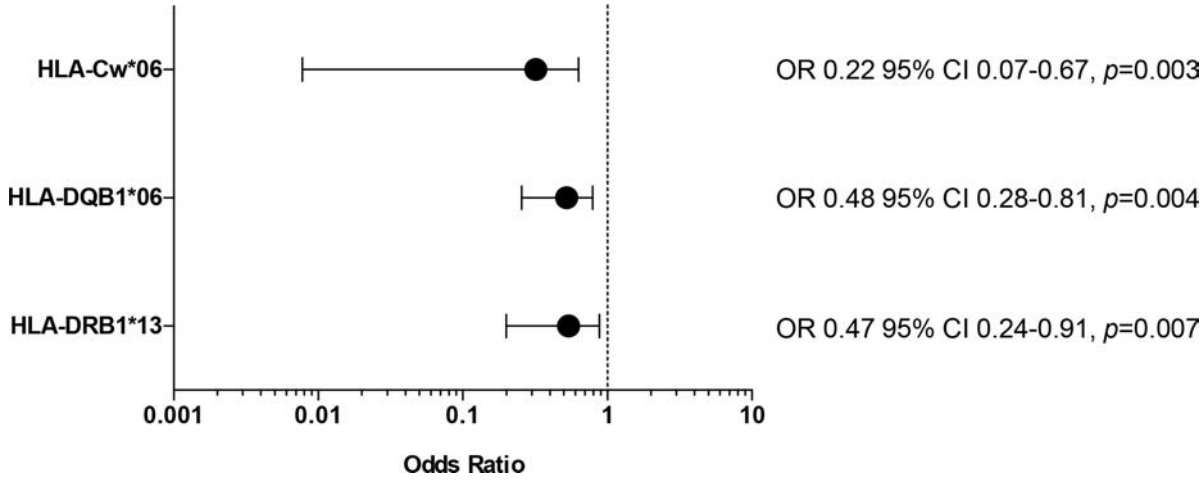

Figure 1 OR and 95% CI of the risk of CHB when carrying specific HLA alleles. Alleles with P values under the threshold of Bonferroni correction are included.

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Contributors SM, NK, IK and MWH conceived the study. AH, LM, SS, HK, CE, SCHBSG, ME, SDC, TK, AR, and JK identified and provided samples from CHB patients and family members. AD imputed HLA allele genotypes. NK and SM analyzed the data with input from IK and MWH. SM and NK wrote the manuscript together with IK and MWH. All authors edited and approved the final manuscript.

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Tracking digital ulcers in systemic sclerosis: a feasibility study assessing lesion area in patient-recorded smartphone photographs

Systemic sclerosis (SSc)-related digital ulcers (DUs) are painful and disabling,[1–3] and DU burden is often the primary outcome measure in clinical trials of SSc-related digital vasculopathy.[4] This is despite several studies showing a lack of agreement between rheumatologists as to what constitutes a DU.[5–8]

Objective outcome measures of SSc-related DUs for tracking change over time are therefore urgently required for clinical practice and research studies. The application of digital planimetry to clinical DU photographs has shown the possibility of fine-grained measurement of DU characteristics (area).[9] Our aims were to (1) demonstrate the feasibility of patients with SSc-related DUs/digital lesions photographing their lesions using smartphone cameras, and (2) use digital planimetry-style software analysis on images collected from patients to measure and track lesion area as a marker of healing or progression.

Patients with SSc-related digital lesions (judged to be ulcers by an experienced clinician) were asked to photograph their lesion(s) daily, using their own smartphone, for a maximum of 35 days. All patients gave written, informed consent. All patients were taking vasodilators, and one was on immunosuppressant therapy (methotrexate). The patients received normal clinical wound care throughout the study period, after which images were collected in-person, and stored securely for further analysis (eg, see figure 1).

Time and date stamps were extracted for each patient image sequence to accurately describe chronology. Images were loaded into custom digital planimetry software[9] and initially calibrated using a fixed-size object (often the finger width) to allow comparison between images in the sequence. For each image, the lesion area was measured by fitting an elliptical shape to the outline of the lesion by a single observer (figure 1). Using the calibration information, areas from each image were finally normalised to the area measured in the first image in the sequence.

Image sequences were collected from four patients describing a total of seven lesions (one patient with three lesions, one patient with two lesions, two patients with one lesion). The median (range) sequence duration was 29 (13–35) days, and for number of images recorded/day 0.63 (0.31–1.00). The relative area time course for each lesion is shown in figure 2. On average, lesion areas had, by study's end, reduced to 56% of the area measured on day 1, with six out of seven lesions reducing in size over the time course.

This pilot study confirms that it is feasible for patients to monitor their own lesions over an extended period (weeks)

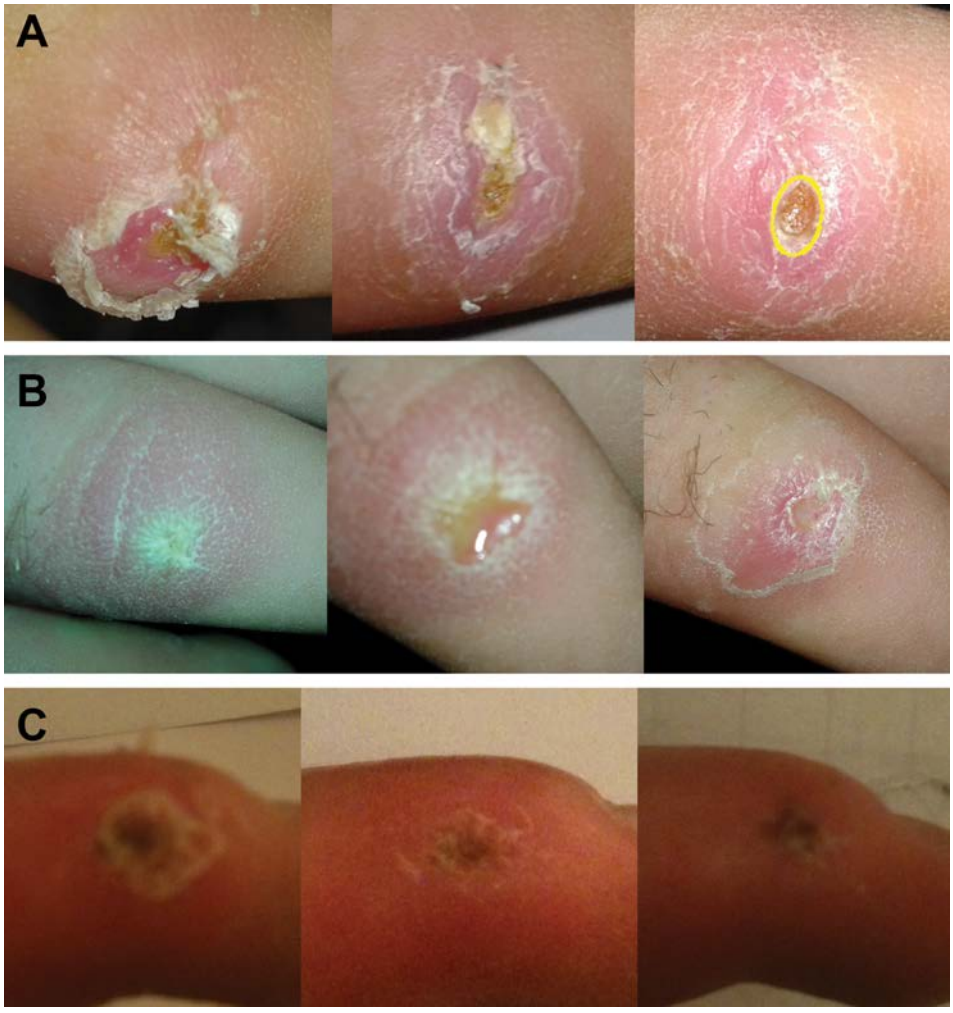

Figure 1 Selected examples of digital ulcer/lesion images taken from three sequences. Sequences demonstrate the varying quality of images captured by patients (particularly the bottom sequence where there are focus issues), although all were acceptable for further quantitative analysis. (A) (left to right): days 1, 24 and 35; (B) (left to right): days 1, 4 and 12; (C) (left to right): days 2, 7 and 18. Lesions are represented by sequences 4, 5 and 6 in figure 2 (top to bottom, respectively). Top-right image includes example of fitted ellipse shape (yellow outline) from software analysis.

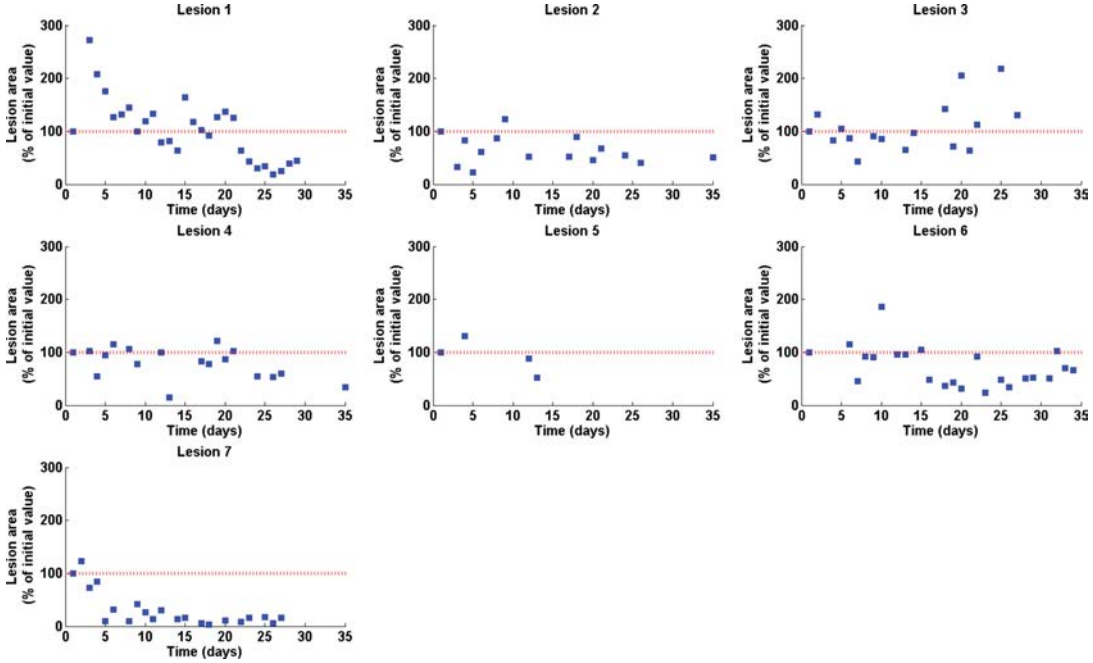

Figure 2 Relative area time course plots for each of seven digital lesions. Dashed red lines indicate 100% area, relative to the area measured on day 1. Lesion areas all reduced except for lesion 3 (top right).

by taking photographs with their smartphone camera. Photographs were taken on approximately two out of every three days during the study period, suggesting patients were highly engaged in the process. Collected photographs were of analysable quality.

This study therefore suggests a potential new tool for monitoring of lesion status/healing, both in the clinical setting, and as an outcome measure in clinical trials of SSc-related digital vasculopathy. Further work involving larger numbers of patients is now required to validate measurements produced and to improve data collection by integrating imaging into a smartphone application.

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IgG4-related disease of the mitral valve demonstrated by immunohistochemistry

INTRODUCTION

IgG4-related disease (IgG4-RD) is known to involve the cardiovascular system as aortitis or periaortitis[1]; however, the number of reported valvulopathies are exceptionally rare. This case is the first with immunohistochemistry proven mitral valve involvement.

CASE REPORT

A 72-year-old Korean woman with paroxysmal atrial fibrillation and end-stage renal disease due to long-standing diabetes and hypertension presented to the emergency department with near-syncope and dyspnoea with exertion. Further evaluation revealed three-vessel coronary artery disease and severe mitral valve stenosis due to suspected rheumatic mitral valve disease with a mitral valve area of 0.62 cm^2, peak gradient of 15.5 mm Hg and mean velocity of 136 cm/s. The patient underwent three-vessel coronary artery bypass graft surgery and mitral valve replacement with a 27 mm pericardial bioprosthesis. Both the anterior and posterior chordae tendineae were preserved. Intraoperative findings showed severe calcification of the posterior annulus of the native mitral valve. Pathological examination of the excised mitral valve leaflets revealed dense fibrosis and calcification (figure 1). Histological studies demonstrated prominent plasma cell-rich lymphoplasmacytic infiltrates (figure 2A,B). Immunohistochemistry exhibited an IgG4-positive plasma cell/IgG-positive plasma cell ratio of 70%, with greater than 100 IgG4-positive plasma cells per high-power field (figure 2C,D). Immunostains for kappa and lambda light chains demonstrated polyclonality. Further serological work up was significant for a serum IgG4 concentration of 413 mg/dL, or four times the upper limit of normal (reference range 1–123 mg/dL), an elevated lipase at 162 U/L (reference range 9–63 U/L) and an elevated erythrocyte sedimentation rate at 43 mm/h (reference range ≤25 mm/h) and C reactive protein at 3.7 mg/dL (reference range <0.8 mg/dL). Thoracoabdominal CT angiography showed no aortitis or retroperitoneal fibrosis; however, there was a 4.8 cm left kidney mass that was radiographically consistent with malignancy. The patient's postoperative course was complicated by an intracranial haemorrhage

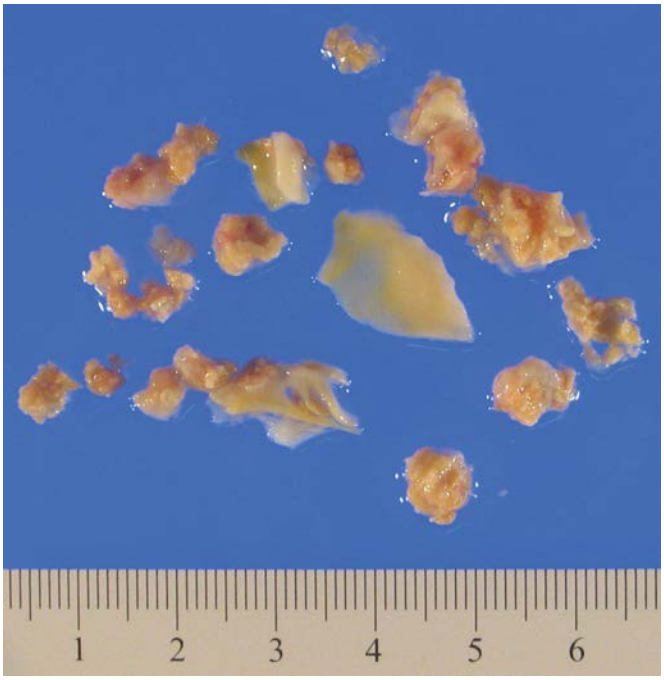

Figure 1 Gross photograph of the mitral valve leaflets. Fragments of densely calcified and fibrotic mitral valve tissue are evident.

due to coagulopathy and subsequent aspiration pneumonia. She opted to defer any further invasive diagnostic procedures due to operative risks.

DISCUSSION

This case demonstrates IgG4-RD manifesting as a valvulopathy in a patient with mitral valve stenosis. Two prior cases reported IgG4-RD presenting with an intracardiac mass,[2 3] and another reported an extensive cardiac pseudotumour infiltrating both the aortic and mitral leaflets requiring double valve replacement,[4] but the current case is the first to demonstrably show mitral valve disease with immunohistochemistry. A review of the literature described two cases of IgG4-RD involving the aortic valve requiring valve replacement.[5] The challenge in obtaining a tissue diagnosis for IgG4-RD involving the cardiovascular system is the risk of the procedure, leading some to question whether IgG4-RD valvulopathies are underdiagnosed.[6] Furthermore, the subsequent finding of a concomitant malignancy in our patient reflects prior observations showing the association of IgG4-RD and cancer. In prior studies, IgG4-RD patients had a malignancy rate threefold higher than matched controls.[7] The meticulous evaluation of the mitral valve in the current case underscores how IgG4-RD can be a confounding mimicker of other disease processes and reinforces the importance of careful pathological evaluation to make the correct diagnosis.

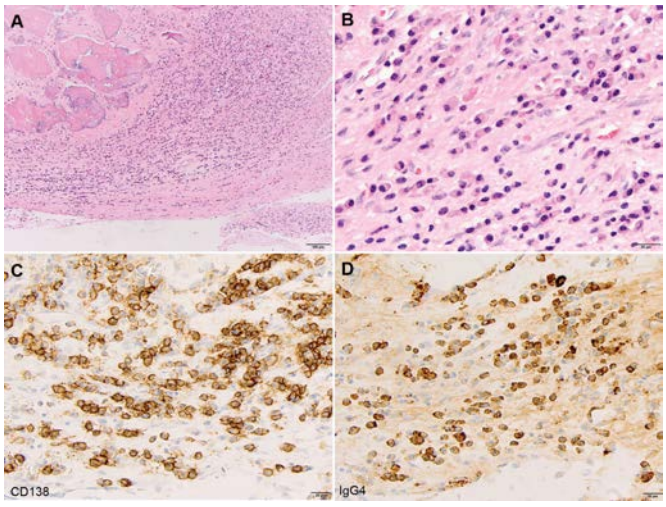

Figure 2 Microscopic findings. (A) H&E staining of histological sections of the mitral valve leaflets show calcification and dense fibrosis with an exuberant lymphoplasmacytic infiltrate (40×). (B) The infiltrate consists primarily of plasma cells; eosinophils are also present (H&E, original magnification 600×). (C and D) Immunohistochemistry using the plasma cell marker CD138 and IgG4 demonstrates approximately 70% of the plasma cells are of the IgG4 subclass; greater than 100 IgG4-positive plasma cells can be seen in a single high power field (original magnification 400× for both).

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CC-chemokine ligand 18 is a useful biomarker associated with disease activity in IgG4-related disease

IgG4-related disease (IgG4-RD) is a systemic disorder characterised by elevated serum IgG4 levels, tissue infiltration by $IgG4^+$ plasma cells and severe fibrosis.[1 2] However, biomarkers for IgG4-RD disease activity are lacking.[3] A recent report demonstrated that CC-chemokine ligand 18 (CCL18) was a substantial biomarker for fibrotic diseases.[4] Here, we investigated the correlation between serum CCL18 levels and clinical features of patients with IgG4-RD.

Written informed consent was obtained from all patients and healthy controls. Twenty-eight consecutive patients with active, untreated IgG4-RD diagnosed based on the 2011 comprehensive diagnostic criteria[5] and 16 healthy controls were enrolled. Diagnosis of IgG4-RD was biopsy proven in 26 patients (93%). Disease activity was assessed using the IgG4-RD responder index (IgG4-RD RI).[6] Healthy controls had no autoimmune diseases, atopic diseases or active infections at enrolment. Serum CCL18 levels were measured using a human CCL18/PARC Quantikine ELISA Kit (R&D Systems, Minneapolis, Minnesota, USA).

Characteristics of patients are shown in table 1. The mean age of patients with IgG4-RD and healthy controls was 59.7 and 47.3 years, and the proportion of females were 50% (14/28) and 69% (11/16), respectively.

Serum CCL18 levels in patients with IgG4-RD (median 44.5 ng/mL, range 3.6–120.9 ng/mL) were significantly higher than those in healthy controls (median 13.0 ng/mL, range 0.1–63.8 ng/mL; p=0.01) (figure 1A). The number of IgG4-RD patients with above normal levels of serum CCL18 (mean+1.96 SD of the healthy controls: 27.79 ng/mL) was 17 (61%), which was significantly higher than that of healthy controls (n=3, 19%; p=0.01). Of note, serum CCL18 levels were positively correlated with IgG4-RD RI score (ρ=0.54, p<0.005), number of affected organs (ρ=0.56, p<0.005), serum IgG4 level (ρ=0.50, p<0.01) and soluble Interleukin (IL)-2 receptor level (ρ=0.56, p<0.005), but not serum IgE level (ρ=−0.05, p=0.79) or blood eosinophil count (ρ=0.18, p=0.38), suggesting that serum CCL18 level is associated with IgG4-RD disease status rather than allergic condition (figure 1B, C). In line with this observation, serum CCL18 levels were similar between IgG4-RD patients with and without an atopic history (mean 47.8 vs 40.0 ng/mL, p=0.51; figure 1D). There was no statistically significant correlation between specific organ involvement and higher serum CCL18 levels. Serum CCL18 levels significantly decreased after glucocorticoid treatment (44.7 ng/mL vs 12.7 ng/mL, p<0.01; figure 1E) with declining disease activity (IgG4-RD RI: 12 vs 2, p<0.01).

Recent reports suggest that M2 macrophages are involved in the process of fibrosis.[7] CCL18 is primarily secreted from activated M2 macrophages induced by T helper type 2-associated cytokines such as IL-4 and IL-13,[8] and plays a role in the stimulation of collagen production by fibroblasts.[9] Importantly, DNA microarray analysis showed that CCL18 was upregulated in IgG4-RD-affected tissues.[10] Moreover, CCL18 expression was colocalised with massive infiltration of M2 macrophages and positively correlated with the fibrosis scores at affected IgG4-RD sites.[7] Thus, CCL18 secreted by M2 macrophages plays a significant role in the fibrotic process in IgG4-RD.[7]

Table 1 Characteristics of patients with IgG4-related disease

Clinical parameters	
IgG4-RD responder index, median (range)	12 (6–21)
Serum IgG (mg/dl), median (range)	1729 (934–3593)
Serum IgG4 (mg/dL), median (range)	387.5 (65–2178)
Soluble IL-2 receptor (U/mL), median (range)	448 (202–1963)
Atopic features	
Past atopic history, n (%)	17 (61%)
Serum IgE (IU/mL), median (range)	310 (38–3300)
Blood eosinophil count (cells/μL), median (range)	231 (62–1568)
Organs involved	
Number of affected organs, median (range)	3 (1–6)
Lacrimal gland and orbit, n (%)	22 (79%)
Salivary gland, n (%)	19 (68%)
Lymph node, n (%)	16 (57%)
Lung, n (%)	7 (25%)
Pancreas, n (%)	7 (25%)
Kidney, n (%)	5 (18%)
Retroperitoneum, n (%)	3 (11%)
Aorta, n (%)	2 (7%)
Skin, n (%)	2 (7%)
Breast, n (%)	1 (4%)
Paravertebral mass, n (%)	1 (4%)

IgG4-RD, IgG4-related disease; IL-2, interleukin-2.

Our study has several limitations. For example, serum CCL18 levels after glucocorticoid treatment may be a direct result of the medication rather than being a secondary marker of disease activity like other biomarkers. To eliminate the possible confounding effects of glucocorticoids on the decline in CCL18 levels, further longitudinal studies in patients with relapsing or glucocorticoid-resistant IgG4-RD are required. Such studies can clarify whether a preceding elevation in serum CCL18 level is a predictive indicator for subsequent relapse of IgG4-RD activity in guiding treatment decisions.

In conclusion, our results indicate that CCL18 is a useful biomarker for evaluating not only the disease activity of IgG4-RD, but also patient response to therapy. Our data suggest that CCL18 may be a novel therapeutic target for IgG4-RD.

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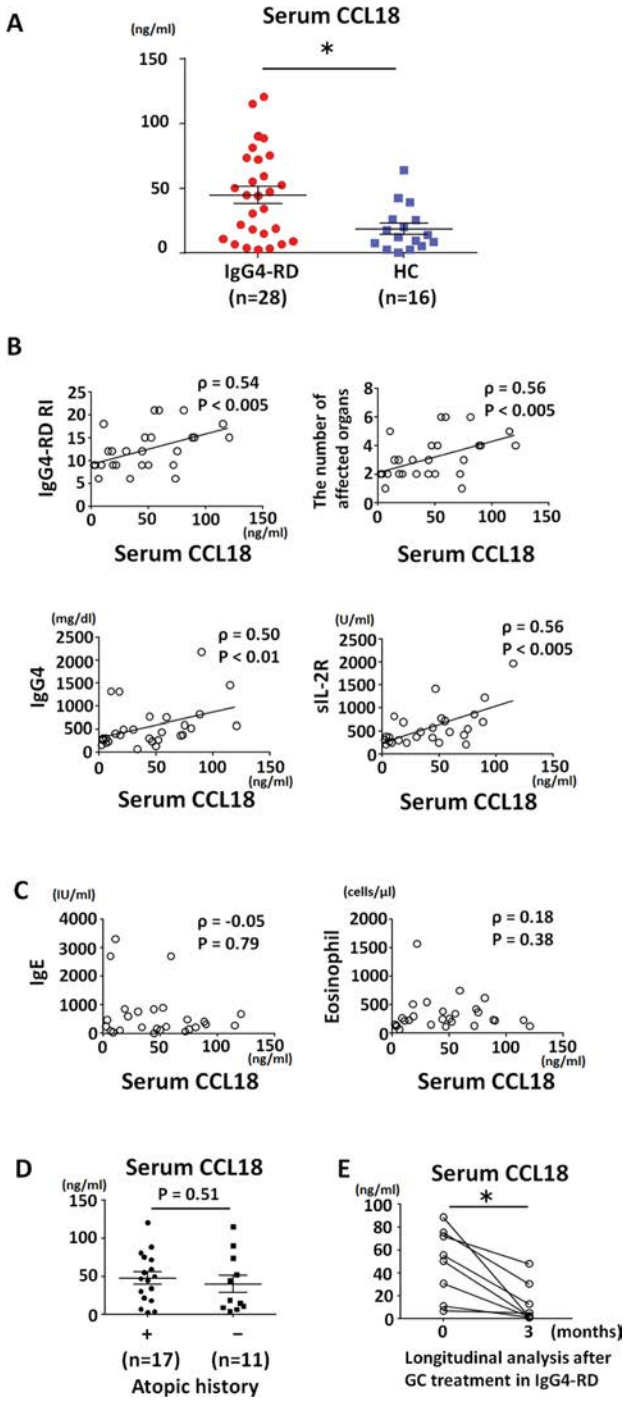

Figure 1 Serum CC-chemokine ligand 18 (CCL18) levels were elevated and correlated with disease activity in IgG4-RD. Serum CCL18 levels (A); correlation between serum CCL18 levels and disease activity (B) and allergic condition (C); correlation between serum CCL18 levels and atopic history (D); and longitudinal analysis of serum CCL18 levels after glucocorticoid treatment (E). Group-wise comparisons were performed using the Mann-Whitney U test. The correlation between serum CCL18 level and clinical parameters including IgG4-RD responder index; number of affected organs; levels of IgG4, sIL-2R, and IgE; and eosinophil count was analysed using Spearman's correlation coefficient. Differences before and after glucocorticoid treatment were determined using the Wilcoxon rank sum test for paired samples. A two-sided p value <0.05 was considered significant. All statistical analyses were performed using GraphPad Prism V.6.0 (GraphPad, La Jolla, California, USA). GC, glucocorticoid; HC, healthy controls; IgG4-RD, IgG4-related disease; sIL-2R, soluble IL-2 receptor.

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Efficacy of infliximab in the treatment of Erdheim-Chester disease

Erdheim-Chester disease (ECD) is a rare non-Langerhans cell histiocytosis characterised by long bones and various other organs' involvements.[1] Tissues are infiltrated by CD68+ CD1a− foamy histiocytes.[2] Therapeutic options are pegylated interferon-α,[3] cladribine,[4] mTOR inhibitors[5] and anakinra in mild forms of ECD.[6 7] Conversely, targeted BRAF or MEK inhibitor therapies are used for refractory or life-threatening ECD manifestations.[8 9]

All these therapies have frequent side effects. Interferon-α leads to fatigue, cytopenias and autoimmune diseases. Anakinra needs subcutaneous daily injections, with frequent local intolerance. Targeted therapies cause skin cancers, QT allongement, rhabdomyolysis and haemorrhage,[10] and we lack long-term safety data with these drugs. Moreover, targeted therapies are not available in all countries. Thus, there is an unmet need for improving treatments in many patients with ECD, particularly in countries where access to targeted therapies is complicated or impossible.

Although our comprehension of this disease has recently moved from inflammatory to clonal myeloproliferative disease,[11] the accumulation of pathological histiocytes leads to an increase of several cytokines in blood and affected tissues. Cytokine/chemokine network has been described in ECD lesions, and most of these factors are regulated by tumour necrosis factor alpha (TNF-α), which is also expressed in ECD lesions and serous fluids like pericardial effusion[12–14] and has been implicated in ECD pathogenesis in in vitro models.[15] Two ECD patients with cardiovascular involvement were successfully treated with infliximab, a TNF-α monoclonal chimeric antibody, achieving an improvement of symptoms, cardiac involvement and function.[14] We aim to determine the efficacy of infliximab in a larger series of patients with ECD.

We retrospectively reviewed the medical records of patients with ECD who received at least one infusion of infliximab between 2011 and 2016 in our tertiary care centre. ECD diagnosis was based on clinical and radiological presentation consistent with ECD and histological confirmation as described elsewhere.[1] All patients underwent *BRAF* mutation detection on codon V600 by pyrosequencing as previously described,[16] and whenever negative were then analysed by multiplex picodroplet digital PCR as previously described.[17] The eligible patients received infliximab 5 mg/kg D0–D15 then every 4 weeks. After 6 months, infliximab was administered every 6–8 weeks. All patients (except those who had a contraindication to this treatment) also received weekly oral methotrexate at the dosage of 10 mg, to prevent immunisation against infliximab chimeric antibodies.

Since ECD is a multisystemic heterogeneous disease, and due to the fact that response assessment was not uniformly performed, we used several criteria for assessing the efficacy: blood C reactive protein (CRP) levels, metabolic responses and radiographic evolution. The metabolic response was classified as complete metabolic remission (CMR), partial metabolic remission (PMR), stable disease or progression. The PERCIST criteria were used to evaluate this response and is extensively described elsewhere.[8] All the ^{18}FDG PET-CT scans were centrally reviewed. Radiographic evolution was classified in progression, stable or regression in successive assessments. All adverse events were noted. The infliximab treatment was continued until severe adverse event, death or disease progression occurred.

Qualitative data were described with numbers and percentages and quantitative values with the medians and ranges. Quantitative values (standard uptake value (SUV) and CRP levels) were compared with the non-parametric Wilcoxon rank-sum test for paired values. All tests were two-sided, and a P value 0.05 was considered to be statistically significant. Statistical analyses were performed using GraphPad Prism V.6.0 software (GraphPad Software, San Diego, California, USA).

Sixteen patients (11 men, 5 women, median age at ECD diagnosis 67; range 16–72) received at least one infliximab infusion. The patients previously received interferon-α (n=11), anakinra (n=3) and/or cladribine (n=2). Seven patients (64%) harboured the *BRAF*^{V600E} mutation (*BRAF* status was not determined in five patients). All patients had multisystemic involvement with aortic (n=12), retroperitoneal (n=10), cardiac (n=7), central nervous system (CNS) (n=5), skin (n=4) and retro-orbital (n=3) infiltrations. Two patients died before treatment efficacy was assessed: one died rapidly after treatment initiation due to a progression of multisystemic involvement, and another one died from stroke complications 6 months after infliximab was started. One patient prematurely stopped the treatment for personal convenience, and another one was lost to follow-up. Thus the treatment efficacy was evaluated in 12 patients (table 1). The median duration of infliximab treatment was 20 months (11–60). Five patients (42%) achieved PMR, three (25%) had stable disease and four (33%) experienced disease progression under treatment. Median SUVmax in target lesions was not significantly improved between baseline and last evaluation (figure 1A). The blood CRP level was not different between baseline and last visit (median CRP at baseline 17 mg/L; median CRP at last infusion 15 mg/L, P=0.85) (figure 1B). Cardiac involvement, which was observed in 5/12 patients, was stable in 3 patients, worsened in 1 and improved in 1 in radiographic assessments. CNS involvement, which was present in four patients, was stable in two (pseudo-meningioma) and improved in two (cerebellar infiltration). Retro-orbital infiltration was stable in two and worsened in one. Retroperitoneal infiltration was stable in nine and

Table 1 Clinical characteristics and evaluation of the efficacy of infliximab in 12 patients with Erdheim-Chester disease

Case	Age*, sex	Previous treatments	Metabolic assessment	Cardiac evaluation	CNS evaluation	AE	Duration under infliximab (months)	Reason for treatment interruption
#1	F, 80	IFN, anakinra	Progression	–	–	Urinary tract infection	40	Progression
#2	F, 23	IFN, anakinra	Stable	–	–	Bronchitis	30	Personal reason (mild symptoms)
#3	M, 66	IFN	PMR	Improved	–	None	60	No interruption
#4	M, 72	IFN	Progression	–	Stable	None	18	Personal reason (metabolic progression but no symptoms)
#5	M, 54	IFN, cladribine	PMR	–	Improved	Myelodysplasia	35	AE
#6	M, 76	Anakinra	PMR		Stable	Lung infection	14	Death (ischaemic stroke)
#7	M, 73	IFN	Stable	New	–	Pneumocystis infection	13	Progression
#8	F, 62	IFN	Progression	Worsened		None	12	Progression
#9	F, 55	Cladribine	PMR	Stable	Improved	None	11	Switch to vemurafenib†
#10	M, 67	–	Progression	Stable	–	Lung infection	22	AE and progression
#11	M, 57	IFN	Stable	–	–	Hypogammaglobulinaemia	43	No interruption
#12	M, 69	–	PMR	Stable	New	None	12	Progression

*Age at the initiation of infliximab treatment.
†The patient had neurological involvement, and despite improvement with infliximab, she was given vemurafenib due to severe CNS involvement.
–, no data; AE, adverse event; CNS, central nervous system; F, female; IFN, interferon-alpha (standard or pegylated); M, male; New, appearance of a new localisation; PMR, partial metabolic remission.

worsened in one. Sinus involvement improved in one patient. Eight patients experienced side effects during the treatment: five had infectious events, one myelodysplasia and one hypogammaglobulinaemia. The treatment was discontinued in 10 patients: 1 for death (ischaemic stroke), 2 for serious adverse events (haematological malignancy and severe infection, the latter had also a progressive disease) and 4 for progression of ECD. Additionally, two patients who had stable disease stopped the treatment for personal reason (they had mild or no symptoms and preferred quitting treatment). One patient had severe CNS involvement, and despite a mild improvement observed under infliximab, she was prescribed vemurafenib after 11 months of infliximab because of the presence of *BRAF*^{V600E} mutation.

Our study demonstrates that infliximab has a variable efficacy in ECD. Metabolic response was achieved in 42% of patients. Cardiac efficacy was variable. CNS involvement improved in two patients. Overall, the toxicity profile was overall favourable with only two patients in whom the treatment was discontinued due to adverse events.

We now have increasing evidence that targeting therapies (BRAF and/or MEK inhibitors) leads to dramatic improvement in *BRAF* mutated as well as patients with wild-type ECD.[8] However, the tolerance profile of such drugs is questionable and they should be kept for patients with refractory disease or life-threatening manifestations. Moreover, the majority of patients relapse after BRAF inhibitors interruption.[10] Infliximab could be used in mild forms of ECD or in second line after targeted therapies interruption.

The limitations of our studies are due to the retrospective design and heterogeneity in disease assessment. The ^{18}FDG PET scans were centrally reviewed and objective criteria were used to determine the overall metabolic response, but the metabolic assessment has some limitations for evaluating anticancer response.

In conclusion, infliximab has a moderate although variable efficacy in ECD. Our study suggests that infliximab has little efficacy in ECD and could be considered as a treatment option in patients with mild disease, intolerance to other drugs or after targeted therapies interruption.

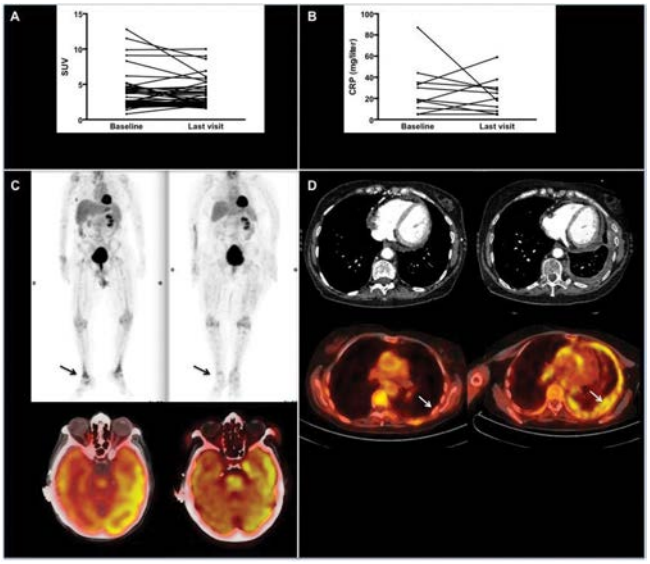

Figure 1 Outcomes of patients with Erdheim-Chester disease under infliximab therapy. Standard uptake values (^{18}FDG PET scans) of target lesions at baseline and end of follow-up are shown in (A), and blood C reactive protein (CRP) levels in (B). (C) The improvement of bone and central nervous system hypermetabolisms in patient #9. (D) The worsening of pleural thickening and effusion under infliximab in patient #8.

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Contributors FC-A, ZA and JH designed the study. FC-A, J-FE, NB, FC, PM and JH collected the data. J-FE and FC centrally reviewed the histological samples. J-FE determined the BRAF status. FC-A and JH conducted the statistical analysis. FC-A, J-FE, FC, ZA, PM, PC and JH analysed and interpreted the data. PM centrally reviewed the PET-CT scans. FC-A, J-FE, ZA and JH wrote the manuscript. All authors critically reviewed and approved the final version of the manuscript.

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Minimal neonatal transfer of certolizumab pegol in a Japanese patient with rheumatoid arthritis

Clowse *et al*[1] have reported minimal to no transfer of certolizumab pegol (CZP) into breast milk, and their findings have supported the continuation of CZP treatment during breast milk feeding. Rheumatoid arthritis (RA) often develops in women of childbearing age. It is generally difficult to treat these patients with methotrexate, which is the anchor drug for RA. Therefore, biologics, such as tumour necrosis factor (TNF) inhibitors, are often considered for active RA during pregnancy. However, the biologics cross the placenta from mother to fetus and transfer into breast milk during lactation. Although a meta-analysis report indicated that anti-TNF-α therapy did not increase the risks, such as congenital malformation or abortion during pregnancy, in patients with inflammatory bowel disease,[2] there have been major concerns for the safety of biologics to the fetus. It is well known that the neonatal Fc receptor for IgG (FcRn) and the Fc portion of antibodies are important for placental transfer of biologics. Novel TNF inhibitor, CZP, lacks the Fc fragment, suggesting its limited transfer through the placenta.[3] Current data on CZP exposure during pregnancy have suggested CZP safety and tolerability during pregnancy.[4 5] Additionally, Clowse *et al* indicated CZP safety of breast milk feeding for infants. We conducted a similar examination in a Japanese patient. A 30-year-old Japanese woman with RA became pregnant. Before pregnancy, her disease activity was low with 5 mg of prednisolone (PRD) use. However, her symptoms got worse and disease activity score 28-erythrocyte sedimentation rate (DAS28-ESR) increased from 3.19 to 3.86 at 8 weeks of pregnancy. Following this, the PRD dose was increased to 10 mg, but PRD treatment could not control her disease activity. DAS28-ESR increased to 5.71 at 28 weeks of pregnancy. At this point, CZP treatment was started under the informed consent of the patient because CZP was considered not to pass through the placenta to the fetus. DAS28-ESR decreased to 3.58, and her symptoms improved. Her fetus showed normal development under CZP treatment. At 40 weeks of pregnancy, she delivered a healthy baby boy. We evaluated the concentration of CZP using ELISA (Sanquin, Amsterdam, Netherlands). Although the trough concentrations of CZP were well maintained during pregnancy (table 1), CZP was not detected in cord blood and neonatal serum on day three after birth. After delivery, she continued CZP therapy. Eight weeks after delivery, the CZP concentration in the mother's serum was maintained. We evaluated the CZP concentration in breast milk at the same time. CZP was not detected in breast milk before or after CZP administration (table 1). We also confirmed CZP in sera and breast milk using western blot analysis. After electrophoresis, PEGylated proteins were detected by anti-PEG antibody (Abcam, clone PEG-B-47). The PEGylated proteins were detected in maternal serum but not in cord blood, foetal serum and breast milk (figure 1).

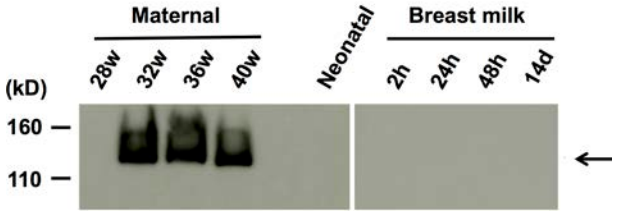

Figure 1 Certolizumab pegol, detected as a PEGyrated protein, was detected in maternal blood but not in cord blood, neonatal blood and breast milk.

Our case supports the hypothesis that the unique structure of CZP limits its transfer to the fetus and breast milk. It provides the possibility of CZP treatment for RA during late gestation and breast milk feeding without potential harm to the newborn.

Table 1 Concentration of CZP in sera and breast milk

CZP administration	Pre	4 weeks	8 weeks	12 weeks	16 weeks	20 weeks
Week of pregnancy	28	32	36	40		
Maternal serum (μg/mL)	<0.1	40	35	22*	26	35
Cord blood (μg/mL)				<0.1†		
Neonatal serum (μg/mL)				0.3‡		
Sampling time after CZP	Before	2 hours after	24 hours	48 hours	7 days	14 days
Breast milk (μg/mL)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1

The concentrations of CZP were measured using ELISA. The detection limit was 1 μg/mL. The concentrations of CZP in breast milk were measured on day 1 after delivery.
*2 days before delivery.
†Delivery day.
‡3 days after delivery.
CZP, certolizumab pegol.

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Rheumatoid arthritis, γδ T cells and bisphosphonates

We read with great interest the results of the study of Mo *et al*.[1] The authors suggest that γδ T cells are involved in the pathogenesis of rheumatoid arthritis (RA). The study showed a significant reduction, in patients with RA, of peripheral total γδ T cells (particularly Vδ2 T cells, the major population of peripheral blood γδ T cells). The percentage of peripheral Vδ2 T cells of RA was negatively correlated with the levels of inflammatory markers, including C-reactive protein, erythrocyte sedimentation rate as well as the Disease Activity Score in 28 joints. The peripheral reduction of Vδ2 T cells in RA did not result from abnormal proliferation or apoptosis capacity, but probably from the migration in the synovia. In human, RA synovial effusions and membranes have been found to contain a high number of γδ T cells,[2 3] and recently Akitsu *et al* found an increased homing of T cells in mice with inflammatory arthritis.[4]

It is known that γδ T cells produce proinflammatory cytokines, including interferon gamma (IFN-γ) and tumour necrosis factor alpha (TNF-α)[5]; Mo *et al* showed that in RA, Vδ2 T cells aberrantly secrete high levels of IFN-γ and interleukin-17. Targeting γδ T cells might be a potential approach for RA. In collagen-induced arthritis, an experimental model of RA, preventive depletion of γδ T cells ameliorated the disease severity.[6]

Interestingly, the use of amino-bisphosphonates (N-BP) for the treatment of various bone diseases is occasionally associated with the appearance within 24–36 hours of fever and musculoskeletal pain.[7] The latter is referred to as the acute phase response (APR) and it is linked to the activation of γδ T cells (in particular their major subpopulation of Vδ2 T cells) and the release of the pyrogenic cytokines such as IFN-γ and TNF-α.[8] We observed that the proportion of circulating γδ T cells is an important determinant of the occurrence of APR after administration of N-BPs,[9] and that N-BP treatment is associated with a decrease in circulating γδ T cells for at least 1 year,[10] in particular in the patients with APR.[11] The decrease in circulating γδ T cells could be attributed to the differentiation and homing at tissue levels of these cells.[12] Moreover, we recently suggested that the homing and the abundance of activated γδ T cells in the enthesis, ciliary body and aortic valve[13] might explain some adverse effects of N-BPs.[14]

The TNF-α-mediated chemotaxis of peripheral Vδ2 T cells, well described by Mo *et al* in patients with RA,[1] could play a role also in some of the immunoeffects of N-BPs.

Given these results, we think that it would be of interest to explore both the incidence of RA in patients exposed to N-BPs and the clinical effects of N-BP use in patients with RA.

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Amino-bisphosphonates, γδT cells, and their roles in Rheumatoid Arthritis

We thank Dr Rossini and colleagues[1] for their interesting comments on our manuscript.[2]

Aminobisphosphonates (N-BPs), the analogue of isopentenyl pyrophosphate, promote the activation and proliferation of peripheral γδ T cells, specifically Vγ9/Vδ2 T cells,[3] which are the cells we showed that play an important role in the pathogenesis of rheumatoid arthritis (RA).[2] We fully agree that γδ T cells are one of the major factors that may contribute to N-BPs-related acute phase response (APR).[4]

The therapeutic effect of N-BPs for RA is controversial. A few studies evaluated the efficacy of N-BPs for RA. One suggested that pamidronate was effective in the amelioration of disease activity and bone resorption,[5] whereas the other studies did not reveal significant clinical benefits.[6 7] In collagen-induced arthritis model, pamidronate also did not show significant treatment efficacy.[8] Although its inhibition effect on the activity of osteoclasts and bone resorption has been well described, N-BPs, on the other hand, could also stimulate macrophages to produce proinflammatory cytokines including interleukin (IL)-1β, tumour necrosis factor-α and IL-6, the driven cytokines of RA.[9] In addition, N-BPs promote the activation of Vδ2 T cells,[3] suggesting N-BPs may also aggravate the inflammation in RA. This might explain the controversial observations of the effect of N-BPs in the treatment of RA.

The lower peripheral γδ T cells after N-BPs treatment is an intriguing observation.[10] It is possible that γδ T cells may accumulate in the tissues like synovium after N-BPs treatment. However, more direct evidences, such as synovial histology from patients exposed to N-BPs, are required to support this hypothesis. Another possible explanation is postactivation apoptosis, given lower γδ T cells are more frequently observed in patients with APR.[11]

The role of N-BPs in the development of RA remains elusive, and very limited data is available at present. Given its undesirable effect on macrophages and γδ T cells, we are inclined to think that N-BPs might probably play an unfavourable role in the development of RA. A prospective controlled study on postmenopausal osteoporosis population is warranted to explore the effect of N-BPs in the development of RA.

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Metabolic and cardiovascular benefits of hydroxychloroquine: exploration in a wider population at high CV risk

We complement the authors on their systematic review and meta-analysis on metabolic and cardiovascular (CV) benefits of hydroxychloroquine (HCQ).[1] The authors have restricted the meta-analysis to studies comparing HCQ users versus non-users in rheumatoid arthritis (RA). This criterion has precluded the inclusion of many studies where metabolic and CV benefits of HCQ have been reported. We would like to highlight these to add to the significant benefits of this molecule, thus widening the evidence base.

Authors did not include studies comparing HCQ with other disease-modifying antirheumatic drugs (DMARDs) and biologicals. In a study cohort of 13 905 participants, majority of whom had RA, multivariate adjusted HRs for diabetes were 0.62 (95% CI 0.42 to 0.91) for tumour necrosis factor (TNF) inhibitors, 0.77 (95% CI 0.53 to 1.13) for methotrexate and 0.54 (95% CI 0.36 to 0.80) for HCQ compared with other non-biologic DMARDs.[2] Thus, incidence of new-onset diabetes was least with HCQ. In another recent study published in this journal,[3] data of 13 669 patients with RA from a longitudinal observational cohort were assessed. The adjusted HRs (95% CI) for diabetes mellitus (DM) were 0.67 (0.57 to 0.80) for HCQ, 1.31 (1.15 to 1.49) for glucocorticoids and 1.56 (1.36 to 1.78) for statins. Other synthetic DMARDs were not associated with any risk change. Interestingly, this increased risk of DM with glucocorticoids and statins,[4] both classes used commonly in patients with RA, was decreased by concomitant use of HCQ. These studies further strengthen the metabolic benefits of using HCQ in RA.

Although authors have analysed the effects of HCQ in patients with RA, the glycaemic and lipid-lowering effects of HCQ have also been reported in other chronic inflammatory conditions like lupus[5] and diabetes. In a double-blind randomised study involving 267 patients with uncontrolled type 2 diabetes, comparing HCQ with pioglitazone as add-on to metformin and sulfonylurea, change in total cholesterol and low-density lipoprotein cholestrol was significant in favour of HCQ while the glycaemic benefits were statistically similar.[6] In another study comparing the effects of the combination of HCQ with statin versus statin monotherapy in 328 patients with primary dyslipidaemia, significantly more reduction in lipid parameters was observed with combination as compared with statin monotherapy, while in an exploratory analysis, significantly lesser subjects showed deterioration from prediabetes to diabetes in the HCQ+statin combination arm.[7]

The role of chronic systemic inflammation in pathogenesis of atherosclerotic CV disease (ASCVD) has been proven in the recent CANTOS trial[8] where anti-inflammatory therapy with canakinumab led to a significantly lower rate of recurrent CV events. The ongoing OXI trial undertaken by the University of Helsinki will shed more light on the cardioprotective role of HCQ in reducing CV risk in patients with myocardial infarction.[9] Thus, the metabolic and CV benefits of HCQ have been firmly established in rheumatological conditions and are now being explored in prospective trials involving larger at-risk population with ASCVD and diabetes.

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Statins in systemic lupus erythematosus

Statins represent an important therapeutic option for prescription by rheumatologists due to their preventive effects related to cardiovascular disease as well as their immunoregulatory activity.

De Jong *et al*[1] recently published a report emphasising the use of statins as safety drugs, whose diverse effects in immune modulation could be related to autoimmune disorders. Although De Jong's cohort shows no association with autoimmune disease development such as systemic lupus erythematosus (SLE), in our view, the data could support potential prevention of SLE.

Fasano *et al*[2] recently ratified that statins are effective in primary prevention of cardiovascular disease, as previously mentioned for rheumatoid arthritis and other rheumatic diseases,[3] and Watanabe *et al*[4] described statin's protective thrombotic effect for SLE and antiphospholipid syndrome.

There is evidence that statins diminish SLE activity, although Artola *et al*[5] in recent meta-analysis were unable to show this effect. Nonetheless, in their paper, statin therapy reduced lipid and C-reactive protein levels, despite failure to alter the SLE Disease Activity Index (SLEDAI).

The efficacy of statins in reducing SLE activity and effecting immune regulation has been shown in case reports.[6] In a small, open-label study we assessed the effect of statin therapy in 19 patients with SLE with at least 1 year of follow-up prior and subsequent to statin use. In our group, with median age of 28 years (8–59) at baseline, 67 months of SLE evolution (28–238), 12 had lupus nephritis. Creatinine clearance maintained stability, while disease activity (MEX-SLEDAI), proteinuria, prednisone dose and relapses decreased during the year after the initiation of statins. We did not observe any adverse events related to the use of statins. This open-label study suggests that statin therapy in Mexican patients with SLE improves disease activity, reduces steroid dosage and lowers the relapse rate, thus stabilising renal function and improving SLEDAI. Hydroxychloroquine is prescribed for SLE as it reduces thrombosis risk as well as relapses, although adverse events are frequently observed, including photosensitivity with hyperpigmentation. It is plausible that statin therapy would have fewer adverse events than the current treatment with hydroxychloroquine.

I would like to request information regarding the demographics of the population studied by De Jong *et al* as well as SLE activity ratings prior to and after receiving statins and if the study includes information regarding statin treatment in patients with SLE younger than 40 years of age. In addition, is there evidence of adverse events related to statin use in the study?

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How useful is PET/CT in the evaluation of fever/inflammation of unknown origin? Comment on the article by Schönau *et al*

We read with interest the article by Schönau *et al*,[1] a rare prospective study performed in the setting of fever/inflammation of unknown origin (FUO/IUO). They concluded that positron emitted tomography (PET)/CT is helpful in the diagnosis of the underlying cause of fever/inflammation not elucidated with a preliminary diagnostic work-up. Notwithstanding the merits of the study, there are aspects that may deserve additional comments.

To fulfil the concept of FUO/IUO, the patient has to have a condition whose diagnosis is not clear after an initial screening including anamnesis and physical examination, complete blood count, routine blood chemistries, blood and urine cultures, and chest X-ray.[2] Although all patients in the study were referred for additional evaluation, it is not clear how far were the patients investigated before referral and if they really configure having FUO/IUO.

The authors inform having performed a diagnostic work-up including chest X-ray and abdominal ultrasonography as part of the study protocol. They did not perform contrast-enhanced chest and abdomen CTs, which are widely available, cost one-fourth to one-eighth the price of a PET/CT scan, and are the standard procedures in this context.[2] So the real added value of undertaking a PET/CT is not addressed in the present study.

The authors state that PET/CT was considered helpful in almost 57% of the patients. However, the definition of 'helpful' is not clearly explained. Just pointing to a right diagnosis (true positive rate) does not necessarily mean helpfulness. For example, demonstrating abnormal uptake in shoulders is not in general necessary to begin treatment of polymyalgia rheumatica diagnosed on clinical and laboratory grounds.[3] To justify the wide use of PET/CT, it is necessary to demonstrate when and how the exam was decisive for correct diagnosis and treatment. The 'inaccuracy' rate (35%) and its consequences must also be taken into consideration.

Despite the good internal validity, the study seems to have some limitations that may reduce its external validity and applicability. In this context, a larger step forward would be to perform a randomised controlled trial comparing strategies with and without PET/CT (on a background of evaluation including chest and abdomen CT), or alternatively comparing directly PET/CT with chest/abdomen CT. Besides obtaining a correct diagnosis, outcomes as disease remission or cure, incidence of adverse events, necessity of additional tests, costs, and duration of in-hospital stay must be considered.

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Uric acid and incident dementia: a population-based cohort study

We read with great interest the article by Latourte and colleagues,[1] regarding the effect of serum uric acid (SUA) level on the incidence of dementia. This large population-based prospective cohort study with 12 years of follow-up demonstrated that high SUA levels were associated with increased risk of dementia, especially vascular or mixed dementia. However, some issues deserve comment. The results reported by Latourte *et al* differ from those of another prospective population-based cohort study,[2] which demonstrated that higher SUA levels are associated with a decreased risk of dementia and better cognitive function later in life, suggesting a possible protective role for uric acid as an antioxidant. First, traditional cardiovascular risk factors, such as age, hypertension, and diabetes mellitus , had a much higher incidence in the group with high SUA levels than in the group without high SUA levels.[1] A positive association between cardiovascular risk factors and dementia has been extensively reported.[3 4] Moreover, a positive association between cardiovascular risk factors and SUA levels has also been reported.[5] SUA is an important risk factor for cardiovascular diseases, which are major risk factors for dementia. Therefore, we cannot exclude the possibility that the positive association between SUA and dementia may depend on the concordant effect of confounding risk factors, even though statistical significance remained after adjustment for traditional cardiovascular risk factors. In addition, the confounding effect of additional unmeasured variables (eg, dietary purine intake, fructose ingestion, exercise and level of education) on the association between SUA and dementia cannot be excluded. In this regard, the association might be a consequence of the confounding effects. Second, as the authors noted, SUA levels were assayed only at baseline. Measuring SUA once may not reflect long-term or lifelong exposure. We cannot be certain that SUA levels remained stable over time based on a single assessment. The lack of multiple assessments during follow-up makes it difficult to rely on the results of the study. Future studies should examine changes in SUA at different periods in life to determine their relationship with cognitive decline and dementia. Although we respect the great work done by the authors, the study should be interpreted with the limitations mentioned above. We believe that no conclusive causal relationship has been established, and that further research is needed to clarify any definitive role of SUA in dementia.

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Response to: 'Uric acid and incident dementia: a population-based cohort study' by Lee and Song

We thank Drs Lee and Song for their interest and comments concerning our recent population-based cohort study investigating the relationship between serum uric acid (SUA) levels and dementia in elderly patients.[1 2] They shared their concerns about the association that we found between higher SUA levels at baseline and an increased risk of incident vascular or mixed dementia. As discussed in our paper, these findings contradict a previous prospective study conducted in the Rotterdam cohort, which suggested that higher SUA levels were associated with a decreased risk of dementia and better cognitive function later in life.[3]

Drs Lee and Song highlighted that the association between higher SUA levels and dementia might be mediated by cardiovascular risk factors. This is indeed one of the key message of our study, in which we found a stronger association with vascular or mixed dementia, a subtype of dementia with a vascular component, than with Alzheimer's disease.[1] Whereas the association was maintained after adjustment for traditional cardiovascular risk factors, it was no longer significant after adjustment for interim strokes, suggesting a mediating effect of cerebrovascular disease. We also found a non-significant trend for an association between higher SUA levels and brain MRI markers of cerebrovascular burden, consistently with previous data.[4–6] Also, a previous study reported that the association between higher SUA levels and cognitive impairment was mediated by cerebral ischaemia.[7] The relationship between uric acid (UA) and vascular dementia is also indirectly supported by the association between hyperuricaemia and several cardiovascular risk factors, such as hypertension.[8] We agree that some dietary factors which were not evaluated in our study might be of importance, and this remains a topic for future research.

Development of dementia results from a long and silent preclinical process. In this perspective, we have also discussed the lack of multiple measurements of SUA levels over time as a limitation of our study. The sample of participants taking urate-lowering therapy (ULT) was also too small to evaluate its impact in our study. These are common issues in the field, which also apply to the Rotterdam study.[3] A recent study by Singh *et al* found no significant association between initiation of ULT and the risk of subsequent dementia, but the specific impact of SUA levels was not investigated.[9] We concur with Drs Lee and Song that future research should consider change in SUA levels over time, especially under ULT, to better understand the impact of UA on dementia.

Finally, we would like to emphasise that the antioxidant properties of UA are still debated.[10 11] A recent preclinical study found that UA might have neurotoxic effects.[12] Two recent meta-analyses of mostly cross-sectional studies did not establish a clear relationship between UA and neurodegenerative dementia.[13 14] Thus, we agree with Drs Lee and Song that no causal relationship between UA and Alzheimer's disease can be formally established to date, but our study supports an association with vascular or mixed dementia. There is undoubtedly a strong need for further research to better understand the role of SUA in the pathogenesis of each subtype of dementia. Until then, we believe that prescribers should continue to treat hyperuricaemia whenever it is appropriate.

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Correction: *European League Against Rheumatism/American College of Rheumatology classification criteria for adult and juvenile idiopathic inflammatory myopathies and their major subgroups*

Lundberg IE, Tjärnlund A, Bottai M, *et al*. 2017 European League Against Rheumatism/American College of Rheumatology classification criteria for adult and juvenile idiopathic inflammatory myopathies and their major subgroups. *Ann of Rheum Dis* 2018;76:1955–64.

The sentence on page 1957, right column:

Probability of IIM including muscle biopsy=1/[1+exponential(5.33–score)]
or,
Probability of IIM without muscle biopsy=1/[1+exponential(6.49–score)]
should read as:
Probability of IIM without muscle biopsy=1/[1+exponential(5.33–score)]
or,
Probability of IIM including muscle biopsy=1/[1+exponential(6.49–score)]

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