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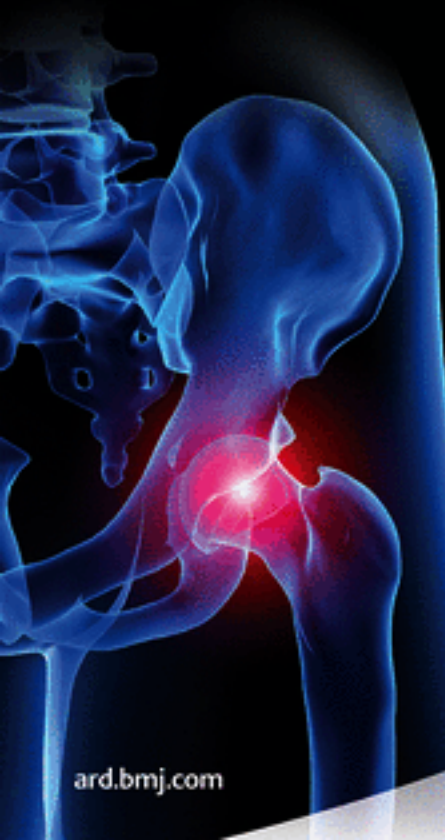

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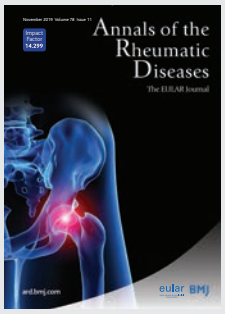

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Shared genetic factors and their causality in autoimmune diseases

Kazuhiko Yamamoto [1], Yukinori Okada[2]

Autoimmune diseases including rheumatic diseases affect 3%–10% of the world population. The frequency of incidence is dependent on the geographical area, ethnicity, sex and environmental factors.[1] Except for rare monogenic diseases, the majority of autoimmune diseases are defined as a complex trait disorder, where multiple genetic and environmental factors interact. For example, twin studies have estimated that the heritability of rheumatoid arthritis (RA) is ~60%.[2 3] RA is classified into anti-citrullinated protein antibodies (ACPA) positive and negative subtypes. The above-mentioned heritability of RA applies primarily to patients with RA who are positive for ACPAs, whereas the heritability of seronegative RA appears to be low,[4] suggesting the differences in the genetic background of these two conditions. Therefore, although the classification criteria are satisfied, each autoimmune disease appears to be heterogeneous. On the other hand, autoimmune diseases share several symptoms, such as Raynaud phenomenon and arthritis. In a clinical situation, the terms 'poly-autoimmunity' and 'familial autoimmunity' are used. The former suggests the presence of multiple autoimmune diseases in a single patient, and the latter implies the situation where diverse autoimmune diseases are persistent in a family.

Genes in the major histocompatibility complex (MHC) have been reported to be the strongest single genetic effect in the majority of autoimmune diseases. Although the precise mechanisms remain to be discovered, these MHC loci are the first and the most robust examples of shared genetic factors among several autoimmune diseases. Since 2007, rapid advances in genome-wide association study (GWAS) have enhanced the identification of hundreds of genetic risk factors for many complex diseases. Thereafter, one possible approach to expanding our understanding of the genetics of autoimmune disease using GWAS data is cross-trait analyses to identify genetic correlations with other complex traits. In fact, it has been reported that genetic variants associated with one autoimmune disease likely confer risk to other diseases (ie, pleiotropy).[5] For example, an interesting approach for a GWAS meta-analysis examining RA and coeliac disease identified a number of loci with pleiotropic effects.[6]

Recently, two large studies combining several diseases were performed. Li *et al* conducted a meta-analysis of 10 paediatric autoimmune diseases.[7] The authors stated that among the associated loci identified, 81% were shared by at least two autoimmune diseases, indicating a relatively high rate of sharing. On the other hand, Ellinghaus *et al* performed a subset-based meta-analysis of five closely associated seronegative inflammatory diseases including ankylosing spondylitis, Crohn's disease, psoriasis, primary sclerosing cholangitis (PSC) and ulcerative colitis.[8] The authors evaluated whether comorbidity is due to pleiotropy (sharing of risk alleles by multiple diseases) or heterogeneity (a subgroup of one disease has a higher load of risk alleles for another disease). Their analysis suggests that the increased comorbidity rates among patients are due to biological pleiotropy rather than heterogeneity. For example, regarding the high prevalence of inflammatory bowel disease (IBD; Crohn's disease and ulcerative colitis) in PSC, the authors speculate that PSC-IBD is a unique disease that shares some genetic factors with ulcerative colitis but distinct from classical IBD phenotypes. These pieces of information support the presence of shared pathological pathways as the basis for clinical co-occurrence. The authors proposed that patients with concomitant syndromes are genetically distinct from patients without concomitant syndromes, indicating classical and non-classical subsets based on genetics in one disease.

In *Annals of Rheumatic Diseases*, Acosta-Herrera *et al* have reported their results from a genome-wide meta-analysis of four systemic seropositive rheumatological immune-mediated inflammatory diseases (IMIDs) including systemic sclerosis (SSc), systemic lupus erythematosus (SLE), RA and idiopathic inflammatory myopathies (IIM).[9] Autoantibody production is one of the main features of these diseases. These diseases also share symptoms, modes of progression, environmental risk factors and high rates of familial aggregation. The authors combined previously published GWAS datasets from European-descent populations to find out shared genetic aetiologies. They identified 27 shared loci, five of which were new.

A subset-based meta-analysis yielded 26 single nucleotide polymorphisms (SNPs) associated with at least two systemic IMIDs. The results indicated that 85% of the associated variants were shared by at least three diseases. SLE exhibited 100% association, SSc and IIM shared the majority, but RA was associated with less than half of the 26 SNPs, suggesting that SLE was more likely to be a prototype of these four systemic IMIDs and that RA shared only a part of the features. The associated SNPs are highly enriched in the functional categories of B and T cells, natural killer cells and monocytes. These cells appear to be relevant in systemic seropositive rheumatic IMIDs. The authors further discuss the significant functional categories of skin cells. This could be related to the nature of connective tissue diseases and the possible involvement of epithelial cells transdifferentiated into mesenchymal cells and further to the fibrotic processes. The authors also focused on reasonable gene products as the target of drug repositioning in systemic IMIDs. In fact, we do not have specific treatments for these diseases except for RA. For example, the gene-product of *TYK2* is targeted by tofacitinib and baricitinib, Janus kinase inhibitors. Since an intronic SNP, rs1185725, within *TYK2* is associated with IIM, SLE and SSc, these drugs are reasonable candidates for therapy repositioning in these diseases. In fact, these drugs are now in clinical trials for SLE, SSc and dermatomyositis.

In the clinical practice of rheumatology, accurate diagnosis is the first step which is followed by the administration of proper treatments based on the diagnosis. This process has been successful for several decades. However, we have been encountering several incomprehensive situations. For example, recent biological therapies do not exhibit similar efficiencies and sometimes are ineffective in different individuals with the same diagnosis.[10] In this regard, the majority of diagnoses have been performed using classification criteria developed on the idea

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of 'one disease, one normal distribution'. However, the concept of 'disease' would be more plastic. As discussed above, RA does not appear to be homogenous at least in terms of ACPA positivity, and an IBD-PSC subtype could exist in IBD. Thus, it is possible to propose that disease definition in rheumatic diseases would be flexible based on several different variables including extensive clinical data, genotype, gene expression and available multiple omics.

The above-mentioned shared genetic factors among different autoimmune diseases illustrate the mechanistic basis of shared disease risks. This idea is based on the premise of a common genetic basis of immune-mediated diseases. However, it is also possible that identical risk factors might have different effects in different diseases.[11] For example, recent GWAS data indicate that substantial proportions of risk variants work as expression quantitative locus (eQTL). Further, this eQTL is likely to work in cell-specific manners and also vary depending on various stimulatory conditions. Therefore, the effects of risk variants can be context specific. It is also important to point out that several variants exhibit opposing risk profiles in different diseases. Perhaps, a locus with the same direction of effect for different diseases would be working in a shared mechanism. On the other hand, a locus with an opposite direction of effect might indicate any counteracting disease mechanisms. For autoimmune diseases and other immune-mediated disorders, a certain locus might be involved in 'polarisation' of the immune response such as the modulation of eQTL effects or changing the balance of several cytokines. Both directions of effect would confer important information and thereby help elucidate the complex mechanistic relationship between these diseases. In addition, assessment of a locus especially having a risk with one disease is also important to elucidate pathophysiology specific to the disease. For example, the functional *PADI4* variants have a strong risk with RA,[12] but not with other immune-mediated diseases. To this end, the application of the meta-analysis methodology to handle risk heterogeneity among the multiple phenotypes is recommended to comprehensively capture both shared and opposite directional effects among phenotypes.[13]

GWAS findings indicate that approximately 80% of autoimmune risk variants occur in non-coding regions.[14] As reported previously, the majority of associated variants are enriched in DNase I hypersensitivity site hotspots. These are the regions where chromatin has lost its condensed structure and thus indicate regulatory DNA regions. In fact, disease-associated variants are enriched in enhancers, within transcription start sites and transcription factor binding sites of lymphocytes. It is also important to note that GWAS data themselves have not conclusively identified a causal gene or a causal variant due to conserved linkage disequilibrium blocks. Further genetic, as well as cellular, studies are necessary to finally define the real causal variants. In this regard, it is interesting to know that two signals in the interleukin (*IL2*)/*IL21* locus on chromosome 4q27 are distinct. Type 1 diabetes mapping to *IL2* and other diseases to *IL21*.[15] We note that cross-phenotype analysis such as that by Acosta-Herrera *et al* can contribute to fine-mapping of the causal variants by combining genetic data from multiple diseases.[9]

Regarding several different autoimmune diseases, more than 100 loci have been reported in one disease. Because genetic information implies a clear causal relationship to the disease, how do these facts imply the pathological mechanisms of diseases? Since the effect size (OR) of each risk variant is usually small (example OR=1.05–1.5), people often argue against the substantial effect of each variant. In this regard, the combination of multiple risk variants could perturb multiple pathways contributing to several alterations of immune functions. Finally, multiple such alterations influence the overall risk of disease. It is also possible that each individual variant can have a large molecular effect in the pathway of immune function but only account for a small fraction of disease susceptibility as a whole.[16] In fact, we have demonstrated that RA biological genes, which were identified by a trans-ethnic meta-analysis of GWAS and subsequently predicted in silico, are the targets of approved therapies for RA, and thus further suggested that drugs approved for other indications may be repurposed for the treatment of RA.[14] Acosta-Herrera *et al* also discuss the possible drug repositioning to SLE, SSc and dermatomyositis as described above. Further, as GWAS predicted that psoriasis, IBD and AS could be the leading disease indications for anti-IL-17 and anti-IL-23 treatments, several of these treatments have been in fact approved and some are now in the midst of ongoing clinical studies.[17 18] All these facts suggest that risk variants with small effect sizes can be really the target of therapy because of their causalities and that genetics of rheumatology should contribute to drug development.

Recently, Witoelar and others have reported genome-wide pleiotropy between Parkinson's disease and autoimmune diseases.[19] The authors performed a systematical investigation of pleiotropy between Parkinson's disease and type 1 diabetes, Crohn's disease, ulcerative colitis, RA, coeliac disease, psoriasis and multiple sclerosis and found 17 novel significant loci. Especially, there was considerable overlap between Parkinson's disease and Crohn's disease, ulcerative colitis and RA. These findings strengthen the hypothesis that risk genes of Parkinson's disease might contribute to the diseases through immune system disturbance. Further, it also emphasises on the importance of cross-phenotype analyses between different complex phenotypes. Through these analyses, we might find common mechanisms among several autoimmune diseases with different phenotypes, such as shared regulatory mechanisms of transcription factor operated with the Epstein-Barr virus EBNA2 protein across risk loci as described by Harley *et al*.[20]

Since the majority of the investigation on shared genetic factors have been conducted using European populations, it would also be important to conduct multi-ethnic cross-phenotype analyses. The distribution of genetic variants and compositions of the disease sub-phenotypes would be heterogeneous among ethnicity, and thus, such efforts should contribute fine-mapping of the causal variants as well as identification of novel risk genes and implicated pathways. Another point that could be assessed is polygenic risk score (PRS) analysis. PRS calculates a disease risk score for each individual according to thousands of the genome-wide SNPs with moderate risk identified by the GWAS. Recent studies demonstrated that PRS estimated from the large-scale GWAS results could be used as a proxy variable to predict disease susceptibility of the individuals,[21] suggesting its critical importance to the future of clinical medicine. Currently, PRS is mostly analysed and applied for every single phenotype separately. As a next step, parallel calculation and phenotype comparison of PRS should reflect both shared and opposite mechanisms of rheumatic diseases, as well as differential diagnosis of the affected diseases of the individuals.

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REFERENCES

1 Cooper GS, Bynum MLK, Somers EC. Recent insights in the epidemiology of autoimmune diseases: improved prevalence estimates and understanding of clustering of diseases. *J Autoimmun* 2009;33:197–207.
2 MacGregor AJ, Snieder H, Rigby AS, *et al*. Characterizing the quantitative genetic contribution to rheumatoid arthritis using data from twins. *Arthritis Rheum* 2000;43:30–7.
3 Cho JH, Gregersen PK. Genomics and the multifactorial nature of human autoimmune disease. *N Engl J Med* 2011;365:1612–23.
4 Padyukov L, Seielstad M, Ong RTH, *et al*. A genome-wide association study suggests contrasting associations in ACPA-positive versus ACPA-negative rheumatoid arthritis. *Ann Rheum Dis* 2011;70:259–65.
5 Pickrell JK, Berisa T, Liu JZ, *et al*. Detection and interpretation of shared genetic influences on 42 human traits. *Nat Genet* 2016;48:709–17.
6 Zhernakova A, Stahl EA, Trynka G, *et al*. Meta-analysis of genome-wide association studies in celiac disease and rheumatoid arthritis identifies fourteen non-HLA shared loci. *PLoS Genet* 2011;7:e1002004.
7 Li YR, Li J, Zhao SD, *et al*. Meta-analysis of shared genetic architecture across ten pediatric autoimmune diseases. *Nat Med* 2015;21:1018–27.
8 Ellinghaus D, Jostins L, Spain SL, *et al*. Analysis of five chronic inflammatory diseases identifies 27 new associations and highlights disease-specific patterns at shared loci. *Nat Genet* 2016;48:510–8.
9 Acosta-Herrera M, Kerick M, González-Serna D, *et al*. Genome-wide meta-analysis reveals shared new *loci* in systemic seropositive rheumatic diseases. *Ann Rheum Dis* 2019;78:311–9.
10 Smolen JS, Aletaha D, Barton A, *et al*. Rheumatoid arthritis. *Nat Rev Dis Primers* 2018;4.
11 Jonkers IH, Wijmenga C. Context-specific effects of genetic variants associated with autoimmune disease. *Hum Mol Genet* 2017;26:R185–R192.
12 Suzuki A, Yamada R, Chang X, *et al*. Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis. *Nat Genet* 2003;34:395–402.
13 Lee CH, Eskin E, Han B. Increasing the power of meta-analysis of genome-wide association studies to detect heterogeneous effects. *Bioinformatics* 2017;33:i379–88.
14 Okada Y, Wu D, Trynka G, *et al*. Genetics of rheumatoid arthritis contributes to biology and drug discovery. *Nature* 2014;506:376–81.
15 Todd JA. Etiology of type 1 diabetes. *Immunity* 2010;32:457–67.
16 Cotsapas C, Hafler DA. Immune-mediated disease genetics: the shared basis of pathogenesis. *Trends Immunol* 2013;34:22–6.
17 Duerr RH, Taylor KD, Brant SR, *et al*. A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. *Science* 2006;314:1461–3.
18 Liu Y, Helms C, Liao W, *et al*. A genome-wide association study of psoriasis and psoriatic arthritis identifies new disease loci. *PLoS Genet* 2008;4:e1000041.
19 Witoelar A, Jansen IE, Wang Y, *et al*. Genome-wide pleiotropy between Parkinson disease and autoimmune diseases. *JAMA Neurol* 2017;74:780–92.
20 Harley JB, Chen X, Pujato M, *et al*. Transcription factors operate across disease loci, with EBNA2 implicated in autoimmunity. *Nat Genet* 2018;50:699–707.
21 Khera AV, Chaffin M, Aragam KG, *et al*. Genome-wide polygenic scores for common diseases identify individuals with risk equivalent to monogenic mutations. *Nat Genet* 2018;50:1219–24.

Getting to the heart of the matter: detecting and managing cardiac complications in systemic sclerosis

John Varga ,[1] Daniel C Lee[2]

Cardiac complications of systemic sclerosis (SSc) are well-deservedly gaining the attention of the medical community. This increased interest is driven by technological advances in non-invasive imaging technologies such as tissue Doppler echocardiography and cardiac MR (CMR) that reveal frequent involvement of the heart and its clinical importance in SSc. Historically, autopsy studies in SSc indicate a high prevalence (>50%) of cardiac fibrosis,[1] and clinically evident cardiac involvement has long been considered a poor prognostic marker in SSc.[2 3] Indeed, the European Clinical Trials and Research Group reported that 26% of SSc disease-related mortality could be attributed to cardiac causes.[4] However, the overall prevalence of cardiac disease in SSc is unknown, in part due to the lack of a consensus definition, classification of 'subclinical' cardiac involvement, and the plethora of diverse approaches employed for its detection.[5]

The cardiac complications of SSc encompass multiple distinct entities including primary cardiac involvement manifested by diastolic dysfunction (an early manifestation of cardiac fibrosis), heart failure with preserved ejection faction, conduction blocks and arrhythmias, myocarditis, as well as pericardial disease; and cardiac involvement secondary to systemic or pulmonary arterial hypertension, renal failure, amyloidosis and other SSc complications. The pathogenesis of SSc-associated primary cardiac involvement is not well understood, and likely encompasses small vessel damage, vasoconstriction and chronic ischaemia-reperfusion injury, cardiac inflammation and fibrosis.

Although systolic dysfunction in SSc is uncommon, diastolic dysfunction is common and predicts poor outcomes. In an observational study of 153 consecutive SSc patients, we showed that 23% had echocardiographically defined left ventricular diastolic dysfunction (often asymptomatic), and its presence was predictive of mortality.[6] A recent study in a large and unselected cohort of SSc patients similarly found that the incidence of diastolic dysfunction was 17% at baseline, and increased to 29% during follow-up of 3.4 years.[7] Significantly, mortality in this group was increased more than fourfold compared with that in SSc patients without evidence of diastolic dysfunction at baseline, underscoring the clinical significance of diastolic dysfunction, and the need for its early recognition.

The prevalence of cardiac involvement in SSc is even higher when screening using CMR. In a study of 62 SSc patients with no prior heart disease or coronary artery disease (CAD) risk factors, 45% had myocardial fibrosis assessed by late gadolinium-enhanced CMR.[8] Moreover, despite the absence of significant epicardial coronary stenosis by CT coronary angiography, stress CMR perfusion imaging revealed subendocardial perfusion defects (a sign of impaired microvascular perfusion) in 79% of the patients. In a prospective study of 201 patients with SSc without known cardiac involvement, cardiac fibrosis was detected by late gadolinium enhancement CMR in 27.9%, most of whom had no evidence of cardiac abnormalities by echocardiography.[9] The presence of late gadolinium enhancement was correlated by ventricular arrhythmias. Even without late gadolinium enhancement, the extracellular volume (ECV) fraction (a CMR index of diffuse myocardial fibrosis) is higher in SSc patients compared with controls.[10] These observations provide strong evidence of the high prevalence of clinical—and subclinical—cardiac involvement in SSc patients.

Given the high prevalence and clinical impact of cardiac involvement in SSc, deeper understanding of its natural history and treatment are sorely needed. In this issue of *Annals of the Rheumatic Diseases*, Valentini *et al* present findings from the DeSScipher (to decipher the optimal treatment of SSc) cohort study evaluating the impact of aspirin and vasodilator therapy on myocardial disease in SSc.[11] This large multicentre observational and event-driven study enrolled 654 SSc patients from 20 sites. Patients underwent thorough cardiac monitoring (exceeding current clinical practice), including history and physical exam at baseline and every 3 months, along with ECG, Holter and echocardiographic evaluations at baseline and every 6 months. Patients were not preselected for cardiac involvement, so the population represents a primary prevention cohort. Patients were classified as receiving vasodilator therapy if they received an ACE inhibitor, an angiotensin II receptor blocker or a calcium channel blocker in some combination. Some 20% also received targeted vasodilator therapy (prostanoids, endothelin receptor antagonists and phosphodiesterase type 5 inhibitors). Outcome measures included occurrence of (1) ventricular arrhythmias (considered a sign of myocardial ischaemia), (2) Q waves, cardiac blocks and/or pacemaker implantation (considered a sign of fibrosis) and (3) left ventricular ejection fraction <55% and/or congestive heart failure (considered a sign of progressive myocardial disease). The results showed that while vasodilator therapy was not associated with measured outcomes on univariate analysis, on multivariate analysis vasodilator treatment was associated with lower incidence of ventricular arrhythmia. Additionally, low-dose aspirin was associated with a lower incidence of the combined endpoint (Q waves, conduction blocks and/or pacemaker implantation) in univariate and multivariate analysis.

These important results need to be placed in the context of the limitations of the study. Foremost is the observational nature of the study. Assignment to treatment groups was not randomised, raising the distinct possibility that unmeasured differences between the treatment groups may have confounded the results. Moreover, the outcome measures selected were not standard for a cardiac outcome study, and the relatively low incidence of individual outcomes required combination into groups based on presumed (although not proven) pathophysiology. Nonetheless, the study raises interesting and timely questions regarding the management of SSc-associated cardiac disease.

First, should low-dose aspirin be routinely prescribed to SSc patients? The mechanism by which Q waves (the sine qua non for diagnosing myocardial infarction by ECG) are prevented by aspirin would presumably be via platelet inhibition or its anti-inflammatory properties. However, for the primary prevention of CAD in non-SSc cohorts, recent large trials and meta-analyses have demonstrated that the benefits of ASA do not outweigh bleeding risks except

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for in high-risk individuals, for example, those with 10-year atherosclrotic cardiovascular disease risk >15%.[12] The limited data on ischaemic events in SSc suggest that incidence is higher than in matched controls, but the 10-year risk of ischaemic events is likely <10%.[13 14] Cardiac blocks and pacemaker implantation are signs of conduction system disease, which in SSc patients is more commonly due to fibrosis of the conduction system but could also be due to ischaemic injury. Did the patients in this study actually have myocardial scar? Because the characteristic patchy, mid-myocardial fibrosis of SSc detected by CMR rarely causes wall motion abnormalities, routine echocardiography is not sufficient to detect its presence.

What about vasodilator therapy? Autopsy studies of SSc patients have demonstrated a high prevalence of contraction band necrosis (a sign of ischaemic injury) despite the absence of epicardial coronary artery disease.[1] The presumed mechanism of ischaemic injury is either vasospasm or small vessel disease. This provides a pathophysiological basis for the potential benefit of vasodilators for prevention of myocardial fibrosis in SSc. The type of ventricular arrhythmias seen in DeSScipher were not sufficiently characterised, but most ventricular arrhythmias can be either ischaemia or scar mediated. The heterogeneous combinations of medications in the vasodilator group makes translation of findings from DeSScipher into clinical recommendations difficult. However, perfusion and scar imaging might help to define the pathophysiological basis of this potential treatment benefit.

These concerns notwithstanding, the DeSScipher investigators should be lauded for embarking on this large, prospective, multicentre observational study of cardiac outcomes in SSc. The results of this study will surely provide more insights into the natural history of cardiovascular complications with continued follow-up of the patients. Hopefully, these observations will inspire further studies of cardiac involvement in SSc, and offer lessons that will enhance future investigations. Although randomised controlled trials evaluating hard events (eg, myocardial infarction, cardiac death) are ultimately required to establish safety and efficacy of therapy, such studies require large numbers and/or long-term follow-up. Prior to embarking on large and expensive pivotal trials, animal studies or smaller patient studies using more sensitive surrogate endpoints may help to better understand underlying biology, and identify the agent (or combination) with maximum therapeutic potential. For example, in a recent study of 44 SSc patients evaluated for haematopoietic stem cell transplantation (HSCT), serial measurement of diffuse myocardial fibrosis by CMR demonstrated significant progression of ECV fraction in patients who did not undergo HSCT, while significant regression was noted in those who did receive HSCT. Furthermore, the changes in ECV were mirrored by changes in modified Rodnan Skin Score.[15] These observations highlight the utility of CMR, and also demonstrate that cardiac fibrosis in SSc is potentially reversible.

Multiple recent studies underscore the high prevalence and significant clinical impact of cardiac involvement in SSc– but also uncover unmet needs. Consensus guidelines for screening, utilisation of advanced imaging and the appropriate management of the entire spectrum of SSc cardiac involvement—from subclinical to overt, from the myocardium to the conduction system to the pericardium—are emerging, but to date are largely based on expert opinion.[16 17] To inform these guidelines, we urge more investigation in this area. Needed are basic research employing animal models of disease to understand the pathogenesis of SSc cardiac disease and identify potential therapeutic targets, and clinical research to understand the risk factors, predictors, natural history and relevant biomarkers of cardiac involvement (including both blood and imaging) for patient stratification and monitoring. Ultimately, controlled clinical trials will be required in order to demonstrate safety and efficacy of novel drugs and treatment strategies for SSc-associated cardiac involvement.

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REFERENCES

1 Bulkley BH, Ridolfi RL, Salyer WR, *et al*. Myocardial lesions of progressive systemic sclerosis. A cause of cardiac dysfunction. *Circulation* 1976;53:483–90.
2 D'Angelo WA, Fries JF, Masi AT, *et al*. Pathologic observations in systemic sclerosis (scleroderma). A study of fifty-eight autopsy cases and fifty-eight matched controls. *Am J Med* 1969;46:428–40.
3 Follansbee WP, Miller TR, Curtiss EI, *et al*. A controlled clinicopathologic study of myocardial fibrosis in systemic sclerosis (scleroderma). *J Rheumatol* 1990;17:656–62.
4 Tyndall AJ, Bannert B, Vonk M, *et al*. Causes and risk factors for death in systemic sclerosis: a study from the EULAR scleroderma trials and research (EUSTAR) database. *Ann Rheum Dis* 2010;69:1809–15.
5 Desai CS, Lee DC, Shah SJ. Systemic sclerosis and the heart: current diagnosis and management. *Curr Opin Rheumatol* 2011;23:545–54.
6 Hinchcliff M, Desai CS, Varga J, *et al*. Prevalence, prognosis, and factors associated with left ventricular diastolic dysfunction in systemic sclerosis. *Clin Exp Rheumatol* 2012;30:S30–7.
7 Tennøe AH, Murbræch K, Andreassen JC, *et al*. Left ventricular diastolic dysfunction predicts mortality in patients with systemic sclerosis. *J Am Coll Cardiol* 2018;72:1804–13.
8 Rodriguez-Reyna TS, Morelos-Guzman M, Hernandez-Reyes P, *et al*. Assessment of myocardial fibrosis and microvascular damage in systemic sclerosis by magnetic resonance imaging and coronary angiotomography. *Rheumatology* 2015;54:647–54.
9 Gargani L, Todiere G, Guiducci S, *et al*. Early Detection of Cardiac Involvement in Systemic Sclerosis: The Added Value of Magnetic Resonance Imaging. *JACC Cardiovasc Imaging* 2019;12:927–8.
10 Lee DC, Hinchcliff ME, Sarnari R, *et al*. Diffuse cardiac fibrosis quantification in early systemic sclerosis by magnetic resonance imaging and correlation with skin fibrosis. *J Scleroderma Relat Disord* 2018;3:159–69.
11 Valentini G, Huscher D, Riccardi A, *et al*. Vasodilators and low-dose acetylsalicylic acid are associated with a lower incidence of distinct primary myocardial disease manifestations in systemic sclerosis: results of the DeSScipher inception cohort study. *Ann Rheum Dis* 2019;78:1576–82.
12 Chiang KF, Shah SJ, Stafford RS. A practical approach to low-dose aspirin for primary prevention. *JAMA* 2019;322.
13 Chu S-Y, Chen Y-J, Liu C-J, *et al*. Increased risk of acute myocardial infarction in systemic sclerosis: a nationwide population-based study. *Am J Med* 2013;126:982–8.
14 Man A, Zhu Y, Zhang Y, *et al*. The risk of cardiovascular disease in systemic sclerosis: a population-based cohort study. *Ann Rheum Dis* 2013;72:1188–93.
15 Lee DC, Shah SJ, Benefield BC, *et al*. *Abstract 17650: myocardial fibrosis in systemic sclerosis, progression and response to therapy measured by cardiovascular magnetic resonance*. Anaheim, California: Circulation, 2017: 136. A17650.
16 Bissell L-A, Anderson M, Burgess M, *et al*. Consensus best practice pathway of the UK systemic sclerosis Study group: management of cardiac disease in systemic sclerosis. *Rheumatology* 2017;56:912–21.
17 Farge D, Burt RK, Oliveira M-C, *et al*. Cardiopulmonary assessment of patients with systemic sclerosis for hematopoietic stem cell transplantation: recommendations from the European Society for blood and marrow transplantation autoimmune diseases Working Party and collaborating partners. *Bone Marrow Transplant* 2017;52:1495–503.

OPEN ACCESS

CLINICAL SCIENCE

Safety and effectiveness of upadacitinib or adalimumab plus methotrexate in patients with rheumatoid arthritis over 48 weeks with switch to alternate therapy in patients with insufficient response

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ABSTRACT

Background In SELECT-COMPARE, a randomised double-blind study, upadacitinib 15 mg once daily was superior to placebo or adalimumab on background methotrexate (MTX) for treating rheumatoid arthritis signs and symptoms and inhibited radiographical progression versus placebo at 26 weeks. Here we report 48-week safety and efficacy in patients who continued their original medication or were rescued to the alternative medication for insufficient response.

Methods Patients on MTX received upadacitinib 15 mg, placebo or adalimumab for 48 weeks. Rescue without washout, from placebo or adalimumab to upadacitinib or upadacitinib to adalimumab occurred if patients had <20% improvement in tender joint count (TJC) or swollen joint count (SJC) (weeks 14/18/22) or Clinical Disease Activity Index (CDAI) >10 (week 26); remaining placebo patients were switched to upadacitinib at week 26. Efficacy was analysed by randomised group (non-responder imputation), as well as separately for rescued patients (as observed). Treatment-emergent adverse events per 100 patient-years were summarised.

Results Consistent with responses through week 26, from weeks 26 to 48, responses by randomised group including low disease activity, clinical remission and improvements in pain and function remained superior for upadacitinib versus adalimumab; radiographical progression remained lower for upadacitinib versus placebo (linear extrapolation). Although both switch groups responded, a higher proportion of patients rescued to upadacitinib from adalimumab achieved CDAI ≤10 at 6 months postswitch versus patients rescued from upadacitinib to adalimumab. Safety at week 48 was comparable to week 26.

Conclusion Upadacitinib+MTX demonstrated superior clinical and functional responses versus adalimumab+MTX and maintained inhibition of structural damage versus placebo+MTX through week 48. Patients with an insufficient response to adalimumab or upadacitinib safely achieved clinically meaningful responses after switching to the alternative medication without washout.

Key messages

What is already known about this subject?

- In patients with rheumatoid arthritis (RA) who do not respond sufficiently to biologic disease-modifying antirheumatic drug (bDMARD) treatment with Janus kinase inhibitors (JAKi) is efficacious.
- Switching of therapies including switching from a JAKi to a bDMARD may be required to achieve treatment goals.
- In the SELECT-COMPARE study, through 6 months, upadacitinib demonstrated significant improvements in RA signs and symptoms versus placebo and adalimumab and inhibited radiographical progression versus placebo.

What does this study add?

- Consistent with responses through week 26, between weeks 26 and 48 of the SELECT-COMPARE study, responses by randomised group including low disease activity, clinical remission and improvements in pain and function remained superior for upadacitinib versus adalimumab and radiographical progression remained lower for upadacitinib versus placebo. Safety at week 48 was comparable to findings through week 26.
- The SELECT-COMPARE study design incorporated blinded treatment switches within the first 6 months that allocated patients who were not sufficiently responding to randomised treatment to the alternative advanced therapy (ie, insufficient responders to upadacitinib were switched without washout to adalimumab and insufficient responders to adalimumab were switched without washout to upadacitinib). Both switch groups responded, but a higher proportion of patients rescued to upadacitinib from adalimumab achieved Clinical Disease Activity Index ≤10 at 6 months postswitch versus patients rescued from upadacitinib to adalimumab.

Key messages

How might this impact on clinical practice?

- A clinically significant number of patients with an insufficient response to adalimumab or upadacitinib safely achieved clinically meaningful responses after switching to the alternative medication without washout.
- Responses were maintained with upadacitinib treatment over 48 weeks and were consistently significantly better with upadacitinib than with adalimumab.
- No new safety findings were observed with longer-term exposure to upadacitinib or in the period following the switch from adalimumab to upadacitinib.

INTRODUCTION

The goal of treatment for rheumatoid arthritis (RA) is to maximise patient outcomes including preventing structural damage and subsequent loss of function. A treat-to-target strategy optimises treatment until clinical remission (or low disease activity (LDA)) is achieved and maintained, which improves long-term prognosis.[1–3] Methotrexate (MTX) is the recommended initial treatment; however, in patients who are intolerant or have an inadequate response, a second conventional synthetic disease-modifying antirheumatic drug (csDMARD), biologic DMARD (bDMARD) or targeted synthetic DMARD (tsDMARD) should be added.[4 5]

Upadacitinib, an oral Janus kinase (JAK) inhibitor engineered to have greater selectivity towards JAK1 than JAK2, JAK3, or TYK2, has demonstrated efficacy in five phase III trials.[6–10] The primary results of SELECT-COMPARE through 26 weeks demonstrated that 15 mg of upadacitinib once daily was superior to placebo and adalimumab for clinical and functional outcomes, including clinical remission and LDA, and was superior to placebo for inhibition of radiographical progression in MTX inadequate responders.[10] Here we report blinded long-term safety and efficacy for upadacitinib versus adalimumab through 48 weeks from SELECT-COMPARE. Importantly, SELECT-COMPARE was uniquely designed to explore a randomised, blinded switch to a JAK inhibitor from a tumour necrosis factor (TNF) inhibitor in patients with insufficient response (and vice versa) without a washout period; similar data have not been reported to date.

PATIENTS AND METHODS

Patients

Inclusion criteria have been described previously.[10] Patients were ≥18 years of age with RA receiving stable MTX background therapy with continued active joint disease, had a high-sensitivity C-reactive protein (hsCRP) ≥5 mg/L and evidence of erosive disease and/or seropositivity.

Study design

Patients were blindly randomised 2:2:1 to upadacitinib 15 mg once daily, placebo or adalimumab (Humira) 40 mg every other week, with stable background MTX (online supplementary figure 1). Blinded rescue treatment, without washout but with background MTX, from placebo and adalimumab to upadacitinib, and upadacitnib to adalimumab occurred at weeks 14, 18 or 22 for patients with <20% improvement from baseline in tender or swollen joints. At week 26, all remaining placebo patients and those not meeting LDA by Clinical Disease Activity Index (CDAI ≤10) receiving adalimumab were rescued to upadacitinib, while those receiving upadacitinib were rescued to adalimumab.

Patients provided written informed consent. AbbVie sponsored the study and collaborated with the academic authors to design the study. AbbVie and the academic authors analysed the data, interpreted the results and prepared, reviewed and approved the final version; AbbVie provided writing support. All the authors approved the final submission and attest to its accuracy.

Assessments

Efficacy through week 48 was assessed by initial randomised group for: meeting improvement in American College of Rheumatology (ACR) response criteria (ACR20/50/70), change from baseline in individual components of ACR response, proportions achieving LDA defined by CDAI ≤10 or Simplified Disease Activity Index (SDAI ≤11); clinical remission defined by CDAI (≤2.8) or SDAI (≤3.3), ACR/European League Against Rheumatism Boolean remission (swollen 28-joint count (SJC28) ≤1, tender 28-joint count (TJC28) ≤1, hsCRP ≤1 (mg/dL), patient's global assessment of disease activity (PGA) ≤1 (on a 0–10 cm Visual Analogue Scale), DAS28(CRP) <2.6 or ≤3.2 and change from baseline in 36-Item Short Form Survey (SF-36) physical component summary (PCS) and morning stiffness duration. For rescued patients, the achievement of CDAI remission and LDA, DAS28(CRP) <2.6 and ≤3.2 were assessed 3 and 6 months (±2 weeks) postswitch.

Assessments of X-rays of hands and feet were conducted at baseline, weeks 14 (for rescued patients), 26 and 48 and included mean change from baseline in van der Heijde's modification of the Total Sharp Score (mTSS),[11 12] joint space narrowing (JSN), Erosion Score (ES) and the proportion of patients with no radiographical progression (defined as change from baseline in mTSS ≤0) versus placebo. Similar to the first reading session of the 6 month X-rays, radiographs were also reviewed by two independent readers blinded to treatment and sequence at a second session at week 48 (reported here).

Safety reports are based on available data as of 6 July 2018, when all patients completed their 48-week visit. As of this cut-off date, safety data for some patients extends past week 48 as patients could subsequently continue into a long-term extension (LTE). Investigator-reported treatment-emergent adverse events (AEs) are summarised for events occurring while exposed to upadacitinib or adalimumab, based on the treatment received at the time of the event ('any upadacitinib', 'any adalimumab'). Exposure-adjusted event rates (EAER) are reported (events/100 patient-years (PY)), and exposure adjusted incidence rates (EAIR) are reported for deaths, major adverse cardiovascular events (MACE) and venous thromboembolic events (VTEs). AEs were coded per the Medical Dictionary for Regulatory Activities (MedDRA), V.19.1, and AEs and laboratory changes were graded using the Rheumatology Common Toxicity Criteria V.2.0 .[13] Changes in creatine phosphokinase (CPK) and creatinine were graded using the Common Toxicity Criteria of the National Cancer Institute.[13 14] Cardiovascular events including MACE and VTE were blindly adjudicated by an independent, external Cardiovascular Adjudication Committee using predefined event definitions. Laboratory changes from baseline to each visit are reported in patients on continuous upadacitinib or adalimumab through the week 48 visit.

Statistical analysis

Efficacy is reported by the three initial randomised groups. Analyses were conducted in the full analysis set, including all randomised patients who had received at least one dose of study drug. For binary endpoints, treatments were compared using the

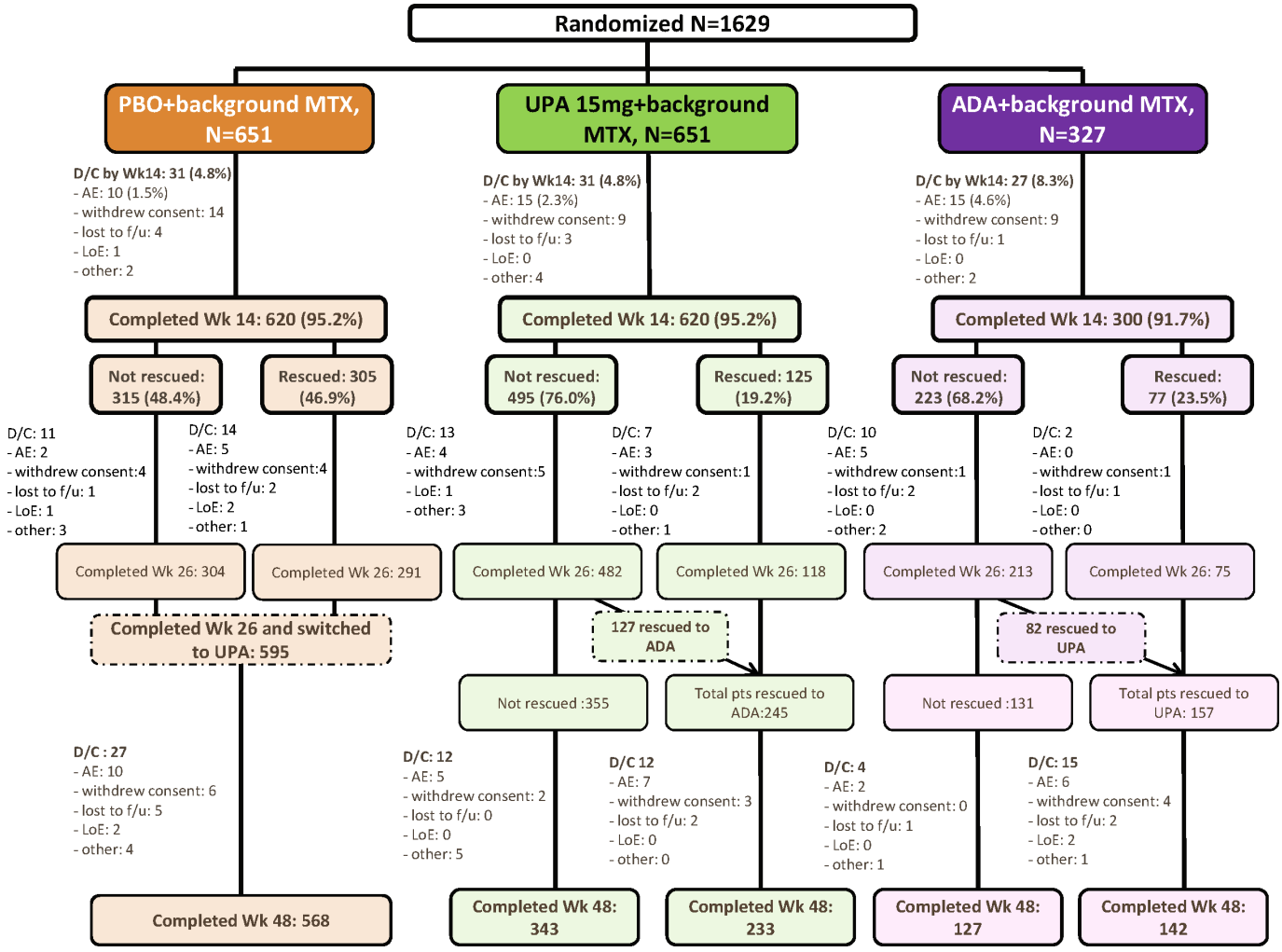

Figure 1 Disposition of patients through week 48. At weeks 14, 18 and 22, patients who had <20% improvement in 66 swollen joint count or 68 tender joint count were rescued. At week 26, patients with CDAI >10 were rescued. Regardless of CDAI low disease activity achievement at week 26, all remaining PBO patients were switched to UPA. ADA, adalimumab; AE, adverse events; CDAI, Clinical Disease Activity Index; PBO, placebo; MTX, methotrexate; UPA, upadacitinib.

Cochran-Mantel-Haenszel (CMH) test, adjusting for the stratification factor of prior bDMARD use (yes, no). Non-responder imputation (NRI) was used for missing data and for observations after rescue for patients rescued at weeks 14, 18 or 22; last observation carried forward (LOCF) was used for observations after rescue for patients rescued at week 26. For continuous endpoints, analyses were conducted using the analysis of covariance (ANCOVA) model including treatment, the corresponding baseline value and the stratification factor of prior bDMARD use (yes, no). LOCF was used for observations after rescue treatment for patients rescued at weeks 14–26. For the radiographical endpoints, ANCOVA and CMH analyses were conducted as above. Linear extrapolation was used for missing data and rescue/switch handling.

For patients who switched treatments, as-observed analyses were conducted. Data on the proportions of patients achieving LDA or remission by CDAI, DAS28(CRP) ≤3.2, DAS28(CRP) <2.6, at 3 and 6 months (±2 weeks) after rescue are summarised.

RESULTS

Disposition

One thousand six hundred and twenty-nine patients were randomised at baseline (figure 1). Among 651 randomised to upadacitinib, 252 (39%) were rescued to adalimumab (125 (19%) for <20% improvement in SJC or TJC between weeks 14 and 22 and 127 (20%) at week 26 for CDAI >10). Among 327 patients randomised to adalimumab, a higher proportion compared with upadacitinib were rescued (159 (49%): 77 (24%) for <20% improvement in SJC or TJC and 82 (25%) for CDAI >10 at week 26). Among 651 randomised to placebo, 305 (47%) were rescued for <20% improvement in SJC or TJC; 303 were switched to upadacitinib 15 mg at week 26 per protocol.

Efficacy by randomised groups

Over 48 weeks, upadacitinib was superior versus adalimumab for ACR20/50/70 responses (online supplementary figure 2); ACR20/50/70 at week 48 was achieved by 65/49/36% and 54/40/23% of patients randomised to upadacitinib and adalimumab (both plus background MTX), respectively ($p<0.01$ for upadacitinib vs adalimumab). Similar significant results were observed for each ACR core component except SJC (online supplementary figure 3). At week 48, mean improvements from baseline in Health Assessment Questionnaire Disability Index (HAQ-DI) were −0.73 and −0.60 ($p<0.01$) with 62% versus 52% achieving the minimum clinically important difference (less than or equal to −0.22, $p<0.01$) for upadacitinib versus

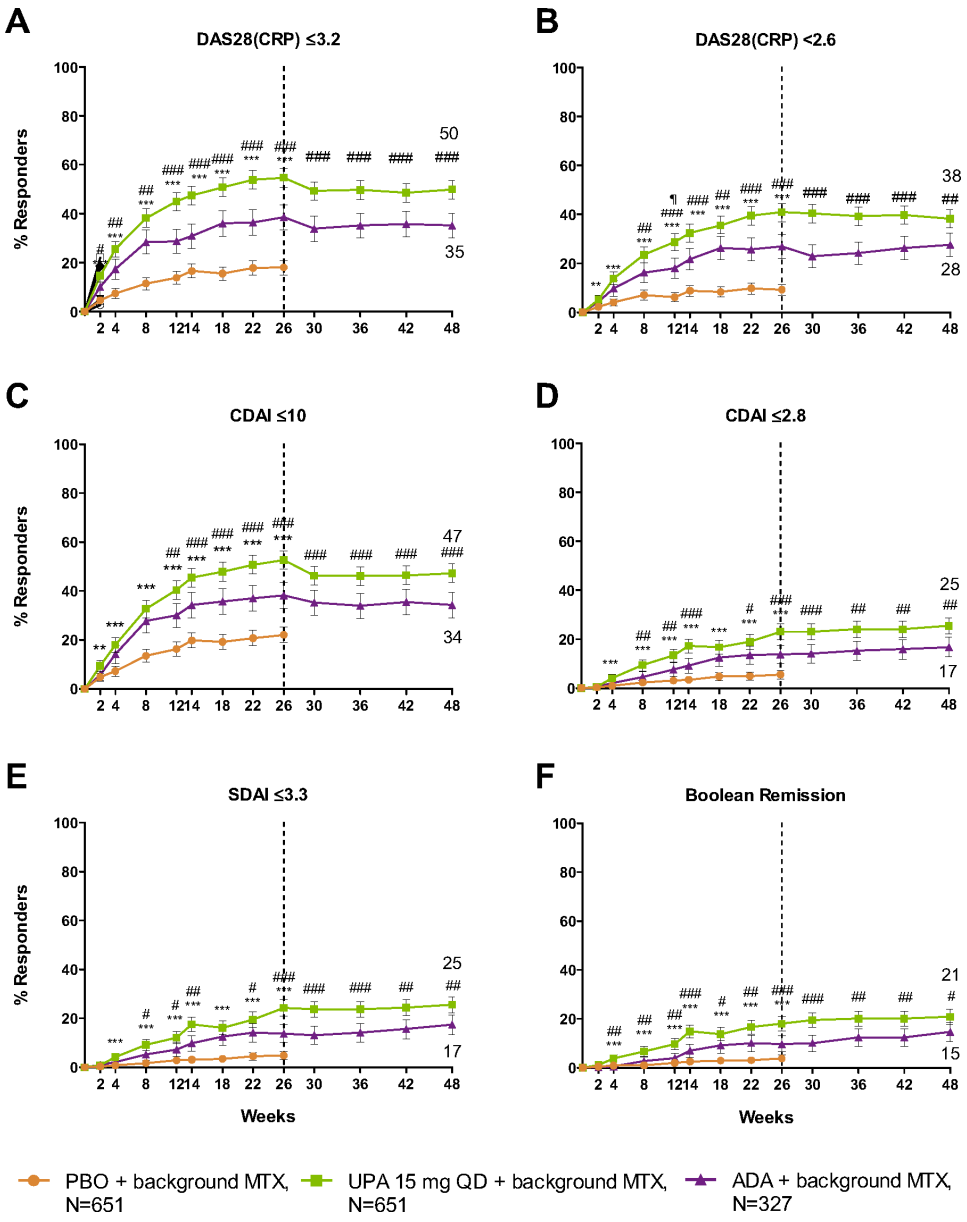

Figure 2 Proportions of patients achieving disease activity states over 48 weeks (NRI). Vertical line at week 26 indicates the end of the PBO-controlled period. Treatment groups are by initial randomisation. *p≤0.05; **p≤0.01; ***p≤0.001 for comparison of UPA versus PBO; #p≤0.05; ##p≤0.01; ###, p≤0.001 for comparison of UPA versus ADA. Error bars reflect 95% CI.¶Indicates multiplicity-controlled comparisons of UPA versus PBO. Observations after rescue were handled using NRI (patients rescued at weeks 14–22) and LOCF (patients rescued at week 26) for binary endpoints. ADA, adalimumab; CDAI, Clinical Disease Activity Index; DAS28(CRP), 28-joint Disease Activity Score based on C-reactive protein; LOCF; last observation carried forward; MTX, methotrexate; NRI, non-responder imputation; PBO, placebo; SDAI, Simplified Disease Activity Index; UPA, upadacitinib.

adalimumab, respectively. At week 48, upadacitinib was superior to adalimumab for the reduction in pain (−36.7 vs −32.1, p<0.05), with continued higher improvements in SF-36 PCS, Functional Assessment of Chronic illnesses Therapy-Fatigue (FACIT-F) and duration of morning stiffness (SF-36 PCS: 9.8 vs 8.1, p<0.01; FACIT-F: 10.2 vs 8.9, p<0.05; morning stiffness: −101.7 vs −95.5; online supplementary figure 4).

LDA and clinical remission by CDAI, SDAI and Boolean remission, as well as DAS28(CRP) <2.6/≤3.2, were consistently and statistically significantly superior for upadacitinib versus adalimumab through week 48 (figure 2 and online supplementary figure 5); at week 48, CDAI LDA was achieved by 47% versus 34% of patients randomised to upadacitinib and adalimumab (p<0.001), respectively, CDAI remission by 25% versus 17% (p<0.01) and Boolean remission by 21% versus 15% (p<0.05). DAS28(CRP) <2.6 was achieved by 38% versus 28% of patients randomised to upadacitinib and adalimumab, respectively (p<0.01).

At week 48, based on initial randomised group, using linear extrapolation, mean change from baseline in mTSS, JSN and ES continued to be significantly lower on upadacitinib versus placebo (p<0.001, figure 3A, B). Significantly more patients randomised to upadacitinib (86%) or adalimumab (88%) had no radiographic progression versus placebo (74%) (p≤0.001). Results were consistent using 'as-observed' analyses (online supplementary figure 6).

Efficacy in the switch population

Of the 651 and 327 patients randomised to upadacitinib and adalimumab, 251 (38.6%) were rescued to adalimumab versus

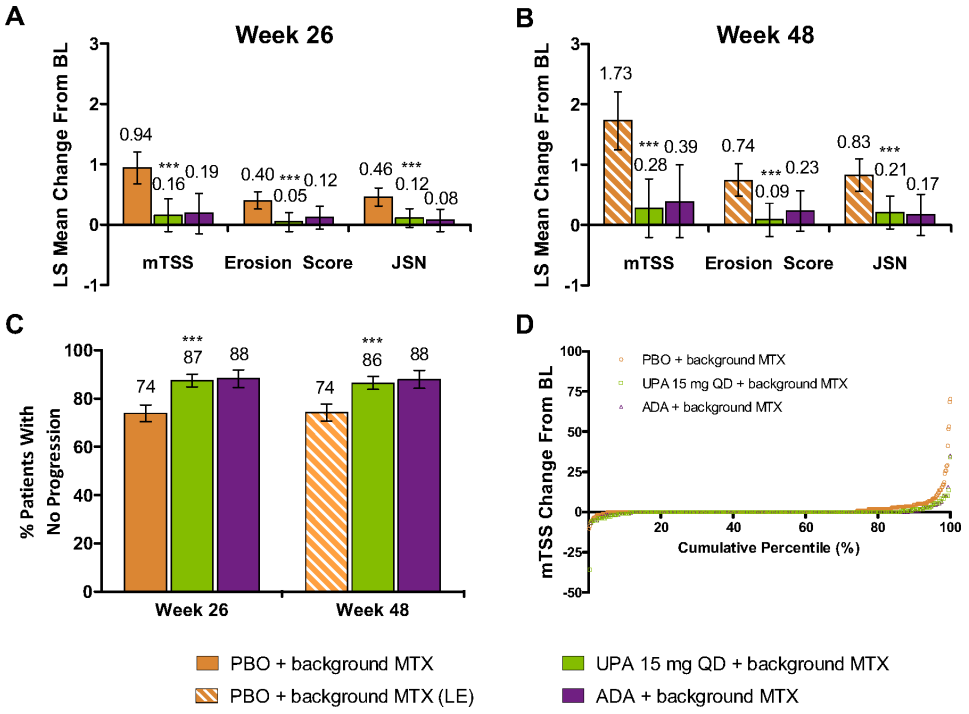

Figure 3 Radiographic progression through week 48 (linear extrapolation). Treatment groups are by initial randomisation. Results based on reading session 2. *p≤0.05; **p≤0.01; ***p≤0.001 for comparison of UPA versus PBO. Error bars reflect 95% CI. For the PBO group, all data at week 48 were imputed by linear extrapolation. X-ray data collected at treatment switching or at discontinuation of PBO (for patients who discontinued PBO) were used for extrapolation. Specifically, for PBO patients who switched to UPA at week 26, the week 26 X-ray was used for extrapolation to impute the data at week 48. For patients randomised to UPA or ADA who were rescued, data at week 48 were also imputed by linear extrapolation using X-ray data collected at treatment switching. Statistical analysis comparing UPA to ADA was not prespecified; nominal p values were not significant. ADA, adalimumab; BL, baseline; JSN, joint space narrowing; PBO, placebo; mTSS, modification of the total Sharp Score; MTX, methotrexate; UPA, upadacitinib.

159 (48.6%) to upadacitinib per the predefined criteria. The demographics of these patients were similar to the overall randomised population. A similar proportion (~90%) of patients in both rescued groups remained in the study at 6 months postswitch (figure 1). Following 6 months of switch treatment in patients rescued from adalimumab to upadacitinib, CDAI remission/LDA was achieved by 15/53% and DAS28(CRP) <2.6/≤3.2 by 35/56%; in patients rescued from upadacitinib to adalimumab, CDAI remission/LDA was achieved in 5/41% and DAS28(CRP) <2.6/≤3.2 was achieved in 21/40%. Improvements in SDAI, HAQ-DI and absolute changes in CDAI, SDAI and DAS28(CRP) were consistent with these results (table 1).

After rescue, responses in many patients were rapid and continued to improve, with a consistently higher magnitude for those switched to upadacitinib from adalimumab versus those switched to adalimumab from upadacitinib (figure 4). Responses in patients who were initially randomised and continued adalimumab or upadacitinib through week 48 were high.

Table 1 Clinical and functional responses in patients who switched treatments, 3 and 6 months postswitch (as observed)

	UPA 15 mg once daily to ADA (N=251)		ADA to UPA 15 mg once daily (N=159)	
n/N (%)	3 months postswitch	6 months postswitch	3 months postswitch	6 months postswitch
DAS28(CRP) ≤3.2	71/233 (30.5)	91/230 (39.6)	77/150 (51.3)	82/147 (55.8)
DAS28(CRP) <2.6	34/233 (14.6)	49/230 (21.3)	45/150 (30.0)	51/147 (34.7)
CDAI ≤10	74/242 (30.6)	95/234 (40.6)	58/148 (39.2)	77/146 (52.7)
CDAI ≤2.8	8/242 (3.3)	12/234 (5.1)	13/148 (8.8)	22/146 (15.1)
SDAI ≤11	69/231 (29.9)	96/229 (41.9)	64/144 (44.4)	77/145 (53.1)
SDAI ≤3.3	9/231 (3.9)	11/229 (4.8)	12/144 (8.3)	26/145 (17.9)
Mean change from baseline (95% CI)*				
HAQ-DI	−0.56 (−0.64 to −0.48)	−0.58 (-0.66 to −0.49)	−0.69 (−0.79 to −0.60)	−0.73 (−0.83 to −0.63)
DAS28(CRP)	−2.13 (−2.3 to −1.96)	−2.40 (−2.58 to −2.22)	−2.74 (−2.96 to −2.52)	−2.88 (−3.11 to −2.65)
CDAI	−24.94 (−26.86 to −23.01)	−27.28 (−29.35 to −25.21)	−27.01 (−29.63 to −24.56)	−29.47 (−32.23 to −26.71)
SDAI	−25.80 (−27.84 to −23.76)	−28.30 (−30.45 to −26.15)	−28.57 (−31.31 to −25.84)	−31.02 (−33.86 to −28.19)

*Mean change from baseline at randomisation.
ADA, adalimumab; CDAI, Clinical Disease Activity Index; DAS28(CRP), 28-joint disease activity score based on C-reactive protein; HAQ-DI, Health Assessment Questionnaire Disability Index; SDAI, Simplified Disease Activity Index;UPA, upadacitinib.

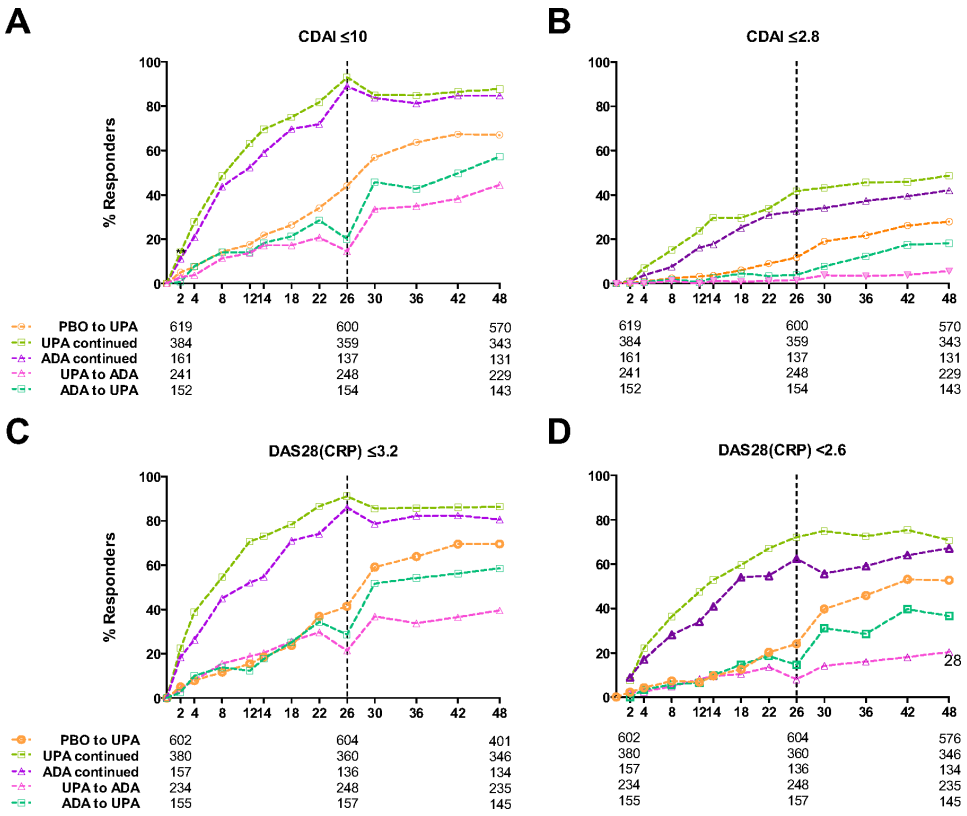

Figure 4 Proportions of patients who were switched from ADA to UPA 15 mg once daily or vice versa achieving disease activity states over 48 weeks (as observed). Vertical line at week 26 indicates the end of the PBO-controlled period. Numbers of patients at selected visits are indicated below the graphs. Groups are by sequence of treatments. ADA, adalimumab; CDAI, Clinical Disease Activity Index; DAS28(CRP), 28-joint Disease Activity Score based on C-reactive protein; PBO, placebo; UPA, upadacitinib.

Safety

The cumulative exposures for upadacitinib and adalimumab were 1243.3 and 467.8 PYs, respectively. Through the cut-off date, the EAER for AEs leading to discontinuation and serious AEs were higher in the adalimumab versus upadacitinib group (table 2). The most frequently reported TEAEs (≥7.5E/100 PY) with upadacitinib were upper respiratory tract infection, urinary tract infection, nasopharyngitis and increased alanine aminotransferase (ALT), while the most frequently reported TEAEs with adalimumab were urinary tract infection and worsening of RA. EAER were similar on upadacitinib and adalimumab for serious infections (4.1 and 4.3, respectively)

Table 2 Exposure adjusted event rates for TEAE (E/100 PYs (95% CI))

	Upadacitinib 15 mg once daily, N=1417, PY=1243.3	Adalimumab, N=579, PY=467.8
Any AE	266.4 (257.4 to 275.6)	294.8 (279.4 to 310.8)
Serious AE	12.9 (11.0 to 15.1)	15.6 (12.2 to 19.6)
AE leading to discontinuation of study drug	7.4 (6.0 to 9.1)	11.1 (8.3 to 14.6)
Infection	86.8 (81.7 to 92.1)	79.1 (71.2 to 87.6)
Serious infection	4.1 (3.1 to 5.4)	4.3 (2.6 to 6.6)
Opportunistic infection	0.7 (0.3 to 1.4)	0.6 (0.1 to 1.9)
Herpes zoster	3.1 (2.2 to 4.2)	1.3 (0.5 to 2.8)
Hepatic disorder*	17.7 (15.4 to 20.2)	13.9 (10.7 to 17.7)
Gastrointestinal perforation†	0.2 (0 to 0.7)	0
Any malignancy (excluding NMSC)	0.4 (0.1 to 0.9)	0.6 (0.1 to 1.9)
NMSC	0.2 (0 to 0.7)	0.2 (0 to 1.2)
MACE (adjudicated)‡	0.4 (0.1 to 0.9)	0.4 (0.1 to 1.5)
Venous thromboembolic events (adjudicated)‡	0.3 (0.1 to 0.8)	1.1 (0.3 to 2.5)
Deaths‡§	0.4 (0.1 to 0.9)	0.9 (0.2 to 2.2)

*Hepatic disorders: majority were based on asymptomatic alanine aminotransferase/aspartate aminotransferase elevations.
†Gastrointestinal perforations were identified through Standardised Medical Dictionary for Regulatory Activities query.
‡Exposure-adjusted incidence rates.
§Deaths included non-treatment emergent deaths.
AE, adverse event; MACE, major adverse cardiovascular event; NMSC, non-melanoma skin cancer; PYs, patient years;TEAE, treatment-emergent adverse events.

and opportunistic infections (0.7 and 0.6), with oral candidiasis being the most common opportunistic infection. The EAER for active tuberculosis were 0.1 and 0.2 for upadacitinib and adalimumab, respectively. The event rate of herpes zoster (HZ) was higher on upadacitinib than adalimumab (table 2). No HZ event was meningoencephalopathic or involved non-cutaneous internal organs except one event on upadacitinib, reported as ophthalmic and led to study drug discontinuation. Most HZ events were non-serious and involved 1–2 dermatomes.

The event rate of malignancies was similar with upadacitinib and adalimumab, and no notable pattern or types of malignancies were observed (online supplementary material). NMSCs were two basal cell carcinomas and one squamous cell carcinoma on upadacitinib, and one basal cell carcinoma on adalimumab; no cases of treatment-emergent lymphoma were reported (table 2). Three events on upadacitinib were classified as 'gastrointestinal (GI) perforation'; however, none were spontaneous GI perforations but rather events of peritonitis with appendicitis in setting of a fallopian tube abscess, anal abscess and anal fistula. Most hepatic events reported were asymptomatic elevations of ALT and AST; most did not lead to premature discontinuation of study drug.

The EAIR for adjudicated MACE were the same on upadacitinib and adalimumab (0.4 n/100 PY) (table 2 and online supplementary material). The EAIR for adjudicated VTE were 0.3 n/100 PY and 1.1 n/100 PY on upadacitinib and adalimumab, respectively; all patients had more than one risk factor besides RA, including family history of VTE, obesity, hypertension and smoking. On upadacitinib, there was one patient with a deep vein thrombosis (DVT), two patients with pulmonary embolism (PE) and one patient with both DVT and PE. On adalimumab, there were four patients with PE and one patient with a DVT. Nine deaths were reported (table 2 and online supplementary material). All patients with events adjudicated as cardiovascular death had known cardiovascular risk factors.

Overall, through week 48 in patients on continuous adalimumab or upadacitinib on a group level, the mean levels of haemoglobin, neutrophils, lymphocytes and platelets continued to remain similar to the first 26 weeks and within the normal ranges (online supplementary figure 7). Mean elevations in low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were observed on upadacitinib versus adalimumab, although ratios of LDL-C:HDL-C and total cholesterol:HDL-C remained steady (online supplementary figure 8 and data not shown). There were a few patients in both arms who had greater than or equal to grade 3 changes in laboratory parameters including decreases in haemoglobin, neutrophils and platelet counts (online supplementary table 1). The proportions of patients with grades 3 and 4 lymphocyte decreases were higher for upadacitinib versus adalimumab; there was no clear association between low lymphocyte counts or low neutrophil counts and the rates of infections, including serious and opportunistic infections, and HZ. Grade 3/4 elevations in ALT/AST were not common, occurring more on upadacitinib than adalimumab or placebo; no Hy's law cases were reported. Greater than or equal to grade 3 increases in CPK occurred in a few patients, more frequently on upadacitinib. Most patients were asymptomatic except two (one with muscle weakness, one with muscle pain) who had single grade 3 CPK increases after vigorous activity; the increases normalised without study drug interruption. No patient had rhabdomyolysis or discontinued due to increased CPK.

Switch safety

Among patients who were rescued from upadacitinib to adalimumab or vice versa, the proportion (95% CI) of patients with serious AEs and serious infections through 6 months postrescue was consistent with those observed for adalimumab and upadacitinib during comparable periods: for serious AEs and serious infections in patients rescued to upadacitinib, 8.8 (95% CI 5.32 to 14.24) and 3.8 (95% CI 1.74 to 7.99), respectively; for patients rescued to adalimumab, it was 6.7 (95% CI 4.25 to 10.54) and 3.8 (95% CI 0.85 to 4.56).

DISCUSSION

In this 48-week trial, clinical responses, including LDA and clinical remission by multiple validated metrics, as well as functional, pain, quality of life and fatigue responses, in patients randomised to upadacitinib plus MTX were superior to adalimumab plus MTX and were maintained consistently through 48 weeks, with a similar impact on radiographic inhibition and consistent with observations up to week 26.[11] Safety over 48 weeks remained consistent with observations during the first 26 weeks, including events observed after protocol-directed immediate treatment switches between upadacitinib and adalimumab despite a lack of washout. Furthermore, patients who had an insufficient response to initial treatment with upadacitinib or adalimumab and switched without washout to the other therapy were able to improve clinically with many able to achieve the treat-to-target goal of either clinical remission or at minimum LDA after 3 or 6 months of therapy.

Through 48 weeks, treatment with upadacitinib was associated with consistently higher levels of LDA and clinical remission than adalimumab, with approximately one-half and one-quarter of patients achieving LDA and clinical remission, respectively, by various composite definitions. Interestingly, the unique rescue rule based on CDAI LDA at week 26, when coupled with NRI imputation, affected the pattern of responses differently across endpoints in a consistent manner between the upadacitinib and adalimumab arms. LDA rates decreased slightly at week 30 before improving again, as patients not meeting CDAI LDA at week 26 had their week 26 (non-responder) status carried forward through week 48. Conversely, remission rates were stable or continued to increase from weeks 26 to 48 as non-rescued patients who had not yet achieved remission at week 26, although in LDA, had a chance to do so through week 48.

The SELECT-COMPARE study design uniquely incorporated a blinded rescue, with an immediate switch, for patients with an insufficient response to upadacitinib, adalimumab or placebo at or before week 26, as advocated by the treat-to-target principles. The proportion of patients rescued at week 14 is consistent with what has been observed in other clinical trials using similar rescue criteria.[15–17] In contrast to those trials, SELECT-COMPARE had three additional rescue visits including rescue for patients who did not meet LDA based on CDAI at week 26 explaining why the overall proportion of patients rescued was higher than in other clinical studies. Importantly, although clinical trials of JAK inhibitors have demonstrated robust outcomes in patients who were switched from a TNF-inhibitor for an inadequate response to a JAK inhibitor,[7 18 19] there is a lack of data on the outcomes of patients who are switched from a JAK inhibitor to a TNF inhibitor for the same reasons. This is the first prospective, blinded, randomised controlled trial which evaluated this scenario. While this study was not powered to assess which switch strategy is more efficacious, among patients who were rescued

from upadacitinib to adalimumab, the response rate increased after switch, although to a lesser extent than for patients rescued from adalimumab to upadacitinib. Still, further appropriately powered studies, including other bDMARDs and tsDMARDs, are needed to answer this important clinical question. In SELECT-COMPARE, the proportions of patients, on a group level, achieving clinical remission and LDA postswitch appeared slightly lower than those observed among the naive populations over the first 26 weeks. However, the results in patients rescued to upadacitinib from adalimumab were consistent with the observations in a previous study of upadacitinib in bDMARD-IR patients.[7]

This study addresses an important question relevant to clinical practice, where patients are often switched between treatments with different mechanisms of action without washout of a prior immunosuppressant. Based on the limited switch data in this study, no additional safety concerns were observed; however, larger studies are needed to more thoroughly evaluate this issue. Adalimumab was included as a long-term safety comparator, and patients and investigators remained blinded to switch between adalimumab and upadacitinib up to week 48. Treatment-emergent AEs, including serious and opportunistic infections, malignancies, MACE and VTE, appeared comparable between upadacitinib and adalimumab in this data set, except for the known HZ signal observed in patients receiving JAK inhibition.[18 20] The types of serious infections reported were generally consistent with those anticipated in a population of patients with moderately to severely active RA. Most opportunistic infections reported with upadacitinib exposure and all opportunistic infections with adalimumab exposure were non-serious mucosal candida infections. Rates of VTEs were within the reported range for the general RA population.[21 22] An integrated analysis across the five phase III SELECT studies will better characterise the overall AE profile of upadacitinib, in particular for rare events such as malignancies, NMSC and VTE.

Limitations of this 48-week study include that placebo was permitted only until week 26 (for ethical reasons) and that the rescue arms were not powered or designed to enable a valid statistical comparison for efficacy between the switch arms. Furthermore, only one bDMARD was used as a comparator; different results may have been observed had other bDMARDs been included.

In summary, responses were maintained with upadacitinib treatment over 48 weeks and were consistently significantly better than with adalimumab. The significant inhibition of radiographical progression at week 26 was maintained at week 48 and was comparable to adalimumab. Patients with insufficient response to either upadacitinib or adalimumab can benefit from switching to the other therapy. No new safety findings were observed with longer term exposure to upadacitinib, or in the period following the switch from adalimumab to upadacitinib.

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Contributors RMF, MG, CP, YL and I-HS participated in the design of the study. RF, EM, LB, CP, PD and AO participated in the acquisition and interpretation of data. MG participated in the interpretation of data. JVE,YL and I-HS participated in the analysis and interpretation of data. CP participated in the analysis of data. RF, YL and I-HS contributed to the drafting of the manuscript. All authors contributed to the critical revision of the manuscript for important intellectual content.

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Competing interests RF has received research grants and consulting fees from AbbVie, Eli Lilly, and Pfizer. MG has served as a consultant for, and has received grants from, AbbVie, Eli Lilly, Pfizer, Galapagos, and Gilead. EM has received research grants and consulting fees from AbbVie, Eli Lilly, Pfizer, Roche, BMS, and Sandoz. LB has served as a speaker on behalf of, and received consulting fees and research grants from, Amgen, BMS, Janssen, Roche, UCB, AbbVie, Pfizer, Merck, Celgene, Sanofi, Eli Lilly and Novartis. CP is an employee and shareholder of Spire Sciences, has served as a speaker on behalf of Amgen, Bristol-Myers Squibb and has served as a consultant for Centrexion, Crescendo Bioscience, Daiichi Sankyo, EMD Serono, Five Prime, Flexion Therapeutics, Genentech, Gilead, GlaxoSmithKline, Pfizer, Plexikkon, Regeneron, Roche and SetPoint. PD has received speaker fees from BMS, Sanofi, Eli Lilly, and Celltrion. AO has served as a speaker on behalf of, and has received consulting fees and/or research grants from BMS, Janssen, Roche, UCB, AbbVie, Pfizer, Merck, Celgene, Sanofi, Eli Lilly and Novartis. JVE, YL, and I-HS are employees of AbbVie and may own stock or stock options.

Patient consent for publication Not required.

Ethics approval The study was conducted per the International Conference on Harmonisation guidelines, applicable regulations, and the Declaration of Helsinki. Study-related documents were approved by the United States Central Institutional Review Board (Quorum #31009) and other local institutional ethics committees and review boards.

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REFERENCES

1 Smolen JS, Breedveld FC, Burmester GR, *et al*. Treating rheumatoid arthritis to target: 2014 update of the recommendations of an international Task force. *Ann Rheum Dis* 2016;75:3–15.
2 Grigor C, Capell H, Stirling A, *et al*. Effect of a treatment strategy of tight control for rheumatoid arthritis (the TICORA study): a single-blind randomised controlled trial. *The Lancet* 2004;364:263–9.
3 Versteeg GA, Steunebrink LMM, Vonkeman HE, *et al*. Long-Term disease and patient-reported outcomes of a continuous treat-to-target approach in patients with early rheumatoid arthritis in daily clinical practice. *Clin Rheumatol* 2018;37:1189–97.
4 Smolen JS, Landewé R, Bijlsma J, *et al*. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2016 update. *Ann Rheum Dis* 2017;76:960–77.
5 Singh JA, Saag KG, Bridges SL, *et al*. 2015 American College of rheumatology guideline for the treatment of rheumatoid arthritis. *Arthritis Rheumatol* 2016;68:1–26.
6 Burmester GR, Kremer JM, Van den Bosch F, *et al*. Safety and efficacy of upadacitinib in patients with rheumatoid arthritis and inadequate response to conventional synthetic disease-modifying anti-rheumatic drugs (SELECT-NEXT): a randomised, double-blind, placebo-controlled phase 3 trial. *The Lancet* 2018;391:2503–12.
7 Genovese MC, Fleischmann R, Combe B, *et al*. Safety and efficacy of upadacitinib in patients with active rheumatoid arthritis refractory to biologic disease-modifying anti-rheumatic drugs (SELECT-BEYOND): a double-blind, randomised controlled phase 3 trial. *The Lancet* 2018;391:2513–24.
8 Smolen JS, Pangan AL, Emery P, *et al*. Upadacitinib as monotherapy in patients with active rheumatoid arthritis and inadequate response to methotrexate (SELECT-MONOTHERAPY): a randomised, placebo-controlled, double-blind phase 3 study. *The Lancet* 2019;393:2303–11.

9 van Vollenhoven RTT, Pangan AL, Friedman A, *et al*. A phase 3, randomized, double-blind study comparing Upadacitinib monotherapy to MTX monotherapy in MTX-Naïve patients with active rheumatoid arthritis. *Arthritis and Rheumatology* 2018.
10 Fleischmann R, Pangan AL, Song In-Ho, *et al*. Upadacitinib versus placebo or adalimumab in patients with rheumatoid arthritis and an inadequate response to methotrexate: results of a phase 3, Double-Blind, randomized controlled trial. *Arthritis Rheumatol* 2019.
11 van der Heijde D. How to read radiographs according to the Sharp/van Der Heijde method. *J Rheumatol* 1999;26:743–5.
12 van der Heijde DM, van Leeuwen MA, van Riel PL, *et al*. Biannual radiographic assessments of hands and feet in a three-year prospective followup of patients with early rheumatoid arthritis. *Arthritis Rheum* 1992;35:26–34.
13 Woodworth T, Furst DE, Alten R, *et al*. Standardizing assessment and reporting of adverse effects in rheumatology clinical trials II: the rheumatology common toxicity criteria v.2.0. *J Rheumatol* 2007;34:1401–14.
14 NCI. Common terminology criteria for adverse events (CTCAE) version 4.0 NIH publication; 2009.
15 van der Heijde D, Tanaka Y, Fleischmann R, *et al*. Tofacitinib (CP-690,550) in patients with rheumatoid arthritis receiving methotrexate: twelve-month data from a twenty-four-month phase III randomized radiographic study. *Arthritis Rheum* 2013;65:559–70.
16 Smolen JS, Beaulieu A, Rubbert-Roth A, *et al*. Effect of interleukin-6 receptor inhibition with tocilizumab in patients with rheumatoid arthritis (option study): a double-blind, placebo-controlled, randomised trial. *The Lancet* 2008;371:987–97.
17 Taylor PC, Keystone EC, van der Heijde D, *et al*. Baricitinib versus placebo or adalimumab in rheumatoid arthritis. *N Engl J Med* 2017;376:652–62.
18 Genovese MC, Kremer J, Zamani O, *et al*. Baricitinib in patients with refractory rheumatoid arthritis. *N Engl J Med* 2016;374:1243–52.
19 Genovese MC, van Vollenhoven RF, Wilkinson B, *et al*. Switching from adalimumab to tofacitinib in the treatment of patients with rheumatoid arthritis. *Arthritis Res Ther* 2016;18.
20 Winthrop KL, Yamanaka H, Valdez H, *et al*. Herpes zoster and tofacitinib therapy in patients with rheumatoid arthritis. *Arthritis Rheumatol* 2014;66:2675–84.
21 Kim SC, Schneeweiss S, Liu J. Risk of venous thromboembolism in patients with rheumatoid arthritis. *Arthritis Care Res* 2013;65:1600–7.
22 Chung W-S, Peng C-L, Lin C-L, *et al*. Rheumatoid arthritis increases the risk of deep vein thrombosis and pulmonary thromboembolism: a nationwide cohort study. *Ann Rheum Dis* 2014;73:1774–80.

EPIDEMIOLOGICAL SCIENCE

Global, regional and national burden of rheumatoid arthritis 1990–2017: a systematic analysis of the Global Burden of Disease study 2017

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ABSTRACT

Objectives To provide the level and trends of prevalence, incidence and disability adjusted life years (DALYs) for rheumatoid arthritis (RA) in 195 countries from 1990 to 2017 by age, sex, Socio-demographic Index (SDI; a composite of sociodemographic factors) and Healthcare Access and Quality (an indicator of health system performance) Index.

Methods Data from the Global Burden of Diseases, Injuries, and Risk Factors study (GBD) 2017 were used. GBD 2017 modelled the burden of RA for 195 countries from 1990 to 2017, through a systematic analysis of mortality and morbidity data to estimate prevalence, incidence and DALYs. All estimates were presented as counts and age-standardised rates per 100 000 population, with uncertainty intervals (UIs).

Results Globally, the age-standardised point prevalence and annual incidence rates of RA were 246.6 (95% UI 222.4 to 270.8) and 14.9 (95% UI 13.3 to 16.4) in 2017, which increased by 7.4% (95% UI 5.3 to 9.4) and 8.2% (95% UI 5.9 to 10.5) from 1990, respectively. However, the age-standardised rate of RA DALYs per 100 000 population was 43.3 (95% UI 33.0 to 54.5) in 2017, which was a 3.6% (95% UI −9.7 to 0.3) decrease from the 1990 rate. The age-standardised prevalence and DALY rates increased with age and were higher in females; the rates peaked at 70–74 and 75–79 age groups for females and males, respectively. A non-linear association was found between age-standardised DALY rate and SDI. The global age-standardised DALY rate decreased from 1990 to 2012 but then increased and reached higher than expected levels in the following 5 years to 2017. The UK had the highest age-standardised prevalence rate (471.8 (95% UI 428.9 to 514.9)) and age-standardised incidence rate (27.5 (95% UI 24.7 to 30.0)) in 2017. Canada, Paraguay and Guatemala showed the largest increases in age-standardised prevalence rates (54.7% (95% UI 49.2 to 59.7), 41.8% (95% UI 35.0 to 48.6) and 37.0% (95% UI 30.9 to 43.9), respectively) and age-standardised incidence rates (48.2% (95% UI 41.5 to 55.1), 43.6% (95% UI 36.6 to 50.7) and 36.8% (95% UI 30.4 to 44.3), respectively) between 1990 and 2017.

Conclusions RA is a major global public health challenge. The age-standardised prevalence and incidence rates are increasing, especially in countries such as Canada, Paraguay and Guatemala. Early identification and treatment of RA is vital especially among females, in order to reduce the ongoing burden of this condition. The quality of health data needs to be improved for better monitoring of disease burden.

Key messages

What is already known about this subject?

- No updated global study on rheumatoid arthritis (RA) has been published after 2010.

What does this study add?

- Globally, the age-standardised point prevalence and annual incidence rates of RA increased by 7.4% (95% uncertainty interval (UI) 5.3 to 9.4) and 8.2% (95% UI 5.9 to 10.5) from 1990, respectively.
- The global age-standardised disability adjusted life year rate decreased from 1990 to 2012 but then increased and reached higher than expected levels in the following 5 years to 2017.
- The UK had the highest age-standardised prevalence and incidence rates in 2017.
- Canada, Paraguay and Guatemala showed the largest increases in age-standardised prevalence and incidence rates between 1990 and 2017.

How might this impact on clinical practice or future developments?

- Early identification and treatment of RA is vital especially among females, in order to reduce the burden and disability associated with this condition and to provide appropriate care for this community.

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic auto-immune disease. Symmetrical inflammatory polyarthritis is the primary clinical manifestation, usually beginning in the small joints of the hands and the feet, spreading later to the larger joints.[1] A number of national studies have examined prevalence, incidence and mortality of RA[2–4]; however, there is a lack of a comprehensive global study. In 2016, the WHO estimated the years lived with disability (YLD), years of life lost (YLL) and disability

adjusted life years (DALYs) of RA by age, sex and country[5] but no paper has been published in this regard. Recently, Sebbag and colleagues reported the global burden of musculoskeletal disease for 2000, 2010 and 2015 using aforementioned WHO database but they have not specifically focused on RA and their estimates rely on 2015 data.[6] An analysis utilising the Global Burden of Diseases, Injuries, and Risk Factors Study (GBD) 2010 reported global and regional burden of RA in terms of prevalence and DALYs,[7] but national-level estimates were not provided. Association of burden of RA with sociodemographic status of countries was not examined in that study. Another study was conducted on GBD 2013 musculoskeletal data combined and were only reported for Eastern Mediterranean region.[8] Overall, no updated global study on RA has been published after 2010. Hence, in the present study, we examined the data from GBD 2017 for global, regional and national prevalence, incidence and DALY in terms of counts and age-standardised rates from 1990 to 2017 by age, sex, Socio-demographic Index (SDI; a composite of sociodemographic factors) and Healthcare Access and Quality (HAQ; an indicator of health system performance) Index to provide comprehensive and comparable analysis of RA burden.

METHODS

Overview

GBD 2017, conducted by Institute of Health Metrics and Evaluation (IHME), involved 195 countries, seven super-regions and 21 regions from 1990 to 2017.[9] Three hundred and fifty-four diseases and injuries, 282 causes of death and 84 risk factors were systematically analysed in the GBD 2017 study. The general methodology of GBD 2017 and its main changes compared with previous years have been described in previous publications.[9–12] The additional information on fatal and non-fatal estimates can be found at https://vizhub.healthdata.org/gbd-compare/ and http://ghdx.healthdata.org/gbd-results-tool. Methods were developed by and most analyses reported here were conducted at IHME.

Case definition and data sources

As stated in many epidemiological studies, RA is a systemic autoimmune disorder that causes pain and swelling of the joints. While RA is known to affect internal organs in addition to the joints, these extra-articular effects are not factored into the disability weights (DW) used in GBD. The reference case definition for RA is based on the 1987 guidelines by the American College of Rheumatology (ACR 1987),[13] which explain seven diagnostic criteria (1. morning stiffness, 2. arthritis of three or more joint areas, 3. symmetric arthritis, 4. arthritis of hand, 5. rheumatoid nodules, 6. serum rheumatoid factor and 7. radiographic changes), of which four need to be satisfied for a diagnosis. Criteria 1 through 4 must have been present for at least 6 weeks.[9 13]

A comprehensive systematic review was conducted on RA prevalence, incidence and mortality in GBD 2010 and updated in GBD 2017. Studies with the following characteristics were excluded: (a) non-representative, (b) non-population-based, (c) inadequate primary data on epidemiological parameters, (d) studying a specific type of RA, for example, seropositive RA and (e) reviews. Finally, prevalence (site-years=499), incidence (site-years=151) and other, including remission (site-years=20), were estimated through literature studies included in GBD 2017. Notably, a site-year is a unique combination of location and calendar year and is defined as a country or other subnational geographical unit contributing data in a given year. Also, the number of countries with data was quite small globally, and these numbers were different for estimating the prevalence (n=42), incidence (n=14) and other (n=9). The number of GBD regions with data was higher for prevalence (n=16), compared with incidence (n=6) and other (n=5). In addition, USA claims data for 2000, and 2010–2014 by US state and Taiwan claims for 2016 were included. It is worth mentioning that hospital inpatient data were not used as it was assumed, they would not be representative of true prevalence.[9]

Data used to estimate RA mortality included vital registration, verbal autopsy, and China disease surveillance data from the cause of death database. Outlier criteria were to exclude data points that were (1) implausibly high or low relative to global or regional patterns, (2) substantially conflicted with established age or temporal patterns, or (3) significantly conflicted with other data sources based from the same locations or locations with similar characteristics that is, SDI.

Disease model

GBD 2017 methods included the standard Cause of Death Ensemble model, which was applied to estimate deaths due to RA. The list of covariates used for the RA model can be found in the appendix methods section of previously published GBD 2017 paper.[10] RA prevalence, incidence and mortality data were analysed within the IHME Bayesian meta-regression tool DisMod-MR 2.1 for modelling and calculation of estimates by pooling the available heterogeneous data to adjust for methodological differences and check for internal consistency.

IHME prior settings in the DisMod-MR 2.1 model included setting remission to 0.009–0.021, which is the assumed remission rate for natural disease (ie, drug-free) remission, and it was assumed that there was no incidence or prevalence of RA before the age of 5 years.[9] Data from all sources were re-extracted to better reflect the range of case definitions. ACR 1987 criteria[13] was set as the reference, with other definitions including Rome 1961,[14] American Rheumatology Association 1958,[15] or European League against Rheumatism[16] criteria identified with a single study covariate 'non-ACR_1987'. Additional study covariates were created for studies using administrative health system data sources; for studies covering regional rather than (sub)-nationally representative populations; and for claims data. R software V.3.5.2 was used to generate figures of the final estimates of prevalence and incidence rates from data available from ghdx.healthdata.org/gbd-results-tool.

Severity and YLD

The International Classification of Diseases version 10 codes were used for RA (M05-M06.9, M08.0-M08.89) with three sequelae (severity levels) where each sequela had specific DW ranging from 0.11 to 0.58 (see online supplementary table 1). GBD 2013 European Disability Weights Measurement Study and GBD 2010 Disability Weights Measurement Study were used as the sources of DW values. More details are described in previous GBD studies.[9 17] Medical Expenditure Panel Surveys were used to specify the proportion of each of the severity levels in patients with RA.[9] Then, these proportions were used to split the overall prevalence of RA into the severity categories. Finally, the prevalence of each severity category was multiplied by severity-specific DWs to calculate YLDs.

Compilation of results

The YLLs were calculated by multiplying the number of deaths in an age group by the remaining life expectancy in that age

group, taken from the GBD standard life table. DALYs were then calculated as the sum of YLLs and YLDs. Uncertainty was propagated by sampling 1000 draws at each computational step, combining uncertainty from multiple sources such as input data, corrections of measurement error and estimates of residual non-sampling error. Uncertainty intervals (UIs) were defined as the 25th and 975th values of the ordered draws. We examined the shape of association of RA burden in terms of DALYs with SDI and HAQ for 21 regions and 195 countries using the Smoothing Splines models.[18] SDI is a composite indicator of lag-dependent income per capita; that is gross domestic product per capita that has been smoothed over the preceding 10 years, average years of schooling for the population older than 15 years of age, and total fertility rate under the age of 25. It ranges from 0 (less developed) to 1 (most developed). HAQ is an indicator of health system performance and reflects personal HAQ for 195 countries and is calculated based on amenable mortality, that is, deaths from causes that should not occur in the presence of effective medical care. This index ranged from 0 (worst-performing health systems) to 100 (best-performing health systems). Additional details for HAQ have been presented previously.[19]

RESULTS

Global level

The present study found that globally there were 19 965 115 (95% UI 17 990 489 to 21 955 673) prevalent cases of RA, with an age-standardised prevalence rate of 246.6 per 100 000 (95% UI 222.4 to 270.8), which increased by 7.4% (95% UI 5.3 to 9.4) between 1990 and 2017. Also, RA was responsible for 1 204 599 (95% UI 1 071 090 to 1 331 694) incident cases globally with an age-standardised incidence rate of 14.9 (95% UI 13.3 to 16.4), an increase of 8.2% (95% UI 5.9 to 10.5) between 1990 and 2017 (table 1).

The global age-standardised DALY rate showed a decreasing trend from 1990 to 2012 but increased and reached higher levels in the following 5 years. Moreover, RA accounted for 3.4 million (95% UI 2.6 to 4.4) DALYs at the global level, with an age-standardised rate of 43.3 (95% UI 33.0 to 54.5) DALYs per 100 000 population. The age-standardised DALY rate reduced by 3.6% (95% UI −9.7 to 0.3) from 1990 to 2017 (table 1).

Regional level

At the regional-level, the age-standardised prevalence of RA was found to be highest in high-income North America (377.6 (95% UI 356.4 to 400.2)), Western Europe (346.8 (95% UI 314.4 to 378.3)) and the Caribbean (338.9 (95% UI 304.6 to 374.1)). In contrast, Southeast Asia (100.9 (95% UI 89.9 to 112.2)), Oceania (135.3 (95% UI 120.9 to 150.8)) and Western Sub-Saharan Africa (135.7 (95% UI 120.6 to 151.6)) showed the lowest age-standardised rates (table 1).

The age-standardised incidence rates were also found to be highest in high-income North America (22.5 (95% UI 20.9 to 24.1)), South Asia (20.7 (95% UI 18.4 to 22.9)) and Western Europe (20.4 (95% UI 18.3 to 22.4)); whereas, Southeast Asia (6.2 (95% UI 5.5 to 6.9)), Oceania (7.9 (95% UI 7.0 to 8.9)) and Western Sub-Saharan Africa (8.5 (95% UI 7.5 to 9.5)) showed the lowest rates (table 1).

The regional-level age-standardised prevalence and incidence estimates for all GBD 2017 regions have been presented by sex in online supplementary figure 1.

This study also found that the percentage change in age-standardised prevalence rates during 1990–2017 was not similar across the GBD 2017 regions. East Asia (25% (95% UI 22 to 29)), high-income North America (19% (95% UI 14 to 25)) and Western Sub-Saharan Africa (14% (95% UI 11 to 17)) showed the most increasing significant trends, while Southern Sub-Saharan Africa (−12% (95% UI −15 to −8)), high-income Asia-Pacific (−7% (95% UI −10 to −4)) and Eastern Sub-Saharan Africa (−5% (95% UI −8 to −2)) showed decreasing significant trends (table 1 and online supplementary figure 2). The number of prevalent cases was found to be doubled from 1990 (10 226 042 (95% UI 9 320 195 to 11 179 199)) to 2017 (19 965 114 (95% UI 17 990 489 to 21 955 673)) but the contribution of GBD 2017 regions was different (see online supplementary figure 3).

East Asia (26% (95% UI 23 to 30)), high-income North America (22% (95% UI 18 to 28)) and North Africa and Middle East (13% (95% UI 10 to 15)) had the top statistically significant increasing trends in age-standardised incidence rates whereas statistically significant decreasing trend was found in Southern Sub-Saharan Africa (−11% (95% UI −14 to −8)), high-income Asia-Pacific (−10% (95% UI −14 to −7)) and Eastern Sub-Saharan Africa (−9% (95% UI −12 to −6)) (table 1 and online supplementary figure 2). The number of incident cases was also found to be doubled from 1990 (650 269 (95% UI 589 738 to 711 375)) to 2017 (1 204 599 (95% UI 1 071 089 to 1 331 694)) with differing contribution of GBD 2017 regions (see online supplementary figure 3).

The top three statistically significant increasing trends in age-standardised DALY rate belonged to high-income North America (13% (95% UI 8 to 18)), Western Sub-Saharan Africa (10% (95% UI 1 to 18)) and Tropical Latin America (9% (95% UI 5 to 13)). Southern Sub-Saharan Africa (−29% (95% UI −35 to −22)), high-income Asia-Pacific (−28% (95% UI −34 to −23)) and Eastern Sub-Saharan Africa (−24% (95% UI −35 to −15)) were considered to have statistically decreasing trend in age-standardised DALY rate among GBD 2017 regions (table 1).

National level

Age-standardised prevalence rate of RA ranged from 91 to 471 cases per 100 000 population. The UK (471.8 (95% UI 428.9 to 514.9)), Trinidad and Tobago (404.4 (95% UI 362.6 to 446.6)) and Barbados (402.6 (95% UI 361.1 to 445.3)) had the three highest age-standardised prevalence rates in 2017, whereas Indonesia (91.1 (95% UI 81.1 to 101.8)), Timor-Leste (91.4 (95% UI 81.5 to 102.4)) and Sri Lanka (97.2 (95% UI 85.9 to 109.6)) showed the lowest rates (figure 1 and online supplementary table 2).

Age-standardised incidence rates varied from 5.6 to 27.5 cases per 100 000 population. UK (27.5 (95% UI 24.7 to 30.0)), Ireland (23.7 (95% UI 21.0 to 26.4)) and Sweden (23.4 (95% UI 21.0 to 25.8)) showed the highest age-standardised incidence rates in 2017; in contrast, Indonesia (5.6 (95% UI 4.9 to 6.3)), Timor-Leste (5.7 (95% UI 5.1 to 6.4)) and Sri Lanka (5.9 (95% UI 5.2 to 6.7)) had the lowest rates (figure 2 and online supplementary table 3).

The percentage change in age-standardised prevalence rates from 1990 to 2017 differed substantially between countries, with Canada (54.7% (95% UI 49.2 to 59.7)), Paraguay (41.8% (95% UI 35.0 to 48.6)) and Guatemala (37.0% (95% UI 30.9 to 43.9)) showing the largest increases. In contrast, South Africa (−16.6% (95% UI −20.3 to −13.0)) Sweden (−15.7% (95% UI −19.9 to −11.4)) and Burundi (−15.6% (95% UI −19.5 to −11.1)) showed decreasing trends (see online supplementary table 2).

The percentage change in age-standardised incidence rates (from 1990 to 2017) also differed between countries. The largest

Table 1 Prevalent cases, incident cases and DALYs for rheumatoid arthritis in 2017 for both sexes and percentage change of age-standardised rates by Global Burden of Disease (GBD) region (generated from data available from: ghdx.healthdata.org/gbd-results-tool/)

	Prevalence (95% UI)			Incidence (95% UI)			DALYs (95% UI)		
	Counts (2017)	Age-standardised rates (2017)	Percentage change in age-standardised rates between 1990 and 2017	Counts (2017)	Age-standardised rates (2017)	Percentage change in age-standardised rates between 1990 and 2017	Counts (2017)	Age-standardised rates (2017)	Percentage change in age-standardised rates between 1990 and 2017
Global	19 965 115 (17 990 489 to 673)	246.6 (222.4 to 270.8)	7.4 (5.3 to 9.4)	1 204 599 (1 071 090 to 1 331 694)	14.9 (13.3 to 16.4)	8.2 (5.9 to 10.5)	3 492 036 (2 658 460 to 4 414 818)	43.3 (33 to 54.5)	−3.6 (−9.7 to 0.3)
High-income Asia-Pacific	592 474 (5 30 417 to 6 54 202)	187.1 (167.4 to 206.2)	−7.2 (−10.3 to −4.1)	29 557 (25 963 to 33 128)	11.2 (9.9 to 12.4)	−10.3 (−13.8 to −7.1)	101 012 (76 168 to 1 27 922)	30.4 (22.3 to 39.1)	−28.2 (−34.4 to −22.9)
High-income North America	1 954 937 (1 837 944 to 616)	377.6 (356.4 to 400.2)	18.9 (13.9 to 24.5)	105 426 (96 864 to 113 713)	22.5 (20.9 to 24.1)	22.2 (17.5 to 27.7)	301 847 (225 327 to 384 825)	57.6 (42.5 to 73.6)	13.3 (8.1 to 18.1)
Western Europe	2 357 945 (2 135 893 to 2 578 942)	346.8 (314.4 to 378.3)	8 (4.8 to 11.1)	118 352 (105 064 to 131 272)	20.4 (18.3 to 22.4)	7.3 (3.9 to 10.9)	356 870 (259 769 to 463 315)	51.5 (36.9 to 67.2)	−4.5 (−10 to 0)
Australasia	108 716 (97 003 to 121 072)	268.2 (238.7 to 297)	4.4 (−2.2 to 11)	6060 (5347 to 6772)	16.5 (14.7 to 18.4)	2.4 (−5.1 to 9.3)	17 932 (13 401 to 22 908)	43.2 (31.8 to 55.7)	−12 (−19.9 to −4)
Andean Latin America	178 171 (160 962 to 196 538)	313.5 (283.2 to 345.9)	6.2 (2.1 to 10.6)	10 656 (9567 to 11 804)	18.2 (16.3 to 20.1)	2.8 (−1.5 to 7.1)	28 123 (20 233 to 36 630)	49.7 (36 to 64.5)	-7 (−14.6 to −0.4)
Tropical Latin America	676 815 (605 733 to 754 902)	281.6 (251.9 to 313.8)	11.6 (8.8 to 14.2)	38 636 (33 960 to 43 375)	16.1 (14.2 to 18)	8 (4.8 to 10.9)	103 214 (74 615 to 134 983)	43 (31.2 to 56.3)	8.9 (5 to 12.6)
Central Latin America	624 874 (565 265 to 688 015)	254.6 (230 to 279.9)	4.6 (1.7 to 7.6)	39 013 (34 971 to 43 048)	15.5 (13.9 to 17.1)	-3 (−6.3 to 0.4)	124 065 (97 205 to 154 064)	51 (40.1 to 63.2)	−10.4 (−15.4 to −6.1)
Southern Latin America	217 872 (195 209 to 2 603)	283.9 (255.1 to 311.7)	3.6 (−0.8 to 8.5)	12 377 (11 090 to 13 651)	16.9 (15.1 to 18.6)	1.8 (−2.7 to 7)	36 371 (27 270 to 46 577)	47.2 (35.2 to 60.6)	−6.8 (−13.3 to −0.6)
Caribbean	170 721 (153 068 to 188 256)	338.9 (304.6 to 374.1)	10.3 (6.8 to 13.9)	9499 (8428 to 10 601)	19.2 (17 to 21.4)	7.9 (4.1 to 11.5)	26 314 (18 897 to 34 224)	52.3 (37.6 to 67.9)	2.8 (−4.2 to 9)
Central Europe	406 849 (361 770 to 452 447)	226.9 (202.3 to 251.3)	4.7 (1 to 8.4)	20 799 (18 241 to 23 395)	13.3 (11.8 to 14.8)	3.6 (−0.1 to 7.7)	62 355 (45 539 to 80 198)	34.5 (25 to 44.6)	−6.2 (−11.9 to −1.1)
Eastern Europe	644 432 (570 685 to 718 332)	207.5 (185.2 to 230.5)	4.1 (1.7 to 6.4)	34 028 (29 461 to 38 599)	12.1 (10.7 to 13.5)	4.8 (2.4 to 7.2)	105 205 (79 075 to 1 34 521)	33.9 (25.4 to 43.5)	−2.8 (−6.6 to 0.7)
Central Asia	178 222 (158 760 to 198 150)	213.8 (191 to 236.8)	6.3 (3.5 to 9.1)	11 218 (9889 to 12 566)	12.7 (11.2 to 14.1)	7.6 (4.7 to 10.7)	29 748 (22 044 to 38 260)	35.6 (26.4 to 45.4)	−3 (−9.1 to 3.5)
North Africa and Middle East	1 315 587 (1 171 457 to 1 473 984)	259.3 (230.6 to 289.7)	13.3 (10.6 to 16.1)	83 695 (73 497 to 94 434)	15 (13.2 to 16.9)	12.8 (10.2 to 15.5)	186 728 (129 598 to 249 177)	36.7 (25.5 to 48.5)	6 (−1 to 11.6)
South Asia	4 840 760 (4 340 681 to 5 383 020)	323.7 (289.7 to 358.5)	2 (−0.8 to 4.8)	331 161 (295 195 to 366 416)	20.7 (18.4 to 22.9)	2.3 (−0.6 to 5.1)	979 798 (757 468 to 1 201 710)	69.7 (54.3 to 84.9)	−5.6 (−16.9 to 1.7)
Southeast Asia	658 609 (584 675 to 736 927)	100.9 (89.9 to 112.2)	9.8 (6.5 to 13)	41 886 (36 798 to 47 257)	6.2 (5.5 to 6.9)	5.3 (1.9 to 8.6)	114 176 (85 908 to 145 679)	17.9 (13.6 to 22.6)	−15.4 (−23.6 to −6.9)
East Asia	3 972 614 (3535309 to 4422987)	194.8 (173.9 to 216.1)	25.2 (22 to 28.5)	232 716 (2 03 851 to 2 61 978)	11.8 (10.4 to 13.1)	26.4 (23.1 to 29.6)	709 970 (5 33 129 to 9 00 231)	35.1 (26.4 to 44.3)	3.9 (−10.2 to 11.8)
Oceania	12 115 (10 742 to 13 659)	135.3 (120.9 to 150.8)	8.6 (5.2 to 12)	826 (729 to 930)	7.9 (7 to 8.9)	8.3 (5 to 11.8)	2305 (1703 to 3038)	25.7 (19.1 to 33.7)	7.8 (−1.9 to 18.6)
Western Sub-Saharan Africa	333 819 (294 662 to 375 452)	135.7 (120.6 to 151.6)	13.9 (10.8 to 17.1)	24 519 (21 667 to 27 531)	8.5 (7.5 to 9.5)	12.2 (9 to 15.4)	55 004 (40 010 to 71 924)	22.9 (17 to 29.6)	9.8 (0.8 to 18.3)
Eastern Sub-Saharan Africa	421 220 (379 258 to 467 912)	212 (190.9 to 233.1)	−4.8 (−7.9 to −1.8)	33 425 (30 086 to 36 797)	14.5 (13 to 16.1)	−8.8 (−11.7 to −5.8)	94 182 (74 288 to 117 235)	51.6 (41 to 63)	−24.5 (−34.6 to −14.6)
Central Sub-Saharan Africa	150 128 (134 719 to 166 777)	219 (197.8 to 242.3)	−4 (−8.3 to 0.3)	11 077 (9956 to 12 257)	13.5 (12.1 to 15)	−5.6 (−9.8 to −1.5)	29 915 (22 826 to 38 220)	45.9 (35.2 to 58.2)	−20.1 (−31.1 to −8.5)
Southern Sub-Saharan Africa	148 235 (132 691 to 164 329)	231.1 (207.6 to 256.9)	−11.7 (−15.1 to −8.3)	9675 (8639 to 10 760)	13.9 (12.4 to 15.5)	−10.8 (−13.8 to −7.6)	26 901 (20 342 to 34 074)	42.6 (32.5 to 53.5)	−28.9 (−34.9 to −21.6)

UI, uncertainty interval.

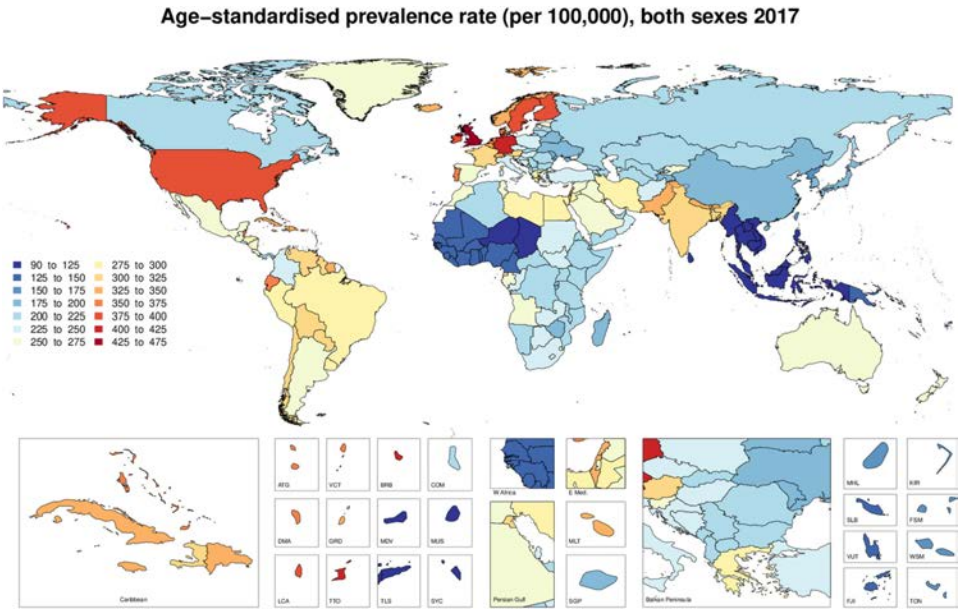

Figure 1 Age-standardised prevalence rate of rheumatoid arthritis per 100 000 population in 2017, by country. ATG, Antigua and Barbuda; BRB, Barbados; COM, Comoros; DMA, Dominica; FJI, Fiji; FSM, Federated states of Micronesia; GRD, Grenada; KIR, Kiribati; LCA, Saint Lucia; MDV, Maldives; MHL, Marshall Islands; MLT, Malta; MUS, Mauritius; SGP, Singapore; SLB, Solomon Islands; SYC, Seychelles; TLS, Timor-Leste; TON, Tonga; TTO, Trinidad and Tobago; VCT, Saint Vincent and the Grenadines; VUT, Vanuatu; WSM, Samoa (generated from data available from ghdx.healthdata.org/gbd-results-tool/).

increases were seen in Canada (48.2% (95% UI 41.5 to 55.1)), Paraguay (43.6% (95% UI 36.6 to 50.7)) and Guatemala (36.8% (95% UI 30.4 to 44.3)). The largest decreases during this period were found in Burundi (−17.0% (95% UI −21.1 to −12.5)), Ethiopia (−16.6% (95% UI −20.1 to −13.6)) and Sweden (−16.2% (95% UI −20.3 to −11.9)) (see online supplementary table 3). The national-level detailed information on DALYs due to RA can be found in online supplementary table 4.

Age and sex patterns

Globally, age-standardised prevalence rate was higher in females and increased with age, peaking at 70–74 and 75–79 age groups among females and males, respectively in 2017. Also, the number of prevalent cases increased with age and peaked in the 60–64 age group for both males and females; after this age, the trend declined (figure 3). In 2017, the global age-standardised

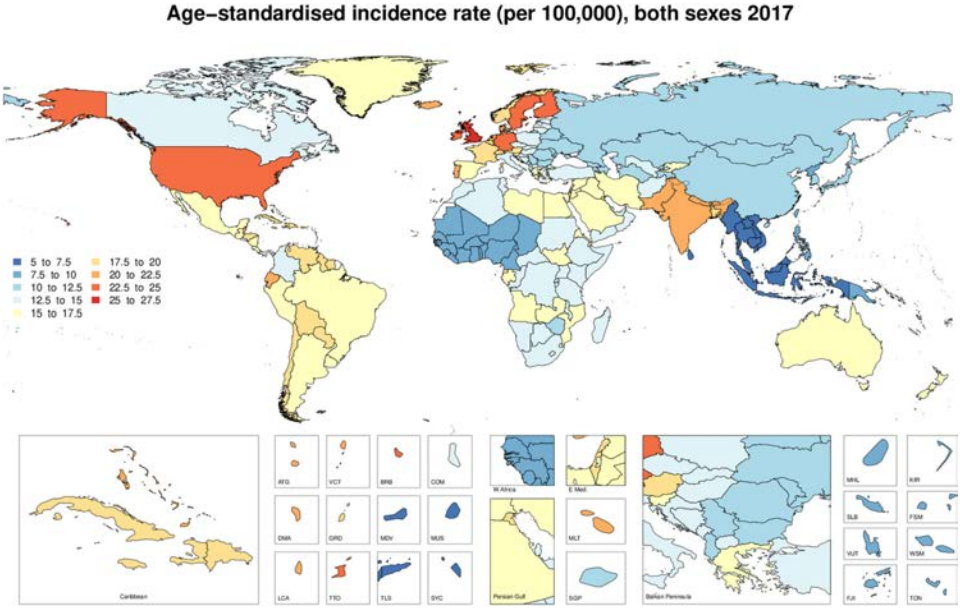

Figure 2 Age-standardised incidence rate of rheumatoid arthritis per 100 000 population in 2017, by country. ATG, Antigua and Barbuda; BRB, Barbados; COM, Comoros; DMA, Dominica; FJI, Fiji; FSM, Federated states of Micronesia; GRD, Grenada; KIR, Kiribati; LCA, Saint Lucia; MDV, Maldives; MHL, Marshall Islands; MLT, Malta; MUS, Mauritius; SGP, Singapore; SLB, Solomon Islands; SYC, Seychelles; TLS, Timor-Leste; TON, Tonga; TTO, Trinidad and Tobago; VCT, Saint Vincent and the Grenadines; VUT, Vanuatu; WSM, Samoa (generated from data available from: ghdx.healthdata.org/gbd-results-tool/).

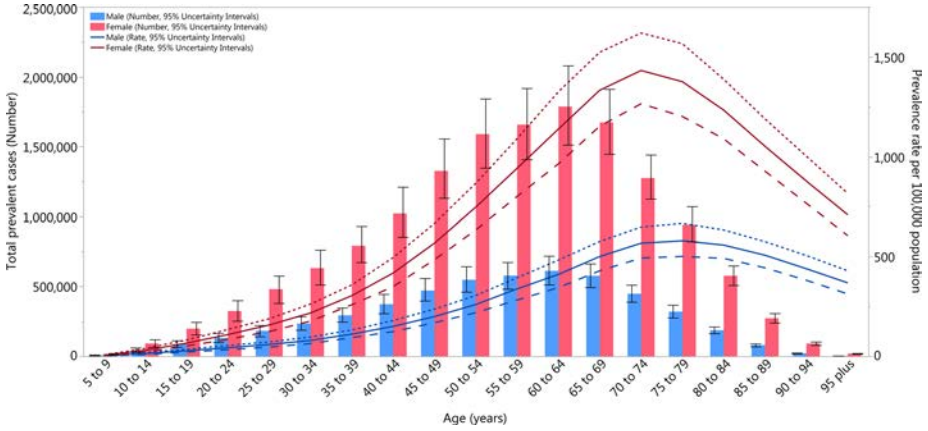

Figure 3 Global number of prevalent cases and prevalence rate of rheumatoid arthritis per 100 000 population by age and sex, 2017; dotted and dashed lines indicate 95% upper and lower uncertainty intervals, respectively

incidence rate was also found to be higher in females and increased with population ageing but there was no statistically significant difference between males and females in the 70+ age groups. The number of incident cases reached the highest level at 50–54 age group then a declining trend was observed to the oldest group (see online supplementary figure 4). The pattern of age-standardised DALY rate by sex across the age groups was relatively similar to the age-standardised prevalence rate (see online supplementary figure 5). Decomposition of DALY rate into YLL and YLD also showed that the YLL rate was significantly lower than YLD up to 70–74 age group. Moreover, it was found 60–64 and 65–69 age groups had the highest number of YLD and YLL, respectively (see online supplementary figure 6).

Burden of RA by SDI and HAQ

At the regional-level, a non-linear association was found between the age-standardised DALY rate and the SDI. The lowest age-standardised DALY rate was seen at an SDI of around 0.43; it then increased and decreased intermittently with SDI improvement (figure 4). In the high-income super region, only high-income North America showed an increasing level during 1990–2017. Despite a declining trend in Western Europe, this region still showed a higher than expected level of age-standardised DALY rate during the measurement period. In the Latin-America super-region, Caribbean and Tropical Latin America showed increasing trend during 1990–2017 and all regions had higher than expected level of age-standardised DALY rate in the recent 6 years. Central Europe, Eastern Europe, Central Asia, North Africa and Middle East, Southeast Asia, East Asia and Oceania had lower than expected age-standardised DALY rates during 1990–2017. Finally, in the Sub-Saharan Africa super-region, only Western Sub-Saharan Africa showed lower than expected age-standardised DALY rate during 1990–2017 (figure 4).

National-level analysis found there was a non-linear association between age-standardised DALY rate and SDI and the high burden of RA was not limited to the most developed or less developed countries. UK, India, Pakistan, Nepal, Honduras, Barbados, Trinidad and Tobago and many other countries showed much higher than expected level of age-standardised DALY rates. In contrast, Singapore, Malaysia, Sri Lanka, Vietnam, Timor-Leste showed and many other countries showed much lower than expected age-standardised DALY rates (figure 5). The association of age-standardised DALY rates and countries' HAQ also was also non-linear (see online supplementary figure 7).

DISCUSSION

In this paper, we presented the prevalence, incidence and DALY counts and age-standardised rates for RA in 195 countries from 1990 to 2017, as reported in GBD 2017. Globally, there were almost 20 million prevalent cases, 1.2 million incident cases and

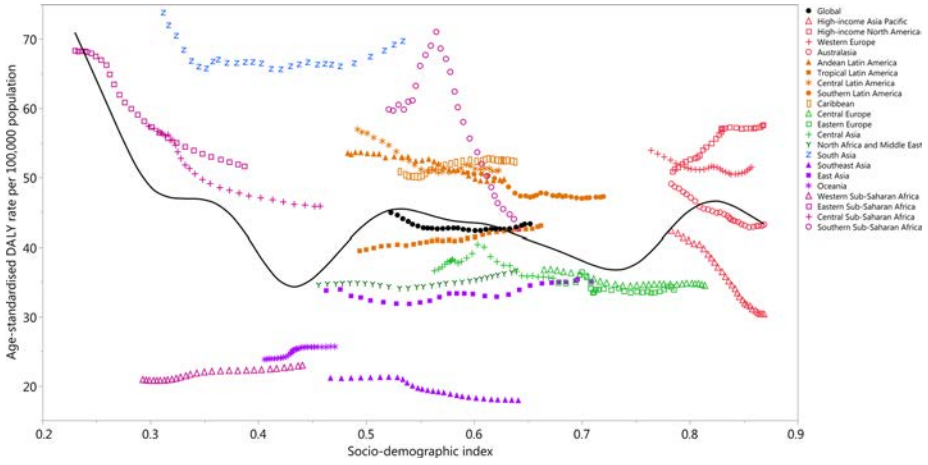

Figure 4 Age-standardised DALY rates for rheumatoid arthritis for 21 Global Burden of Disease (GBD) regions by Socio-Demographic Index (SDI), 1990–2017; expected values based on SDI and disease rates in all locations are shown as the black line. Twenty-eight points are plotted for each GBD region and show observed age-standardised DALY rates from 1990 to 2017 for that region. DALY, disability adjusted life year

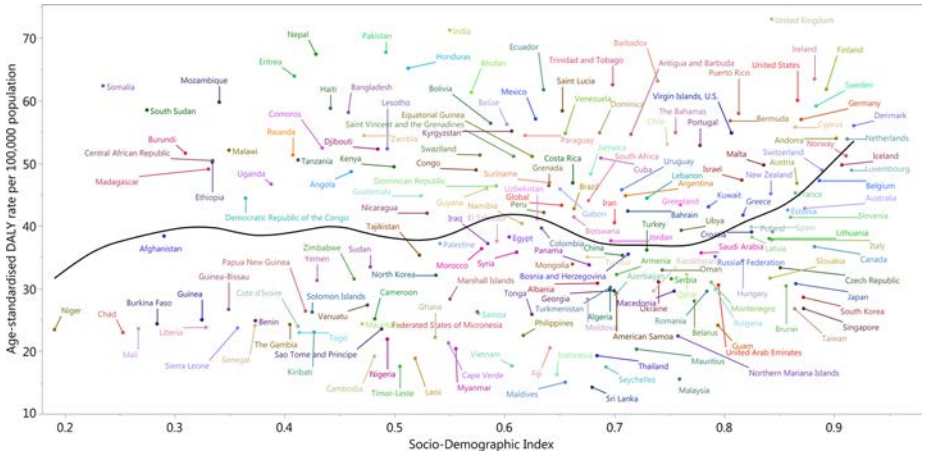

Figure 5 Age-standardised DALY rates of rheumatoid arthritis by 195 countries and Socio-Demographic Index, 2017; expected values are shown as the black line. Each point shows observed age-standardised DALY rate for specified country in 2017. DALY, disability adjusted life year.

3.4 million DALYs. These data serve to highlight the significant, yet under-recognised, global burden of RA.

A previous systematic review on RA prevalence found that regional crude prevalence rates were 400 for Southeast Asia, 370 for the Eastern Mediterranean, 620 for Europe, 1250 for North America, and 420 for the Western Pacific.[4] While estimates from this review could not be directly compared with the GBD 2017 estimates, it is clear that North America and the Eastern Mediterranean are consistently among the highest regions in terms of prevalence. In GBD 2010,[7] RA was found to be responsible for 4.8 million DALYs in 2010, which is higher than the updated GBD 2017 estimates. This difference may in part be explained by additional data sources and new methodologies applied in GBD 2017. The global prevalence of RA was similar between GBD 2010 and GBD 2017, at 240 per 100 000 population.[7] The crude DALYs for RA was higher in 2017 than in GBD 2013 (45.7 vs 37.6).[8]

The GBD 2017 data showed that the age-standardised prevalence and incidence rates have increased during the period 1990 to 2017 but age-standardised DALY rate decreased by 3.6%. An 11.6% increase in crude DALY rate from 1990 to 2013 has been reported[8]; whereas, GBD 2017 found that the crude DALY rate increased by 25% during 1990–2017. Other studies have not investigated the trend of age-standardised rate of prevalence, incidence and DALYS comprehensively and hence, findings cannot be compared.[20 21]

Differences in incidence and prevalence in regions that are within the same super-region should be noted. South Asia, which includes Bangladesh, India, Nepal, Bhutan and Pakistan, is among the regions with the highest incidence and prevalence of RA, yet South-East Asia, including Cambodia Myanmar and Thailand among others, is among the lowest incidence and prevalence rates globally.

GBD 2017 also showed that age-standardised prevalence and DALY rates were higher in females and increased with age and peaked at 70–74 and 75–79 age groups among females and males, respectively. Moreover, the age-standardised incidence rate was also found to be higher in females and increased with age, but there was no statistically significant difference between males and females in those aged over 70 years. The prevalence rate of RA across age groups was also examined in the previous studies and prevalence rates have reported to be higher in females than males[4 7 8]; but the association between prevalence rate and age and sex such as the monotonic positive association of prevalence rate with age found in prior studies[7] were not similar to the GBD 2017 findings.

It must be noted that the data presented here were primarily derived from modelled data through the processes in DISMOD-MR 2.1. True population-based national data on incidence and prevalence of RA were available from very few countries, thus the present study relies on modelling. As such, these national estimates should be interpreted with caution. Greater inclusion of musculoskeletal conditions, including RA, is encouraged in national health data collections.

To the best of our knowledge, the associations of prevalence, incidence and DALYs due to RA with developing status of region and countries have not been examined in the previous studies and the present study has some important findings. First, the association between burden of RA and SDI should not be assumed to be simplistic and linear; GBD 2017 showed a complex and non-linear association. In fact, burden of RA is not limited to developed or less developed countries and a high burden of RA was reported in countries with various SDI. Second, the global burden of RA has reached higher than expected levels during recent years and awareness of the importance of early identification and treatment should be encouraged in the countries with high incidence, prevalence and DALY rates to reduce future burden. Third, the effectiveness of prevention programmes should not be only judged based on the observed values but also the expected levels in each region and country need to be addressed.

One of the approaches in prevention programme is to focus on risk factors. However, previous observational studies found only smoking to be clearly associated with RA.[12] Although some risk factors such as genetic factors, hormones, stress, obesity, infections, gut bacteria and diet have been studied but the findings have been inconclusive. Smoking, as one of the important risk factors, need to be monitored precisely and specific prevention programme need to be applied in each country. According to a previous study,[22] the global age-standardised prevalence of daily smoking decreased by 28.4% and 34.4% in men and women since 1990, respectively. This study also shows the pace of progress as a function of geographies, development status and sex is heterogeneous. Greater success in tobacco control can be achieved through using effective and comprehensive policies introduced in previous papers.[23 24]

Effective treatment of RA needs a policy and health service response, such as the WHO Global Strategy, which aims to

develop and maintain functional ability that enables well-being in older age. In areas where there is problematic access to specialists, delayed diagnosis of RA may impede the effective treatment of RA and difficulties adhering to treat-to-target principles that aim to prevent RA-related disability by reducing disease activity and achieving remission. Early diagnosis and treatment prevent progression of joint damage in 90% of patients with early RA,[25] and increased levels of remission can be gained from effective early treatment with more complex disease modifying and biological drugs that are currently available,[25] although with their higher cost, this is primarily in high-income regions. However, greater awareness is needed of RA burden and dissemination of knowledge of the large body of evidence of the important improvements in morbidity and mortality that can be achieved with early clinical diagnosis and treatment with relatively low-cost drugs such as methotrexate.[26–28] With this early treatment of RA, the adverse consequences and increasing burden of RA can be prevented.

CONCLUSIONS

RA is a major global public health challenge; however, the burden of RA varies geographically. The age-standardised prevalence and incidence rates are overall increasing globally. Increasing population awareness regarding RA, its risk factors and the importance of early diagnosis and treatment with disease modifying agents is warranted to reduce the future burden of this condition. Improving health data for better monitoring of disease burden and health outcomes are strongly suggested.

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REFERENCES

1 Mathers DSC, Pfleger B. The global burden of rheumatoid arthritis in the year 2000. *Criterion* 2006;1.
2 Jean S, Hudson M, Gamache P, *et al*. Temporal trends in prevalence, incidence, and mortality for rheumatoid arthritis in Quebec, Canada: a population-based study. *Clin Rheumatol* 2017;36:2667–71.
3 Won S, Cho S-K, Kim D, *et al*. Update on the prevalence and incidence of rheumatoid arthritis in Korea and an analysis of medical care and drug utilization. *Rheumatol Int* 2018;38:649–56.
4 Rudan I, Sidhu S, Papana A, *et al*. Prevalence of rheumatoid arthritis in low– and middle–income countries: a systematic review and analysis. *Journal of Global Health* 2015;5.
5 World Health Organization. *Global health estimates 2016: disease burden by cause, age, sex, by country and by region, 2000–2016*. Geneva: World Health Organization, 2018.
6 Sebbag E, Felten R, Sagez F, *et al*. The world-wide burden of musculoskeletal diseases: a systematic analysis of the World Health Organization burden of diseases database. *Ann Rheum Dis* 2019;78:844–8.
7 Cross M, Smith E, Hoy D, *et al*. The global burden of rheumatoid arthritis: estimates from the Global Burden of Disease 2010 study. *Ann Rheum Dis* 2014;73:1316–22.
8 Moradi-Lakeh M, Forouzanfar MH, Vollset SE, *et al*. Burden of musculoskeletal disorders in the eastern Mediterranean region, 1990–2013: findings from the Global Burden of Disease study 2013. *Ann Rheum Dis* 2017;76:1365–73.
9 James SL, Abate D, Abate KH, *et al*. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease study 2017. *The Lancet* 2018;392:1789–858.
10 Roth GA, Abate D, Abate KH, *et al*. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: a systematic analysis for the Global Burden of Disease study 2017. *The Lancet* 2018;392:1736–88.
11 Kyu HH, Abate D, Abate KH, *et al*. Global, regional, and national disability-adjusted life-years (DALYs) for 359 diseases and injuries and healthy life expectancy (HALE) for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease study 2017. *The Lancet* 2018;392:1859–922.
12 Stanaway JD, Afshin A, Gakidou E, *et al*. Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease study 2017. *The Lancet* 2018;392:1923–94.
13 Arnett FC, Edworthy SM, Bloch DA, *et al*. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;31:315–24.
14 Kellgren JH. Diagnostic criteria for population studies. *Bull Rheum Dis* 1962;13:291.
15 Ropes MW, Bennett GA, Cobb S, *et al*. Proposed diagnostic criteria for rheumatoid arthritis: report of a study by a Committee of the American Rheumatism Association. *Ann Rheum Dis* 1957;16:118–25.
16 Vonkeman HE, van de Laar MAFJ. The new European League Against Rheumatism/American College of Rheumatology diagnostic criteria for rheumatoid arthritis: how are they performing? *Curr Opin Rheumatol* 2013;25:354–9.
17 Salomon JA, Haagsma JA, Davis A, *et al*. Disability weights for the Global Burden of Disease 2013 study. *Lancet Glob Health* 2015;3:e712–23.
18 Wang Y. *Smoothing splines: methods and applications*. Chapman and Hall/CRC, 2011.
19 Fullman N, Yearwood J, Abay SM, *et al*. Measuring performance on the healthcare access and quality index for 195 countries and territories and selected subnational locations: a systematic analysis from the Global Burden of Disease study 2016. *The Lancet* 2018;391:2236–71.

20 Alamanos Y, Voulgari PV, Drosos AA. Incidence and prevalence of rheumatoid arthritis, based on the 1987 American College of Rheumatology criteria: a systematic review. *Semin Arthritis Rheum* 2006;36:182–8.
21 Myasoedova E, Crowson CS, Kremers HM, *et al*. Is the incidence of rheumatoid arthritis rising?: results from Olmsted County, Minnesota, 1955-2007. *Arthritis Rheum* 2010;62:1576–82.
22 Reitsma MB, Fullman N, Ng M, *et al*. Smoking prevalence and attributable disease burden in 195 countries and territories, 1990–2015: a systematic analysis from the Global Burden of Disease study 2015. *The Lancet* 2017;389:1885–906.
23 Pierce JP, White VM, Emery SL. What public health strategies are needed to reduce smoking initiation? *Tob Control* 2012;21:258–64.
24 Golechha M. Health promotion methods for smoking prevention and cessation: a comprehensive review of effectiveness and the way forward. *Int J Prev Med* 2016;7:7.
25 Aletaha D, Smolen JS. Diagnosis and management of rheumatoid arthritis. *JAMA* 2018;320:1360–72.
26 Choi HK, Hernán MA, Seeger JD, *et al*. Methotrexate and mortality in patients with rheumatoid arthritis: a prospective study. *The Lancet* 2002;359:1173–7.
27 Lopez-Olivo MA, Siddhanamatha HR, Shea B, *et al*. Methotrexate for treating rheumatoid arthritis. *Cochrane Database of Systematic Reviews* 2014;103.
28 Rau R, Herborn G. Benefit and risk of methotrexate treatment in rheumatoid arthritis. *Clin Exp Rheumatol* 2004;22:S83–S94.

EPIDEMIOLOGICAL SCIENCE

Patients with rheumatoid arthritis facing sick leave or work disability meet varying regulations: a study among rheumatologists and patients from 44 European countries

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ABSTRACT

Objectives To describe and explore differences in formal regulations around sick leave and work disability (WD) for patients with rheumatoid arthritis (RA), as well as perceptions by rheumatologists and patients on the system's performance, across European countries.

Methods We conducted three cross-sectional surveys in 50 European countries: one on work (re-)integration and social security (SS) system arrangements in case of sick leave and long-term WD due to RA (one rheumatologist per country), and two among approximately 15 rheumatologists and 15 patients per country on perceptions regarding SS arrangements on work participation. Differences in regulations and perceptions were compared across categories defined by gross domestic product (GDP), type of social welfare regime, European Union (EU) membership and country RA WD rates.

Results Forty-four (88%) countries provided data on regulations, 33 (75%) on perceptions of rheumatologists (n=539) and 34 (77%) on perceptions of patients (n=719). While large variation was observed across all regulations across countries, no relationship was found between most of regulations or income compensation and GDP, type of SS system or rates of WD. Regarding perceptions, rheumatologists in high GDP and EU-member countries felt less confident in their role in the decision process towards WD (β=−0.5 (95% CI −0.9 to −0.2) and β=−0.5 (95% CI −1.0 to −0.1), respectively). The Scandinavian and Bismarckian system scored best on patients' and rheumatologists' perceptions of regulations and system performance.

Conclusions There is large heterogeneity in rules and regulations of SS systems across Europe in relation to WD of patients with RA, and it cannot be explained by existing welfare regimes, EU membership or country's wealth.

Key messages

What is already known about this subject?
- Rheumatoid arthritis (RA) has a high impact on functional ability and work participation.

What does this study add?
- Large variation in social security regulations for sick leave and work disability for patients with RA was observed across countries.
- This heterogeneity cannot be explained by existing welfare regimes, European Union membership or country's wealth.

How might this impact on clinical practice or future developments?
- Heterogeneity between countries regarding regulations for sick leave and work disability can affect patients' chances to return to work.
- These differences call for a platform to consider harmonisation of policies for patients with RA who experience restrictions in work participation.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory joint disease that often starts during working life of

patients.[1–3] RA has a high impact on functioning and ability to participate in social roles.[4 5] Despite substantial improvements in treatment options in the last decades, still 20% of the patients are unable to continue to work in the first 3 years of disease and over 30% of patients become work disabled after 10 years.[6–9]

For patients, work disability (WD) implies exclusion from an essential role in society, but also loss of income and reduced economic self-sufficiency.[5 10] To prevent individuals from poverty in case of WD, substitution of income has been introduced in some European countries in the 19th century. By now, most countries have some form of social security (SS) system in place to regulate income substitution. However, these systems are not uniform, as they have been shaped by national political and social developments throughout the 20th century. Policies targeted at income substitution balance between provision of a fair income, on one hand, and control of expenditures by restricting social security benefits only to individuals with specific levels of work restrictions, on the other hand.[11] More recently, the increasing economic burden of WD, and also the insight into social and health benefits of work participation stimulated in many countries stricter gate-keeping on the one hand and stronger employment support to enhance endurable work participation of persons with chronic disease on the other hand.[12]

SS arrangements have been suggested to result in differences in overall patterns of employment, WD and retirement.[13 14] A recent worldwide multinational study (COMORA) has shown that lower economic wealth and human development of countries is associated with higher rates of unemployment and higher absenteeism.[15] Earlier, QUEST RA study has observed that in low gross domestic product (GDP) countries, people remain working with higher levels of disability and disease activity compared with high GDP countries.[16] The regulations around the sick leave and WD may be, at least partially, responsible for these differences; however, they have not been studied.

Policies with regard to work participation, however, go far beyond income substitution only. Criteria for access to WD benefits as well as levels of income substitution likely depend, among others, on economic, political and cultural factors. While the European Union (EU) and WHO accept the historical differences in the way health and social systems are organised and function in their member states, there is a universal agreement that differences should not result in inequalities in health and quality of life of people across nations.[17] It is unclear whether EU-member states achieved any degree of homogeneity in the key regulations around social policies with regards to WD. In addition to system-level factors, there is evidence that personal contextual and disease-related factors[6 18 19] influence the decision of an individual to take sick leave or apply for long-term WD.

The aim of this study was to describe and explore differences in formal regulations around sick leave and WD for patients with RA, as well as perceptions by rheumatologists and patients on the system's performance, across European countries. We hypothesised that (1) lower GDP countries have stricter rules with regard to obtaining WD and lower income substitution once WD is granted, (2) EU countries have more homogeneous regulations compared with non-EU countries, and (3) patients and rheumatologists in high GDP and EU-member states are more satisfied with the performance of the social security system.

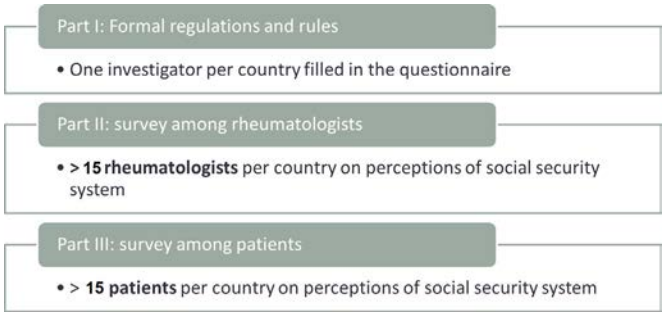

Figure 1 Study design.

METHODS

Design and framework

We conducted a cross-sectional observational study consisting of three surveys in 50 of the 53 countries of the European WHO Region (in three countries, no contact person could be found), in 2014–2016 (figure 1). The questionnaires were designed following the framework of access (originally applied in healthcare[20 21]), with three dimensions: (1) *availability* of re-integration plans or other systems/policies to facilitate work and/or prevent WD, as well as the eligibility criteria that a patient should meet to receive SS benefits; (2) *affordability*, that is, the level of income substitution granted in case of sick leave and permanent disability; (3) *acceptability*, that is, professional and individual perceptions of rheumatologists and patients, respectively, around the system performance on these issues.

Participants

For each of the 50 European countries, one rheumatologist was invited as principal investigator (PI) to complete the questionnaire on SS arrangements in his/her country in case of sick leave and long-term WD due to RA (survey 1; availability and affordability). Additionally, each PI was asked to invite at least 15 rheumatologists (survey 2; acceptability) and at least 15 patients (survey 3; acceptability) to complete a questionnaire on professional and individual perceptions of the system. To recruit rheumatologists, PIs were instructed to ensure a diverse sample in terms of gender, years of professional experience and clinical setting. Patients could also be recruited via patient organisations and aimed at representing the spectrum of patients with RA, assuring that at least half of them had experience with (applying for a) WD pension.

Questionnaires

The questionnaire for PIs addressed national regulations (in 2014) on benefits separately for sick leave and WD (online supplementary text S1a: availability and affordability), as well as calculations of the level of income for nine prespecified scenarios (vignettes), one on sick leave and two for long-term WD across the three levels of income (online supplementary text S1b). In countries where a patient research partner was available (n=21), he/she was invited to comment on any inconsistencies in the answers on the main questionnaire about the formal regulations. In this case, answers were double checked with the PI.

The questionnaire for rheumatologists (online supplementary text s2; acceptability) contained questions about perceptions on appropriateness of the SS arrangements, practical aspects of the application process for benefits and the role of rheumatologists in the process. The questionnaire for patients (online supplementary text S3; acceptability) addressed perceptions about the importance and adequacy of the existing arrangements. Additional questions

were included on age, gender and work environment (non-university hospital, university hospital, private practice, other) for rheumatologists; and on age, gender, disease duration, work status (paid work, no paid work but not work disabled, partially or fully work disabled) and history of sick leave and WD. The PI decided on whether questionnaires could be applied in English, otherwise translated them, wherever possible, with patient partners involved in checking the translation.

Data on GDP per capita (in international dollars, 2013) were extracted from the World Development Indicators report by the World Bank[22] and used as a continuous variable or dichotomised around the median (27 000 int.$). The welfare regimes taxonomy included five groups, namely the Anglo-Saxon, Bismarckian, Mediterranean, Post-Communist and Scandinavian type of system.[23] Rates of WD among patients with RA have been collected by the QUEST-RA study (2009) and were available for 21 countries from our sample.[16 24]

Statistical analysis

Arrangements to support work and SS regulations in case of sick leave or WD due to RA (questionnaire 1)

Collected data on formal rules and policies were first presented through descriptive statistics. To investigate whether the regulations differed by the type of welfare regime,[23] GDP, EU membership (EU-15, new EU-member states and non-EU countries) or were associated with country-level WD rates among patients with RA,[16] subgroup comparisons were performed using Pearson correlations, t-test/Mann-Whitney U test and χ^2/Fisher's test, as appropriate.

Patients' and rheumatologists' perceptions of the SS system (questionnaires 2 and 3)

Answers of rheumatologists on their perspective of SS arrangements were summarised and scored (the higher is the better) around the two domains: (1) 'Performance of the system' (score 0–4) and (2) 'Role of the rheumatologists' (score 0–4). Additionally, a single item on the perceived standardisation in the decision-making process was analysed separately. Input from patients was summarised following three domains: (1) 'Importance and support to remain employed' (score 0–5); (2) 'Process of applying for WD' (score 0–4); (3) 'Obtaining and living with work disability pension' (score 0–6) (complete questionnaires are provided in online supplementary texts S2 and S3). Each domain consisted of four to six questions (each on a 1 (totally agree) to 5 (totally disagree) Likert scale, dichotomised as 1 ("(totally) agree") and 0 ("not agree/not disagree", "(totally) disagree"). The dichotomised scores per question were summed into the five domain scores (two for rheumatologists and three for patients).

Rheumatologists' and patients' characteristics were compared across the type of SS system, EU membership and GDP. Small numbers of surveyed patients and rheumatologists in each country hindered analyses of country-level means and thus were not related to national RA WD rates. The domain 'Importance and support to remain employed' was assessed in patients currently or ever having worked. Analyses in domains 'Process of applying for work disability' and 'Obtaining and living with work disability' were limited to patients currently work disabled or ever considered applying for WD.

Finally, we conducted multilevel (with individuals clustered in countries) multiple regression analysis with each of the domains as an outcome and type of SS system, EU membership or GDP as the independent variable of interest. Models with patient perceptions as outcome were adjusted for age, gender, education, disease duration and ever having had sick leave due to RA. When rheumatologists' perceptions were the outcome, analyses were adjusted for age, gender and work setting.

RESULTS

Forty-four (28 EU and 16 non–EU-member states) countries (88%) provided data on formal rules and regulations for sick leave and WD. Of these, 33 (75%) countries collected data from rheumatologists (n=539), and 34 (77%) countries collected data from patients (n=719) (missing countries were all non-EU members except Luxembourg).

Arrangements to support work and SS regulations in case of sick leave of WD due to RA

While nearly all countries had arrangements to support patients with restrictions to work, a large heterogeneity was observed in the type of arrangements (table 1, online supplementary tables S1–3). All except for 12 countries had facilities to support patients with RA in paid employment (n=32, 73%), but only in a quarter of countries (n=11, 25%) rehabilitation efforts were obligatory prior to the decision about long-term WD. Twenty-five (57%) and 30 (68%) countries had a requirement for employment history or social insurance contributions in order to be eligible for sick leave or long-term WD compensation, respectively. The maximum sick leave length before transition to long-term WD varied from 3 to 36 months (mean (SD) 13 (9)). In eight (18%) and five (11%) of the countries, participation of a rheumatologist was mandatory in the process of application or decision-making process on long-term WD, respectively. In addition to a functional assessment (degree of (dis)ability), prior profession (n=25 (57%) of countries), diagnosis (n=32 (73%)), earning capacity (n=12 (27%)), age (n=20 (45%)) and gender (n=5 (11%)) were reported to be accounted for when a decision was taken about (the degree of) WD. Prognosis, education and place of residence were mentioned as additional factors by few countries.

All countries except the former Yugoslav Republic of Macedonia, Bosnia-Herzegovina and Serbia reported to recognise partial WD, a status that partially substitutes income while the person can continue in (reduced) employment. Of the 26 countries providing data on income substitution, in eight countries (31%) income substitution (averaged over the first 6 months of disability) in case of sick leave was less than 70% of previous income. Income substitution averaged over the first 12 months of disability was less than 70% in 18 (69%) and 15 (58%) in case of moderate (partial) or severe (full) long-term disability, respectively (table 2). While wealthier countries as expected provided higher benefits in absolute terms, when converted to percentage of the previously earned income, no relationship was found between income compensation and neither GDP nor the type of SS system, or rates of WD. In richer countries and in countries with the Bismarckian type of welfare regime, the WD pension burden was more likely to be shared between SS and a private insurance, while countries with lower GDP and other welfare regimes had social insurance as the main source of WD allowances (data not shown). In countries with lower GDP, a rheumatologist was more frequently necessarily involved in the application and decision-making. Other aspects of the system revealed no statistically significant patterns with country-level characteristics or national WD rates.

Table 1 Summary of social security regulations to support stay at work and work disability arrangements for persons with rheumatoid arthritis (RA)

Regulation		N (%)*	Anglo-Saxon	Scandinavian	Bismarckian	Mediterranean	Post-Communist
Support to stay at work							
Facilities for persons with RA that aimed at keeping the patient in paid employment are available	Yes	32 (73%)	**IE**, **GB**	**DK**, **FI**, IS, NO, **SE**	**AU**, **BE**, **DE**, IL, **LU**, CH, **NL**,	**CY**, **IT**, **PT**	AL, BY, **BG**, **CR**, **CZ**, **LT**, MK, MD, ME, **PL**, **RO**, RS, **SK**, **SL**, TJ
	No	12 (27%)	–	–	**FR**	**GR**, **MT**, **ES**, TR	BA, **EE**, GE, **HU**, RU, **LV**, UA
Access to sick leave or short-term absence							
Requirement for length of previous employment AND/OR certain income earned AND/OR certain amount of social insurance tax paid in order to be eligible for sick-leave compensation	Yes	25 (57%)	**IE**, **GB**	**DK**, NO, **SE**	**BE**, **FR**, IL, CH	**CY**, **GR**, **PT**, **ES**	AL, BY, BA, **BG**, **HR**, **CZ**, **LT**, MD, **PL**, **RO**, **SK**, TJ
	No	18 (41%)	–	**FI**, IS	**AU**, **DE**, **LU**, **NL**	**IT**, **MT**, TR	**EE**, GE, **HU**, **LV**, MK, ME, RU, RS, **SL**, UA
The rheumatologist is authorised to certify short-term absence from work due to RA	Yes	33 (75%)	**IE**	**DK**, **FI**, IS, NO, **SE**	**AU**, **BE**, **FR**, **DE**, IL, **LU**, CH	**CY**, **GR**, **MT**, **PT**, **ES**, TR	AL, BY, BA, **CZ**, **EE**, **HU**, **LT**, MK, ME, **PL**, **RO**, RU, TJ, UA
	No	10 (23%)	**GB**		**NL**	**IT**	**BG**, **CR**, GE, **LV**, MD, RS, **SK**, **SL**
First 1–3 days of sick leave are unpaid	Yes	11 (25%)	–	**SE**	**FR**, IL	**CY**, **IT**, **PT**, **ES**, TR	**CZ**, **EE**, **LV**
	No	32 (73%)	**IE**, **GB**	**DK**, **FI**, IS, NO	**AU**, **BE**, **DE**, **LU**, CH, **NL**	**GR**, **MT**	AL, BY, BA, **BG**, **CR**, GE, **HU**, **LT**, MK, MD, ME, **PL**, **RO**, RU, RS, **SK**, **SL**, TJ, UA
Decision about long-term work disability							
Maximum length of sick leave before a WD can be granted	<12 months	35 (80%)	**GB**	**DK**, IS, **SE**	**AU**, **FR**, **DE**, IL, **LU**	**CY**, **GR**, **IT**, **MT**, **PT**, **ES**, TR	AL, BY, **BG**, **CR**, **EE**, **LV**, **LT**, **HU**, MK, MD, **ME**, **PL**, **RO**, RU, RS, **SL**, TJ, UA
	≥12 months	8 (18%)	**IE**	**FI**, NO	**BE**, CH, **NL**	–	BA, **CZ**, **SK**
Is it obligatory that a rheumatologist is involved in application process (ie, providing information about the disease)?†	Yes	10 (23%)	–	–	–	**CY**, **GR**	AL, BY, **BG**, **LT**, MK,**SK**,RU, TJ
	No	31 (70%)	**IE**, **GB** **SE**	**DK**, **FI**, NO	**AU**, **BE**, **FR**, IL, **LU**, CH, **NL**, **DE**	**IT**, **MT**, **PT**, **ES**, TR	BA, **CR**, **CZ**, **EE**, **HU**, **LV**, MD, **PL**, **RO**, RS, **SL**, UA
Is the rheumatologist also in an obligatory way involved in the decision-taking about the long-term work disability? (ie, is part of the commission or expert committee)†	Yes	5 (11%)	–	–	–	**CY**	AL, **BG**, **SK**, TJ
	No	36 (82%)	**IE**, **GB** **SE**	**DK**, **FI**, NO	**AU**, **BE**, **FR**, IL, **LU**, CH, **NL**, **DE**	**GR**, **IT**, **MT**, **PT**, **ES**, TR	BY, BA, **CR**, **CZ**, **EE**, **HU**, **LV**, **LT**, MK, MD, **PL**, **RO**, RU, RS, **SL**, UA
Rehabilitation efforts are obligatory before a patient could be assessed for long-term disability	Yes	11 (25%)		**DK**, **FI**, **SE**, NO	**AU**, CH, **NL**	–	**LT**, MD, ME, **RO**, **SL**
	No	32 (73%)	**IE**, **GB**	IS	BE, **FR**, **DE**, IL, **LU**	**CY**, **GR**, **IT**, **MT**, **PT**, **ES**, TR	AL, BY, BA, **BG**, **CR**, **CZ**, **EE**, **HU**, **LV**, MK, **PL**, RU, RS, **SK**, TJ, UA

European Union countries are in **bold,** high GDP (>27 000 int.$, based on median) countries are underscored.
*Data provided by 44 countries, Georgia reported to not have any effective social security system in place.
†Data missing for IS, ME.
AL, Albania; AT, Austria; BA, Bosnia and Herzegovina; BE, Belgium; BG, Bulgaria; BY, Belarus;CH, Switzerland; CY, Cyprus; CZ, Czech Republic; DE, Germany; DK, Denmark; EE, Estonia; ES, Spain; FI, Finland; FR, France; GE, Georgia; GR, Greece; HR, Croatia; HU, Hungary; IE, Ireland; IL, Israel; IS, Iceland; IT, Italy; LT, Lithuania; LU, Luxembourg; LV, Latvia; MD, Moldova; ME, Montenegro; MK, Macedonia (the former Yugoslav Republic of Macedonia); MT, Malta; NL, The Netherlands; NO, Norway; PL, Poland; RO, Romania; RS, Serbia; RU, Russia; SE, Sweden; SK, Slovakia; SL, Slovenia; TJ, Tajikistan; TR, Turkey; UA, Ukraine; UK, United Kingdom.

Table 2 Income compensation in case of work disability for an employee with average income*

Type of disability	Income compensation		Anglo-Saxon	Scandinavian	Bismarckian	Mediterranean	Post-Communist
In case of sick leave	≤70% of earned income	8 (31%)	**IE**	–	**BE**	**CY**, **PT**, TR	**CZ**, **EE**, **SK**
	>70% of earned income	18 (69%)	–	**FI**, NO	**LU**, **NL**, SE	**FR**, **IT**, **ES**	**BG**, **LT**, **LV**, MD, **PL**, **RO**, RS, **SL**
In case of moderate (50%) work disability†	≤70% of earned income	18 (69%)	**IE**	**FI**, NO	**BE**, SE, CH	**CY**, TR	BY, **BG**, **CZ**, **EE**, **LV**, **LT**, MD, **PL**, **RO**, RS, **SK**
	>70% of earned income	7 (27%)	–	–	**LU**, **NL**	**FR**, **IT**, **PT**, **ES**	AL
In case of severe (75%) work disability†	≤70% of earned income	15 (58%)	–	**FI**, NO	**BE**, **LU**, SE, CH	**CY**,	**CZ**, **EE**, **LV**, **LT**, MD, **PL**, **RO**, RS, **SK**
	>70% of earned income	10 (38%)	**IE**	–	**NL**	**FR**, **IT**, **PT**, **ES**, TR	AL, BY, **BG**

European Union countries are in **bold,** high GDP (>27 000 int.$, based on median) countries are underscored.
*A person 50 years old recently diagnosed with rheumatoid arthritis, who is a citizen and has worked for 25 years full time.
†SL did not provide data for long-term work disability.
AL, Albania;BE, Belgium; BG, Bulgaria; BY, Belarus; CH, Switzerland;CY, Cyprus; CZ, Czech Republic; EE, Estonia; ES, Spain; FI, Finland; FR, France; IE, Ireland; IT, Italy; LT, Lithuania; LU, Luxembourg; LV, Latvia; MD, Moldova; NL, The Netherlands; NO, Norway;PL, Poland; PT, Portugal; RO, Romania; RS, Serbia; SK, Slovakia; SL, Slovenia; TR, Turkey.

Rheumatologists' and patients' perceptions of SS system

In total, 539 rheumatologists (mean age (SD) 48 (10), 284 (53%) female, 278 (51%) working in university hospitals) from 33 countries filled in the questionnaires (online supplementary table S4). Scores on 'Role of the rheumatologists' (0–4) and 'Performance of the system' (0–4) ranged from 1.4 (SD 0.9) (Anglo-Saxon) to 2.4 (1.1) (Post-Communist) and 0.8 (0.9) (Anglo-Saxon) to 2.3 (1.2) (Scandinavian), respectively. Perceived level of standardisation around decision-taking revealed that only 26% (n=135) of rheumatologists consider decisions on WD to be objective. Of note, those who perceived standardisation as poor (vs good) scored worse on both 'Role of the rheumatologists' (−0.4 points) and 'Performance of the system' (−2.6 points) domains (t-test p value for both scores <0.05). Multilevel analyses revealed that rheumatologists in high GDP (vs low GDP) and EU-member (vs non–EU-member) countries felt less confident in having an active role in WD decisions (β=−0.5 (95% CI −0.9 to −0.2) and β=−0.5 (95% CI −1.0 to −0.1), respectively). In addition, significant differences were observed across the system types with the Scandinavian type (Denmark, Iceland, Sweden, Norway, Finland) consistently scoring higher than the others on domains 'Importance and support to remain employed' and 'Process of applying for work disability pension' (table 4 and online supplementary table S5).

The patient sample consisted of 719 patients from 34 countries (mean age (SD) 53 (12), 76% female, 519 (78%) ever worked). The highest (=most satisfied) patient scores on all three domains were consistently observed in countries with Scandinavian and Bismarckian type of security system (table 3). In multilevel adjusted regression models, neither country wealth nor EU status were associated with patients' perceptions (table 4 and online supplementary table S5). The findings across the system type were notably consistent across patient and rheumatologist domains.

Table 3 Patients' and rheumatologists' characteristics per type of security system

	Total	Anglo-Saxon	Scandinavian	Bismarckian	Mediterranean	Post-Communist	P value
Rheumatologists' characteristics (questionnaire 2)							
N of rheumatologists (N of countries)	539 (33)	22 (2)	58 (5)	88 (6)	87 (6)	284 (14)	
Role of the rheumatologists (0–4)	2.1 (1.1)	1.4 (0.9)	1.3 (0.9)	2.1 (1.3)	1.9 (1.0)	2.4 (1.1)	<0.001
Performance of the system (0–4)	1.5 (1.1)	0.8 (0.9)	2.3 (1.2)	2.0 (1.1)	1.1 (1.0)	1.4 (1.1)	<0.001
Patient characteristics (questionnaire 3)							
N of patients (N of countries)	719 (34)	47 (2)	60 (4)	137 (5)	119 (7)	356 (16)	
Importance and support to remain employed (0–5)	2.1 (1.2)	1.6 (0.8)	2.6 (1.2)	2.3 (1.4)	2.1 (1.2)	2.0 (1.1)	<0.001
Process of applying for work disability (WD) (0–4)	2.2 (1.8)	1.3 (1.8)	3.2 (3.0)	2.4 (1.9)	2.0 (1.6)	2.3 (1.7)	<0.001
Obtaining and living with WD pension (0–6)	1.1 (1.1)	0.7 (0.8)	1.2 (1.1)	1.2 (1.4)	1.0 (1.1)	1.1 (1.1)	0.22

Anglo-Saxon: UK, Ireland; Scandinavian: Denmark, Iceland, Sweden, Norway, Finland; Bismarckian: Austria, Belgium, Germany, France, Israel, Netherlands, Switzerland; Mediterranean: Cyprus, Greece, Italy, Portugal, Spain, Turkey; Post-Communist: Albania, Bulgaria, Czech Republic, Croatia, Estonia, Georgia, Hungary, Latvia, Lithuania, Poland, Romania, Russian Federation, Serbia, Tajikistan, Slovak Republic, Slovenia.

Table 4 Patients' and rheumatologists' perceptions across several domains according to (1) GDP per capita purchasing power parity, (2) EU membership status and (3) type of social security system (models adjusted for sociodemographic confounders)

	Patients' perceptions (N of countries=34)*			Rheumatologists' perceptions (N of countries=33)*	
	Importance and support to remain employed (0–5) n=491	Process of applying for work disability pension (0–4) n=342	Obtaining and living with work disability pension (0–6) n=341	Performance of the system (0–4) n=390	Role of the rheumatologists (0–4) n=393
GDP per capita (int.$) High vs low GDP	0.21 (–0.07 to 0.50)	0.39 (–0.20 to 0.98)	–0.09 (–0.44 to 0.25)	0.30 (–0.08 to 0.72)	**–0.55 (–0.94 to –0.16)**
EU membership EU vs non-EU member	0.20 (–0.14 to 0.53)	–0.41 (–1.10 to 0.29)	–0.16 (–0.57 to 0.26)	–0.12 (–0.56 to 0.32)	**–0.54 (–0.95 to –0.13)**
Type of system					
Scandinavian	Reference	Reference	Reference	Reference	Reference
Anglo-Saxon/liberal	**–1.02 (–1.64 to –0.39)**	**–1.41 (–2.73 to –0.09)**	–0.47 (–1.30 to 0.36)	**–1.49 (–2.22 to –0.77)**	–0.57 (–1.49 to 0.35)
Bismarckian/conservative	–0.40 (–0.90 to 0.10)	–0.74 (–1.84 to 0.40)	–0.04 (–0.73 to 0.65)	–0.41 (–0.94 to 1.12)	–0.39 (–1.09 to 0.31)
Mediterranean/southern	**–0.56 (–1.06 to –0.07)**	**–1.29 (–2.33 to –0.25)**	–0.20 (–0.86 to 0.46)	**–1.19 (–1.70 to –0.69)**	–0.11 (–0.78 to 0.56)
Post-Communist/eastern	**–0.63 (–1.06 to –0.19)**	**–1.02 (–1.91 to –0.13)**	**–0.04 (–0.62 to 0.53)**	**–0.98 (–1.44 to –0.51)**	0.21 (–0.40 to 0.83)

Coefficients are derived from separate multilevel multiple models (with individuals clustered in countries and each independent variable (ie, gross domestic product (GDP), European Union (EU) membership or type of system), adjusted for age, gender, education, disease duration and ever having had sick leave due to rheumatoid arthritis; analyses on the rheumatologist domains were adjusted for age, gender and work setting.
Statistically significant (p<0.05) regression estimates are in **bold**.
Anglo-Saxon: UK, Ireland; Scandinavian: Denmark, Iceland, Sweden, Norway, Finland; Bismarckian: Austria, Belgium, Germany, France, Israel, Netherlands, Switzerland; Mediterranean: Cyprus, Greece, Italy, Portugal, Spain, Turkey; Post-Communist: Albania, Bulgaria, Czech Republic, Croatia, Estonia, Georgia, Hungary, Latvia, Lithuania, Poland, Romania, Russian Federation, Serbia, Tajikistan, Slovak Republic, Slovenia.
*The higher the score, the more positive are the perceptions.

DISCUSSION

To our knowledge, this is the first study to provide an extended overview of systems to support work or WD in RA. With regard to the system rules and regulations, that is, availability and affordability of WD arrangements, a large heterogeneity across countries was observed for most regulations including income compensation. While research and judgement on which system is preferable is complex and beyond the aim of this study, it is striking that a person with RA, on becoming disabled, will face very different perspectives on future work participation, depending on his/her country of residence. Only in a minority of countries work re-integration plans were obligatory before starting a procedure towards WD.

Despite important variation, we could not detect patterns explaining differences in the formal rules and regulations of the SS systems, with only few exceptions. We could not find support to our first hypothesis that lower GDP countries would have stricter rules around WD or lower relative income substation when WD is granted, nor did we find any common features of systems in lower GDP countries or EU-member versus non–EU-member states. Moreover, the rules of the SS system did not seem to have a large role in explaining differences in WD in RA. Earlier research—mainly in general population and above 50 years of age in a number of European countries and dating back to 2007—suggested that the national SS system and, in particular, generosity of benefits explains a large proportion of between-country variations in disability rates.[25] We could not reproduce these results in our study among patients with RA. It is worth noting that data on WD rates in RA were available for 21 countries only and collected almost a decade earlier, and was based on a sample of patients rather than national statistics.[16]

In contrast to findings on formal regulations (availability and affordability), patients' and rheumatologists' perceptions of systems to support persons with RA encountering work restrictions (acceptability) showed an apparent variation according to the type of the social security system: the Scandinavian and Bismarckian employment support and social security system consistently appeared to most adequately meet the expectations of patients and rheumatologists regarding remaining at work and application for a WD pension. At the same time, little differences were related to country's wealth or EU membership, and only a weak signal suggested that rheumatologists in lower-income countries are more confident in their role to support patient in WD issues. The latter may indicate that rheumatologists in low-income countries interact more intensively with patients on these issues and accept WD questions as part of their responsibility. Alternatively, other cultural and system factors could be considered, for example, patients referring to other professionals within the system (such as primary care or state agencies) for advice and support regarding WD decisions.[26] Overall, a lack of standardisation in the decision-making process on WD was reported by nearly three quarters of the rheumatologists, and reinforces earlier calls for efforts for standardisation and homogenisation.[27] Therefore, our third hypothesis that levels of satisfaction with the system is higher in EU-member states and higher GDP countries was not supported by the available data, while initial insight was generated with respect to the SS system type where no hypotheses were formed a priori.

This study has some notable limitations. First, the complexity of access to SS related to work and disability is hard to capture with a questionnaire that unavoidably simplifies reality. In absence of a validated tool to measure formal social security regulations and perceptions around the system performance, we used self-developed questionnaires. These happened in intensive collaboration with several international experts on work participation studies. We did not follow a formal translation procedure and the decision and responsibility to translate was left with the PI; however, the translations were double checked by patient partners. While government authorities would be the most knowledgeable parties to enquire about rules and regulations, the feasibility issues around establishing a direct contact with agencies from >30 European countries, in different languages,

and where they likely have little or no interest to contribute to a research project, the well-established network of rheumatologists active in research was approached instead. To improve data quality and accuracy, PIs were encouraged to seek help from other experts in their country; the summary was sent to available patient partners for a face-validity check of data in their country, and the results showed a good agreement. It is emphasised in the discussion that this study has an explorative nature and limited conclusions should be drawn.

We have to acknowledge that *application* of the formal rules and regulations can differ substantially from the formal rules, and hence patients in countries with similar rules could potentially have different experiences when rules are applied. For example, partial WD (which formally implies that a patient can work part of the time) may be a barrier to any employment in some countries and thus perceived differently compared with countries where patients deemed partially disabled are able to use their right for part-time work. We have attempted to get the initial insight into this through the surveys among patients and rheumatologists. Furthermore, it was challenging to select the best country-level characteristics that should be related to indicators of access. On this line, the SS system taxonomy used[23] is most likely an oversimplification of the complex systems that are constantly changing and developing, but to our knowledge, no alternative taxonomy exists. Recourse to (official) disability is a complex construct in which social security has a limited role. Alternative approaches to gain insight into international variation should be considered to study the performance and impact or social security systems. One of the potentially promising methods could consist of a series of clinical vignettes. By considering the rules and regulations that are to be applied to a hypothetical patient with given characteristics in terms of disease, work situation and disability, as well as attitudes and values about role of work in life and society, countries could be compared and further classified. Despite limitations, we present the first attempt to understand whether patterns in regulations can be found to help to understand differences in employment, sick leave and WD. We found differences in regulations and income substitution that are challenging our perceptions of equity and call for further research to justify them or for efforts to define the acceptable standards. Cost-effectiveness models in RA often count with an improvement of WD in parallel with the improvement of the health status of the patient (eg, Health Assessment Questionnaire–based Markov models). Our study suggests that large variations exist between countries regarding regulations of short-term absence or WD that can affect their chances to return to work, a point to consider in economic evaluations. Although we did not find clear relations between regulations and work participation rates, we should keep in mind the previous research that suggested that patients continue to work at different health status across countries.[16] As system type appears to have a rather limited impact on regulations and perceptions on them, this might indicate that interventions to support work retention in patients with RA could in principle be considered irrespective of SS system. Moreover, the ageing of the population worldwide urges policy-makers to increase the age of retirement, meaning that people with RA will also be expected to work longer in the years to come.

In conclusion, we observed large heterogeneity in rules and regulations of SS systems across Europe in relation to WD of patients with RA, and these cannot be explained by existing welfare regimes, EU membership or country's wealth. These differences call for a platform to consider harmonisation of policies for patients with RA who experience restrictions in work participation. While remarkably little differences of patients' and rheumatologists' perceptions are related to country's wealth and membership in EU, Scandinavian employment support and SS system appears to most adequately meet the expectations of patients and rheumatologists regarding endurable work participation and access to WD pension.

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REFERENCES

1 Innala L, Berglin E, Möller B, *et al*. Age at onset determines severity and choice of treatment in early rheumatoid arthritis: a prospective study. *Arthritis Res Ther* 2014;16.
2 Cross M, Smith E, Hoy D, *et al*. The global burden of rheumatoid arthritis: estimates from the Global Burden of Disease 2010 study. *Ann Rheum Dis* 2014;73:1316–22.
3 Eriksson JK, Neovius M, Ernestam S, *et al*. Incidence of rheumatoid arthritis in Sweden: a nationwide population-based assessment of incidence, its determinants, and treatment penetration. *Arthritis Care Res* 2013;65:870–8.
4 Matcham F, Scott IC, Rayner L, *et al*. The impact of rheumatoid arthritis on quality-of-life assessed using the SF-36: a systematic review and meta-analysis. *Semin Arthritis Rheum* 2014;44:123–30.
5 Lenssinck M-LB, Burdorf A, Boonen A, *et al*. Consequences of inflammatory arthritis for workplace productivity loss and sick leave: a systematic review. *Ann Rheum Dis* 2013;72:493–505.
6 Backman CL. Employment and work disability in rheumatoid arthritis. *Curr Opin Rheumatol* 2004;16:148–52.
7 Sokka T. Work disability in early rheumatoid arthritis. *Clin Exp Rheumatol* 2003;21(5 Suppl 31):S71–4.
8 Tiippana-Kinnunen T, Paimela L, Peltomaa R, *et al*. Work disability in Finnish patients with rheumatoid arthritis: a 15-year follow-up. *Clin Exp Rheumatol* 2014;32:88–94.
9 Scott DL, Smith C, Kingsley G. What are the consequences of early rheumatoid arthritis for the individual? *Best Pract Res Clin Rheumatol* 2005;19:117–36.
10 Wolfe F, Michaud K, Choi HK, *et al*. Household income and earnings losses among 6,396 persons with rheumatoid arthritis. *J Rheumatol* 2005;32:1875–83.
11 Angelini V, Cavapozzi D, Paccagnella O. *Dynamics of work disability reporting in Europe. 'Marco Fanno' working paper (104)*. University of Padua, 2009.
12 OECD. *Sickness, disability and work: breaking the barriers. A synthesis of findings across OECD countries*, 2010.
13 Börsch-Supan A, Brugiavini A, Croda E. The role of institutions and health in European patterns of work and retirement. *J Eur Soc Policy* 2009;19:341–58.
14 Chung CP, Sokka T, Arbogast PG, *et al*. Work disability in early rheumatoid arthritis: higher rates but better clinical status in Finland compared with the US. *Ann Rheum Dis* 2006;65:1653–7.
15 van der Zee-Neuen A, Putrik P, Ramiro S, *et al*. Large country differences in work outcomes in patients with RA—an analysis in the multinational study COMORA. *Arthritis Res Ther* 2017;19.
16 Sokka T, Kautiainen H, Pincus T, *et al*. Work disability remains a major problem in rheumatoid arthritis in the 2000s: data from 32 countries in the QUEST-RA study. *Arthritis Res Ther* 2010;12.
17 European Commission. *Reducing health inequalities in the European Union*. Luxembourg: Publications Office of the European Union, 2010.
18 de Croon EM, Sluiter JK, Nijssen TF, *et al*. Work ability of Dutch employees with rheumatoid arthritis. *Scand J Rheumatol* 2005;34:277–83.
19 Odegård S, Finset A, Kvien TK, *et al*. Work disability in rheumatoid arthritis is predicted by physical and psychological health status: a 7-year study from the Oslo RA register. *Scand J Rheumatol* 2005;34:441–7.
20 McIntyre D, Thiede M, Birch S. Access as a policy-relevant concept in low- and middle-income countries. *Health Econ Policy Law* 2009;4:179–93.
21 Putrik P, Ramiro S, Kvien TK, *et al*. Inequities in access to biologic and synthetic DMARDs across 46 European countries. *Ann Rheum Dis* 2014;73:198–206.
22 IMF. World Economic Outlook Database, 2011. Available: http://www.imf.org/external/pubs/ft/weo/2011/02/weodata/index.aspx [Accessed Jun 2014].
23 Alvarez-Galvez J, Rodero-Cosano ML, García-Alonso C, *et al*. Changes in socioeconomic determinants of health: comparing the effect of social and economic indicators through European welfare state regimes. *J Public Health* 2014;22:305.
24 Sokka T, Kautiainen H, Toloza S, *et al*. QUEST-RA: quantitative clinical assessment of patients with rheumatoid arthritis seen in standard rheumatology care in 15 countries. *Ann Rheum Dis* 2007;66:1491–6.
25 Börsch-Supan A. *Work disability, health and incentive effects. MEA discussion paper (07135)*. University of Mannheim, 2007.
26 Ledingham JM, Snowden N, Rivett A, *et al*. Patient- and clinician-reported outcomes for patients with new presentation of inflammatory arthritis: observations from the national clinical audit for rheumatoid and early inflammatory arthritis. *Rheumatology* 2017;56:231–8.
27 Barth J, de Boer WEL, Busse JW, *et al*. Inter-rater agreement in evaluation of disability: systematic review of reproducibility studies. *BMJ* 2017;356.

EPIDEMIOLOGICAL SCIENCE

Shared epitope defines distinct associations of cigarette smoking with levels of anticitrullinated protein antibody and rheumatoid factor

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ABSTRACT

Objects Although the association of cigarette smoking (CS) with susceptibility to rheumatoid arthritis (RA) has been established, the impact of CS on anticitrullinated cyclic peptide/protein antibody (ACPA) and rheumatoid factor (RF) levels in RA has yet been clear, especially in relation to shared epitope (SE) alleles.

Methods A total of 6239 subjects, the largest Asian study ever, from two independent Japanese cohorts were enrolled. Precise smoking histories, levels of ACPA and RF, and HLA-DRB1 allele status were withdrawn from databases. Associations between CS and high ACPA or RF levels, defined by the top quartiles, were evaluated. The effect of HLA-DRB1 alleles on the association was further investigated.

Results CS at RA onset conferred the risks of high levels of both antibodies, especially RF (OR 2.06, $p=7.4\times10^{-14}$; ACPA, OR 1.29, p=0.012), suggesting that RF level is more sensitive to CS than ACPA level. The patients who had quitted CS before RA onset showed a trend of decreased risks of developing high levels of ACPA or RF, and the risks steadily decreased according to the cessation years. The association of CS with high ACPA level was observed only in subjects carrying SE alleles, while the association of high RF level was observed regardless of SE.

Conclusions CS confers the risks of high autoantibody levels in RA in different manners; CS interacts with SE alleles on ACPA level, while CS impacts on RF level despite SE allele. These data suggest novel distinct production mechanisms of RF and ACPA.

Key messages

What is already known about this subject?

- Cigarette smoking (CS) is a known risk of rheumatoid arthritis (RA) and development of anticitrullinated cyclic peptide/protein antibody (ACPA) and rheumatoid factor (RF).
- Shared epitope (SE) alleles are strongly associated with seropositive RA and higher ACPA levels in European and Asian populations.

What does this study add?

- CS affects not only positivity but also higher levels of both ACPA and RF.
- The associations gradually decreased by smoking cessation depending cessation years.
- The association of CS with high ACPA level is more apparent in the presence of SE alleles, while the risk of high RF levels is independent of SE presence.

How might this impact on clinical practice or future developments?

- The importance of smoking cessation may be further highlighted especially in patients with SE alleles.
- Research to address mechanisms of formation of RA autoantibodies should take into consideration difference between ACPA and RF especially in the context of SE.

INTRODUCTION

Rheumatoid arthritis (RA) is characterised by chronic inflammation and subsequent proliferation of synovial tissues leading to cartilage and bone destruction.[1] Both environmental and genetic factors are known to contribute to the development of RA.[2 3] Anticitrullinated cyclic peptide/protein antibody (ACPA) and rheumatoid factor (RF) are characteristic autoantibodies found in patients with RA. Previous studies have revealed that the positivity for and high levels of ACPA and RF are associated with joint destruction[4–8] and systemic bone loss even in early phases of disease course,[9 10] suggesting the importance of not only the positivity but also the levels of ACPA and RF on disease outcomes.

Cigarette smoking (CS) is one of the environmental risk factors for RA development.[11 12] Males are reported to be more susceptible to CS on RA.[12] CS is also known to affect both RF and ACPA formation.[13] Importantly, both CS intensity and duration are directly related to the risk of RA development with prolonged increased risk even after CS cessation.[12]

HLA-DRB1 is the gene most strongly associated with RA.[14] Most of the RA-associated HLA-DRB1 alleles share similar amino acid (AA) sequences at position 70–74 on HLA-DR β chain called the shared epitope (SE).[15] Large-scale association studies have revealed that AA positions 11 or 13, 71 and 74 of HLA-DRB1 are strongly associated with RA in European population.[16 17] A previous Asian study revealed a very similar genetic architecture to the

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European population, with AA position 57 unique to the Asian. HLA-DRB1 is also associated with positivity of RF and ACPA[18] and the levels of ACPA,[19 20] but not of RF.[21] HLA-DRB1*09:01 and other alleles also showed associations of the levels of ACPA and the associations of HLA-DRB1 were mainly explained by 74th AA alanine.[19 20]

The association between CS and ACPA formation in the context of HLA-DRB1 alleles, especially SE alleles, has been investigated in several studies.[22–27] Most of them including two Asian cohort studies reported an interaction between CS and SE with regard to ACPA-positive RA development with exception of single north American cohort. However, the interactive association of SE and CS with ACPA levels (not positivity) have not been well-studied. The impact of CS cessation on ACPA levels, instead of risk of RA, have never been explored as well. Furthermore, since most of the recent studies focused on ACPA, recent knowledge about associations of CS and SE with RF levels has been lacking.

In the present study, we investigated impacts of CS and its cessation, especially at the time of RA onset, on future levels of ACPA and RF in 6239 RA patients from two independent single centre cohorts, which was the largest Asian study ever. We also investigated the associations of HLA-DRB1 alleles, especially SE alleles and HLA-DRB1*09:01 allele, and AA position in HLA-DRB1 with high levels of autoantibodies in relation to CS.

METHODS

Patients

The study participants were recruited from two independent prospective comprehensive single-centre cohorts (545 from Kyoto University Rheumatoid Arthritis Management Alliance: KURAMA cohort[28] and 5694 from Tokyo Women's Medical University Institute of Rheumatology, Rheumatoid Arthritis: IORRA cohort).[29] All patients are Japanese RA patients, and each had a diagnosis of RA based on the 1987 American College of Rheumatology (ACR) criteria and/or 2010 ACR/European League Against Rheumatism classification criteria for RA.[30 31]

Detailed smoking history were collected from the participants using questionnaire sheets. Pack-years, a standardised numerical value of lifetime tobacco exposure, was calculated by multiplying the number of cigarette packs smoked per day by the number of years the individual has smoked (cigarette packs per day × years of smoking).

Written informed consent was obtained from each participant. This study was approved by local ethical committee of each institute (online supplementary notes). Clinical information was obtained from the database of the two cohorts in an unbiased manner.

Study design

A schematic view of the study designs are illustrated in online supplementary figure S1. In brief, patients were first stratified based on their smoking histories at the time of last visits. Patients who had ever smoked and who had never smoked were defined as ever-smokers and never-smokers, respectively. Then, ever-smokers were further stratified into three categories; smokers who had smoked at onset of disease (smokers at onset (SaO)), ex-smokers who quitted smoking before onset (ex-smokers at onset (exSaO)) and subjects who started smoking after onset (others).

Quantification of RF and ACPA

ACPA was quantified as second-generation anticyclic citrullinated peptide antibody by MesaCup CCP ELISA kit (Medical and Biological Laboratories).[19 32] IgM-RF was quantified by latex-turbidimetric immunoassay, Iatro-RF II (Mitsubishi Kagaku Medicine).[5] The cut-off levels of the antibodies were according to manufacturers' instructions (ACPA <4.5 AU/mL; RF <20 IU/mL for IORRA, 15<IU/mL for KURAMA). High levels of ACPA (≥236 AU/mL) or RF (≥139 IU/mL) were determined according to the top quadrant levels within patients who were positive for these autoantibodies.

Table 1 Demographic features of subjects in each cohort

	IORRA	KURAMA
No of subjects	5694	545
Female	4888 (85.8%)	452 (82.9%)
Age (years old), median (IQR)	64.1 (53.2, 71.7)	65.8 (57.8, 73.2)
Age at onset (years old), median (IQR)	47.0 (36.0, 56.0)	51.0 (39.0, 61.0)
Disease duration (years), median (IQR)	14.1 (7.66, 22.0)	9.22 (4.82, 19.6)
RF positive (%)	4162 (73.8%)	438 (80.4%)
RF, median (IQR)	62 (32, 138)	60 (26, 143)
ACPA, positive (%)	4168 (82.8%)	415 (77.9%)
ACPA, median (IQR)	94.2 (27.7, 223.5)	94.2 (27.7, 329)
SaO (smokers at onset) (%)	978 (17.9%)	97 (17.9%)
Smoking quantity at onset, mean (SD)	18.4 (11.4)	16.5 (9.2)
Smoking years at onset, mean (SD)	25.4 (14.7)	22.5 (12.9)
Pack-years at onset, mean (SD)	23.9 (20.7)	20.5 (17.9)
ExSaO (smokers who had quitted at onset) (%)	694 (12.7%)	76 (14.4%)
Cessation year at onset, mean (SD)	13.7 (10.8)	13.4 (9.24)
Smoking quantity before cessation, mean (SD)	15.5 (11.2)	15.0 (9.82)
Smoking year before cessation, mean (SD)	15.5 (11.2)	17.2 (12.0)
Pack-years before cessation, mean (SD)	14.7 (17.5)	15.2 (14.2)
No of patients with HLA data	1124 (19.7%)	335 (61.5%)

ACPA, anticitrullinated cyclic peptide antibody; RF, rheumatoid factor; exSaO, ex-smokers at onset.

HLA genotyping

A WAKFlow system (Wakunaga) or an AlleleSEQR *HLA-DRB1* typing kit (Abbott) was used for *HLA-DRB1* typing, as previously described.[32] HLA-DRB1 *01:01, *04:01, *04:04, *04:05, *04:10, *10:01, *14:02 and *14:06 were defined as the SE alleles.

Statistical analysis

Descriptive summary statistics are provided in table 1 for all continuous variables with parametric or non-parametric data as appropriate.

We selected the following items as candidates of basic covariates in the association studies for positivity or high levels of ACPA and RF, namely, age, age at onset of RA, disease duration, female gender and institutional variable. Disease duration was natural log-transformed due to their skewed distribution. Using these basic covariates, we first investigated the risks of positivity or high autoantibody levels in ever-smokers, SaO or exSaO using multiple logistic regression models. For high autoantibody levels, patients positive with ACPA or RF were included in the models. We also applied linear regression analysis rather than defining high/not-high autoantibody levels. Then, gender difference of the risk in each model was evaluated. Association of pack-years and their gender difference were analysed and expressed by β coefficients. Finally, we added presence of SE, HLA-DRB1*04:05,

HLA-DRB1 non-*04:05 SE and HLA-DRB1*09:01 alleles in each model to adjust with these known risk-related HLA-DRB1 allele covariates. HLA-DRB1*09:01 allele is frequently observed in Japanese and strongly associated with lowering ACPA levels.[33] Never-smokers were set as reference subjects in all the analyses. Omnibus test was performed as described previously.[16 21 34]

The effect difference of covariates from two separate regressions was calculated in the following manner; the results of two regressions were combined but the estimation was kept separate as a seemingly unrelated estimation (SUE), and the generalised Hausman test was conducted to obtain a one-sided p value (p_{SUE}).

Binominal probability test was conducted to compare the effect sizes between males and females assuming chance of difference was 0.5.

Pearson's correlation coefficient was calculated to test on linear trend of proportions.

Stringent significance levels were set based on Bonferroni correction. P values $<2.4\times10^{-4}$ (0.05/208) were regarded as significant in omnibus tests. For the other analyses, p values <0.05 were set as significant. The missing data (see also online supplementary data) were handled as missing completely at random due to the complete lack of relationship between missingness of the data and observed outcome variables (χ^2 $p>0.05$). All the statistical analyses were performed on STATA/IC V.14.

RESULTS

Enrolment of study participants and definition of subgroups

The demographic features and smoking information of enrolled subjects were summarised in table 1. We stratified subjects based on their smoking in the questionnaires as described in the Methods section.

Significant association between CS and positivity and high levels of ACPA and RF

Consistent with the previous studies, we found that SaO (ones who smoked at onset, see the Methods section) had higher risks of positivity of ACPA and RF (OR 1.39 (95% CI 1.09 to 1.76) and 1.52 (1.26 to 1.85), p=0.0068 and 1.8×10^{-5}, respectively, online supplementary figure S2A). We also found a dose-dependency of the associations (p=0.0074 for both ACPA and RF; online supplementary figure S2B). When we focused on subjects positive for the autoantibodies, we found that SaO had significantly positive associations with high levels of ACPA and RF (OR 1.29 (1.06 to 1.57) and OR 2.06 (1.70 to 2.48), p=0.012 and 7.4×10^{-14}, respectively, figure 1A) where the effect size of RF was higher than that of ACPA ($p_{SUE}=5.1\times10^{-4}$; we also got comparable p-values by permutating obtained β coefficients based on their means and standard errors and comparing the difference of their distribution). Again, we observed dose-dependent association of CS with high RF levels (p=0.0065; figure 1B). These results suggest that CS has a larger effect on RF formation than on ACPA. Furthermore, most of the effect sizes were larger and significant in male than in female patients (binominal probability test: $p=2.8\times10^{-4}$), which was comparable to the previous studies of RA onset reporting males being more susceptible to CS.[12]

We conducted the same analyses in ever-smokers, who were more heterogenous population than SaO and found that ever-smokers had almost the same risks as SaO (online supplementary figure S3A–D).

Smoking cessation before onset may lower risks of future high levels of ACPA and RF depending on the duration

Next, we assessed the association of CS cessation before onset with future ACPA and RF levels to evaluate attenuated influence of CS after cessation on autoantibody formation. We found that exSaO (ones who had quitted smoking before onset of RA, see the Methods section) had reduced risks of future positivity (ACPA, OR 1.29 (1.01 to 1.66), p=0.042; RF, OR 1.07 (0.88 to 1.30), p=0.50; online supplementary figure S4A) and of high levels of ACPA and RF (ACPA, OR 1.11 (0.88 to 1.41), p=0.39; RF, OR 1.23 (0.95 to 1.59), p=0.11; online supplementary figure S4B) compared with SaO or ever-smokers (online supplementary table S2). The effect sizes of smoking amount before smoking cessation were smaller than those of ever-smokers or SaO in the context of RF levels (online supplementary table S3, figure S4C, S4D), again indicating RF is more sensitive to CS than ACPA.

Stratifying the exSaO by their cessation years showed that the associations of CS with both positivity (figure 2A) and high

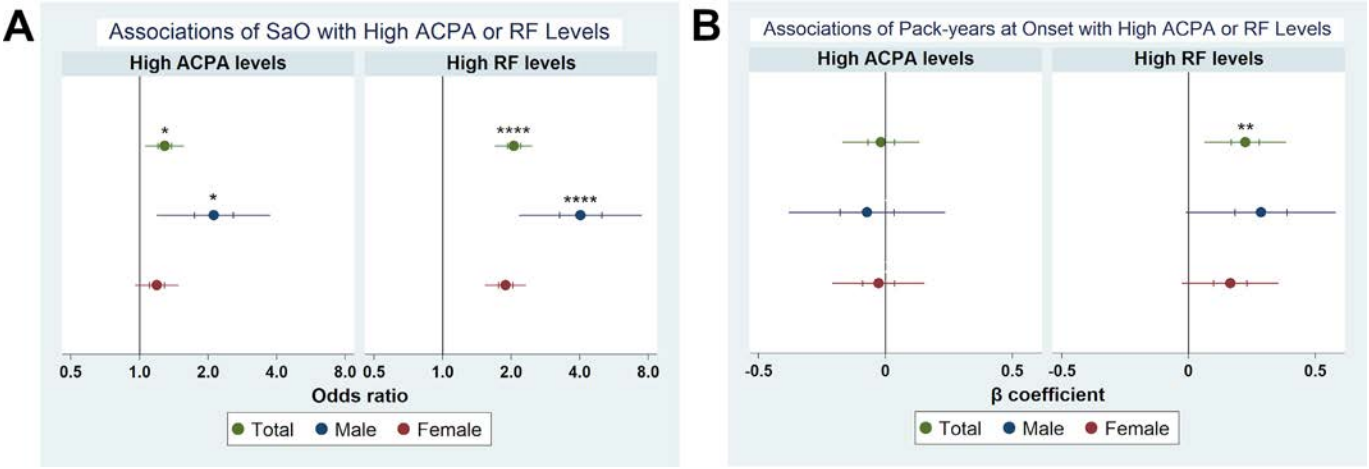

Figure 1 Cigarette smoking at the time of onset is a significant risk of high levels of ACPA and RF. The associations of smokers at the time of onset (SaO) with high levels of ACPA or RF were evaluated referring never-smokers (A). β coefficients of pack-years at the time of onset for high levels of ACPA or RF referring to never-smokers are presented (B). ORs in (A) and β coefficients in (B) are indicated by dots, and 95% CIs are indicated by two-sided lines. *p<0.05, **p<0.01, ****$p<1.0\times10^{-4}$. The numbers of patients, ORs and β coefficients were described in online supplementary notes. High ACPA or RF: top quartile of ACPA or RF-positive patients. ACPA, anticitrullinated cyclic peptide/protein antibody; RF, rheumatoid factor; SaO, smokers at onset.

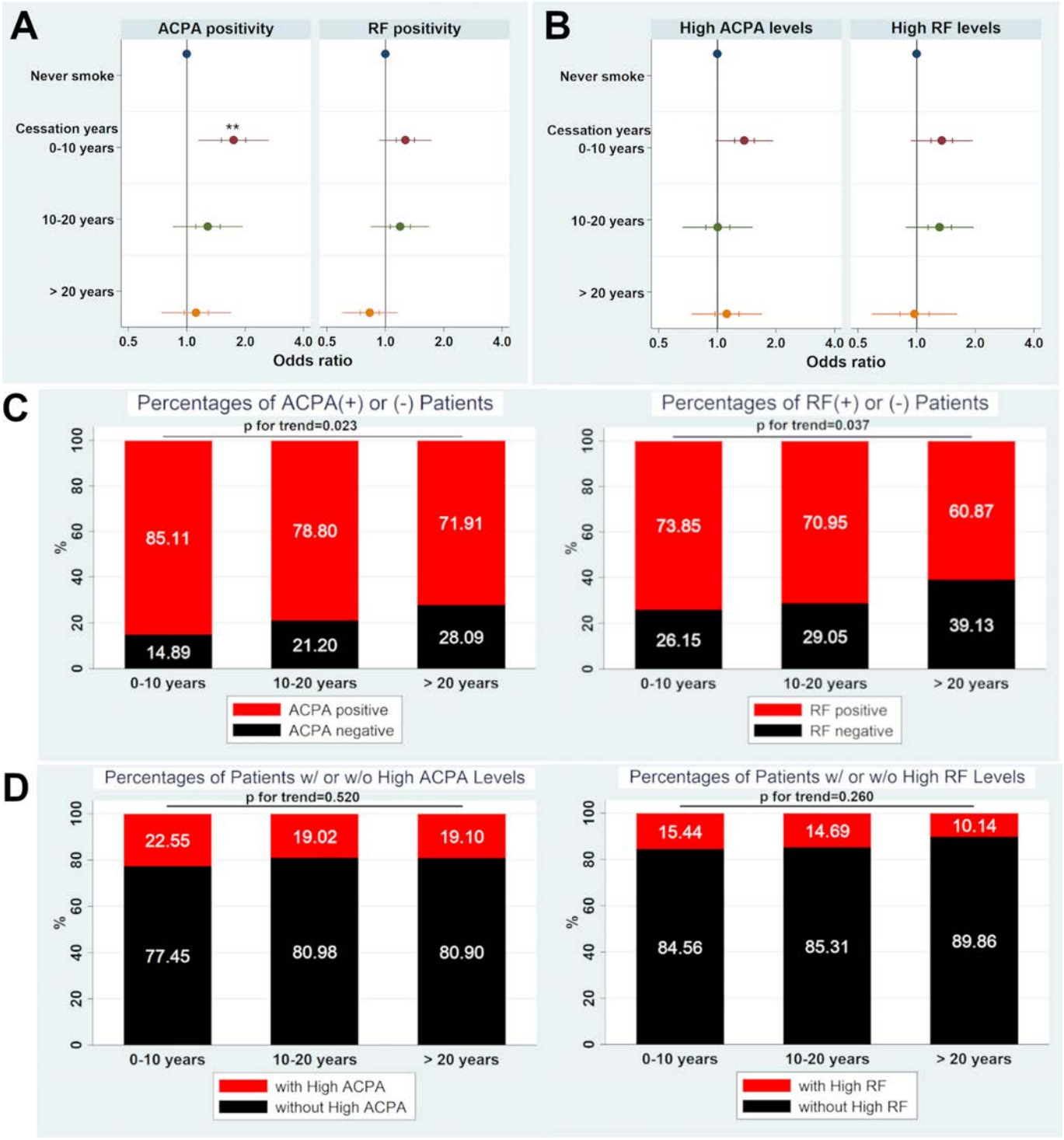

Figure 2 Ex-smokers at onset had no longer significant risk of future high levels or positivity of ACPA and RF depending on the cessation years. Patients who had quitted CS before onset were stratified according to their cessation years, and the association of each category of patients with positivity (A) or high levels (B) of ACPA and RF were evaluated referring that of never-smokers. ORs are indicated by dots, and 95% CIs are indicated by two-sided lines. The numbers of patients and ORs were described in online supplementary notes. Percentages of the patients stratified by the cessation years are presented based on positivity (C) or levels (D) of ACPA and RF. **p<0.01. High ACPA or RF: top quartile of ACPA or RF positive patients. ACPA, anticitrullinated cyclic peptide/protein antibody; CS, cigarette smoking; RF, rheumatoid factor.

levels of ACPA and RF (figure 2B) decreased depending on the cessation years. Likewise, percentages of patients with positive (ACPA, p=0.023; RF, p=0.037; figure 2C) and high levels of ACPA and RF (ACPA, p=0.52; RF, p=0.26; figure 2D) steadily decreased in the same manner.

Together, these results indicated that exSaO had less risks of future positivity and high levels of ACPA and RF than ever-smokers or SaO, and the risks were gradually attenuated depending on duration of smoking cessation.

CS affects ACPA levels only in the presence of SE alleles

Finally, we evaluated the interactive association of CS and SE with ACPA and RF levels (online supplementary table S1). When

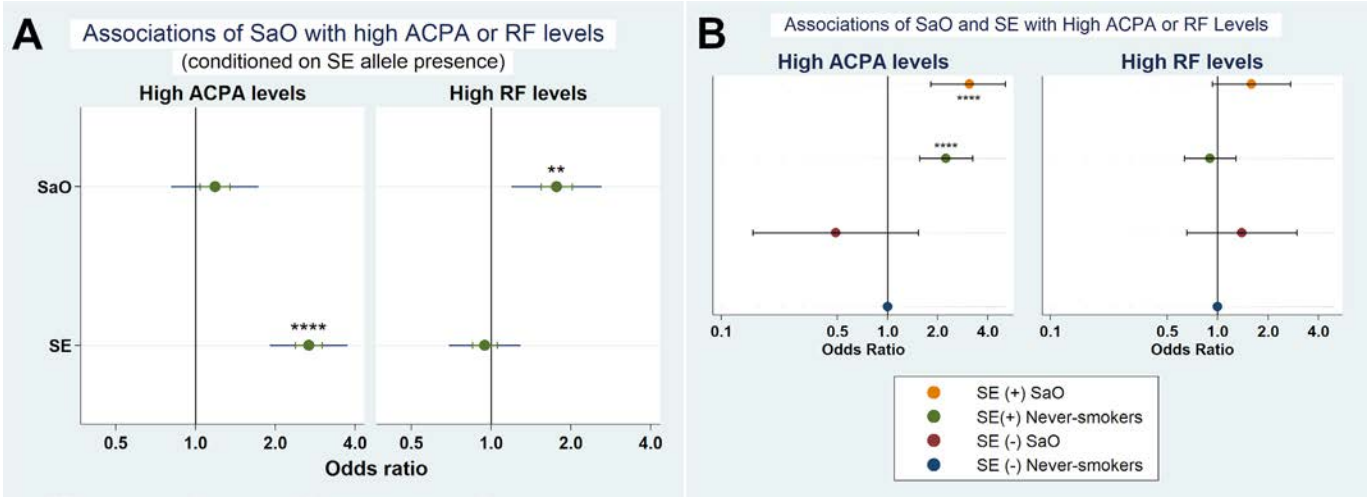

Figure 3 Cigarette smoking affects ACPA levels only in patients with shared epitope alleles while cigarette smoking per se affects high RF levels regardless of shared epitope allele status. (A) The association of SaO with high ACPA or RF levels were evaluated conditioning on presence of shared epitope (SE) alleles. ORs are indicated by dots, and 95% CIs are indicated by two-sided lines. (B) The associations of SaO with high ACPA or RF levels with or without SE alleles were evaluated referring never-smokers without SE alleles. ORs are indicated by dots, and 95% CIs are indicated by two-sided lines. **p<0.01, ***p<0.001, ****p<1.0×10^{-4}. The numbers of patients and ORs were described in online supplementary notes. High ACPA or RF: top quartile of ACPA or RF positive patients. ACPA, anticitrullinated cyclic peptide/protein antibody; RF, rheumatoid factor; SaO, smokers at onset.

conditioned on SE presence, the association between SaO and high ACPA level was no longer significant (preconditioned p=0.012, figure 1A; postconditioned p=0.38, figure 3A). To confirm the finding, we stratified the patients into four categories according to their smoking status and the presence of SE. Neither SE nor smoking history affected the proportions of patients (χ^2 p=0.120, online supplementary table S4). Setting SE(-) never-smokers as a reference, both SE(+) SaO (OR 3.10 (1.91 to 5.03), $p=4.7\times10^{-6}$) and SE(+) never-smokers (OR 2.23 (1.59 to 3.14), $p=4.3\times10^{-6}$) had an increased risk of high ACPA level (figure 3B). Importantly, SE(-) SaO did not show even a trend of positive association, strongly indicating a SE-dependent association between smoking and high ACPA levels. On the contrary, the association between SaO and high RF level remained significant when conditioned on SE allele presence (p=0.0044, figure 3A). SE(+) never-smokers showed a rather negative association (figure 3B, indicated by green), while both SE(-) and SE(+) SaO showed a trend of positive associations (figure 3B, indicated by red and yellow, respectively), indicating an association of CS with high RF level is independent on SE. These association patterns were also observed in ever-smokers and in linear regression analysis where ACPA/RF levels were used as dependent variables (online supplementary figure S5).

Since we previously reported that among SE alleles HLA-DRB1*04:05 has unique features in the context of joint destruction[20 35] and others reported strong interactive associations between CS and SE alleles (mainly non-*04:05 SE) on ACPA positivity in Europeans,[23 27] we assessed whether the associations of SE with autoantibody levels in relation to CS were driven by HLA-DRB1*04:05. However, we did not find a specific association of HLA-DRB1*04:05 (or non-*04:05 SE) (figure 4A,B).

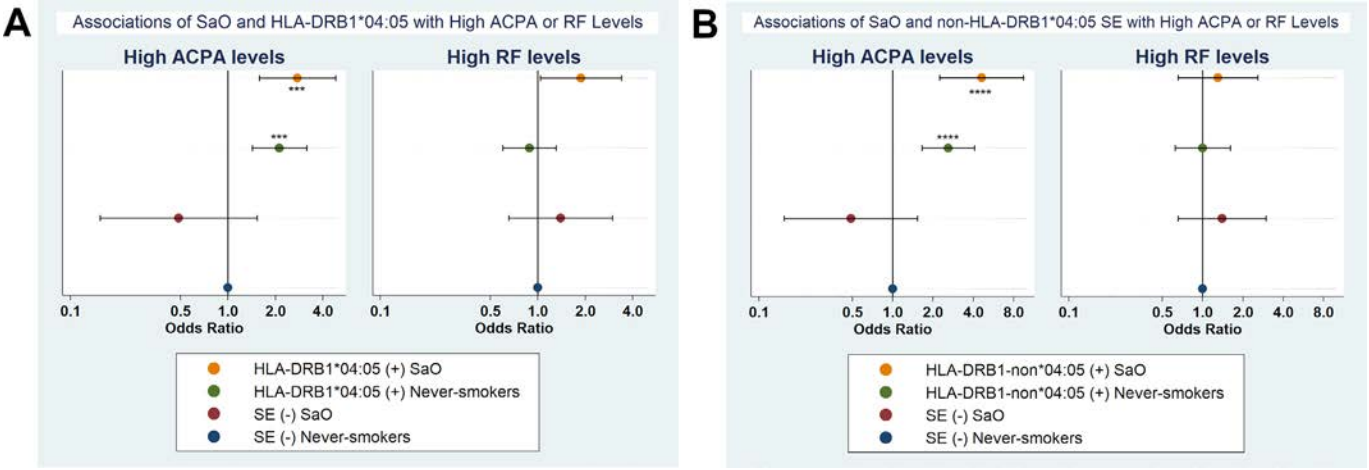

Figure 4 The comparative associations of cigarette smoking with HLA-DRB1*04:05 or non-*04:05 SE alleles on high levels of ACPA and RF. The associations between f high levels of ACPA or RF and SaO with or without HLA-DRB1*04:05 (A) or non-*04:05 SE allele (B) were evaluated referring never-smokers without HLA-DRB1*04:05 or non-*04:05 SE alleles. ORs are indicated by dots, and 95% CIs are indicated by two-sided lines. ***p<0.001, ****p<1.0×10^{-4}. The numbers of patients and ORs were described in online supplementary notes. High ACPA or RF: top quartile of ACPA or RF positive patients. ACPA, anticitrullinated cyclic peptide/protein antibody; RF, rheumatoid factor; SaO, smokers at onset.

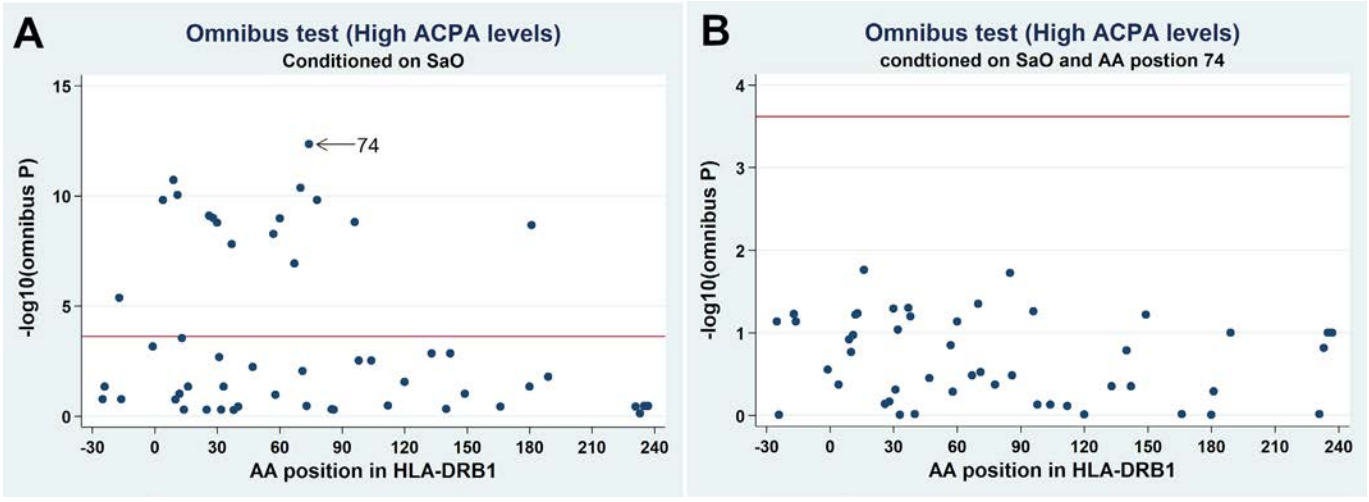

Figure 5 Amino acid position 74 in HLA-DRB1 is most significantly associated with high ACPA levels regardless of smoking status of subjects. Omnibus p values are shown for each HLA-DRB1 amino acid (AA) position of subjects with anticitrullinated cyclic peptide/protein antibody (ACPA). The horizontal red lines indicate the level of significance ($p=2.4\times10^{-4}$). High ACPA or RF: top quartile of ACPA or RF positive patients. RF, rheumatoid factor; SaO, smokers at onset.

We further conducted omnibus tests to investigate associations between AA positions in HLA-DRB1 and high ACPA levels with (figure 5) or without (online supplementary figure S6A,B) conditioning on SaO. We found that AA position 74, a part of SE sequence, had the strongest association with high ACPA levels ($p_{omnibus}=4.5\times10^{-12}$ with conditioning on SaO; $p_{omnibus}=1.1\times10^{-13}$ without conditioning SaO; online supplementary table S5), which is comparable to our previous finding that AA position 74 is strongly associated with both high ACPA levels in ACPA-positive RA and RA susceptibility.[20] While other AAs also showed significant associations (online supplementary table S5), these associations could be explained by AA position 74 (figure 5). On the other hand, none of the AA position in HLA-DRB1 was associated with high RF levels (online supplementary table S6C,D), also consistent with our previous study.[21]

Based on the strongest and unique (a reduced effect size) association of HLA-DRB1*09:01 with levels of ACPA,[19 20] we also evaluated associations among CS, HLA-DRB1*09:01 allele, and high autoantibody levels. However, we found no apparent associations driven by HLA-DRB1*09:01 (online supplementary figure S7).

Taken together, these results show that CS affects high ACPA levels only in the subjects with SE alleles, while CS per se affects high RF levels regardless of SE allele presence.

DISCUSSION

In the present study, we investigated the impact of CS on ACPA and RF levels in the largest Asian study ever. We found that CS, especially at the time of onset, was a significant risk of high levels of both ACPA and RF in a dose-dependent manner. The effect sizes were larger for RF than ACPA suggesting that RF is more sensitive to CS, and were larger in male patients compared with female patients. These risks were attenuated by CS cessation depending on cessation years. The association of CS with ACPA levels was apparent only in the subjects with SE alleles, while CS independently affects RF levels regardless of SE, implying that interaction between CS and SE alleles can impact only on ACPA formation.

Considering that CS is one of risk factors of periodontal disease,[36] our findings together with previous studies further support the idea that CS is strongly associated with RA-related autoantibody formation beyond ethnicities.[13] Of note, we also showed that RF is more sensitive to CS than ACPA, suggesting that the mechanisms of ACPA and RF formation are different as indicated in the previous study.[37]

We showed that associations of CS with autoantibody levels were attenuated by CS cessation depending on the cessation years, but the association with ACPA levels still remained increased 20 years after CS cessation. These long effects of CS seem compatible with the previous study demonstrating that both smoking intensity and duration were directly related to a risk of RA development especially in RF-positive cases, with prolonged (~20 years) increased risk after cessation.[38] The present study stood on intracase analyses lacking ACPA-positive or RF-positive non-RA subjects and thus could not draw any conclusions with respect to the association of CS with RA onset, but still might support the previous observation mentioned above with additional information of the impacts of CS on autoantibody levels.

SE and HLA-DRB1*09:01 are well-established genetic risks for ACPA-positive RA,[34 39] and the latter is also known to lower ACPA levels in Japanese RA patients.[33] Several studies have investigated associations of CS and SE allele presence with ACPA formation, and most of them confirmed the interactive effect of CS and SE on RA development especially in ACPA-positive individuals.[22–27] In addition to these results, we found that CS affected high ACPA level only in the subjects with SE alleles, while the association of CS with RF levels was independent on SE. Furthermore, the omnibus tests indicated that AA position 74 was the most important within SE allele for ACPA development and thus might be a causal AA position to interact with CS. On the other hand, the effect of the interaction between CS and ACPA positivity was reported to be dependent on different SE subtypes.[27] Although we did not observe difference between HLA-DRB1*04:05 and non-HLA-DRB1*04:05 SE possibly due to the different predominance of SE alleles among different ethnicities, other factors not explained by AA position 74 may also be of interest.

Previous studies with small cohort of Caucasian RA patients reported an association between CS and positivity[40] or levels of RF in relation to SE.[41] The former observed an almost independent association of CS with RF positivity, while the latter observed an additive effect of CS on RF levels with dose-dependent SE allele

effect. Although the association of SE with RF levels might be different among different ethnicities partly due to the different SE allele predominance, our study suggests that CS can independently affect RF levels regardless of SE allele at least in Asian populations.

Taken together these findings our study further support that CS significantly affects ACPA and RF formation in RA patients, but in different manners especially in relation to SE alleles. One possible limitation of the present study is that ACPA and RF data used were those had been measured at the time of last visit instead of at onset. Thus, further studies enrolling more numbers of subjects from different races including non-RA subjects with complete sets of data including smoking information and autoantibody data are favourable to confirm the findings and further detailed analyses.

In conclusion, our study suggests that CS, especially at the time of onset, modify the risk of developing positive and higher levels of ACPA and RF in RA patients. CS may interact with SE and affect ACPA formation while the impact of CS on RF formation is independent of SE. Our findings suggest novel distinct mechanisms of RF and ACPA development in RA patients.

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Contributorship Statement YI analysed and interpreted data and wrote the manuscript. KI, MH, KO, MT, HI, AT, HY and TM interpreted data and edited the manuscript. CT conceived the project, analysed and interpreted data, and wrote the manuscript.

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Competing interests KI received speaker fee from fifteen pharmaceutical companies (Asahi-Kasei Pharma, Astellas Pharma, Abbvie GK Inc., AYUMI, Eisai, Otsuka, Kaken, Takeda, Mitsubishi-Tanabe, Chugai, Eli-Lilly, Bristol-Myers Squibb, Pfizer Japan Inc., Janssen and UCB Japan). MH, HI and MT belong to a department that has been financially supported by four pharmaceutical companies (Mitsubishi-Tanabe, Chugai, AYUMI and UCB Japan). TM received personal fees from fourteen pharmaceutical companies (Pfizer Japan Inc., Mitsubishi-Tanabe, Eisai, Astellas, AYUMI, Chugai, Daiichi Sankyo, Nippon Shinyaku, Takeda Pharmaceutical, Eli-Lilly, Asahi-Kasei Pharma, Sanofi, Bristol-Myers Squibb and GlaxoSmithKline). KURAMA cohort study is supported by grant from Daiichi Sankyo Co. Ltd. This study is conducted as investigator initiate study. These companies had no role in the design of the study, the collection or analysis of the data, the writing of the manuscript or decision to submit the manuscript for the publication. IORRA cohort study is supported by grant by twenty pharmaceutical companies (Daiichi Sankyo Co. Ltd., Mitsubishi-Tanabe, Chugai, Bristol-Myers Squibb, AYUMI, Astellas, Pfizer Japan Inc., Takeda Pharmaceutical, Eisai, Nippon Shinyaku, YL Biologics Ltd., AbbVie, Novartis Pharmaceutical K.K., Kaken Pharmaceutical Co., Ltd., UCB Japan, Ono Pharmaceutical Co., Ltd., Taisho Toyama Pharmaceutical Co., Ltd., Teijin Pharma, Torii Pharmaceutical Co., Ltd. and Nippon Boehringer Ingelheim Co., Ltd.). These sponsors were not involved in the: study design; collection, analysis and interpretation of data; writing of the paper; and/or decision to submit for publication.

Patient and public involvement statement This research was done without patient involvement. Patients were not invited to comment on the study design and were not consulted to develop patient relevant outcomes or interpret the results. Patients were not invited to contribute to the writing or editing of this document for readability or accuracy.

Patient consent for publication Not required.

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REFERENCES

1 Calabresi E, Petrelli F, Bonifacio AF, *et al*. One year in review 2018: pathogenesis of rheumatoid arthritis. *Clin Exp Rheumatol* 2018;36:175–84.
2 Terao C, Raychaudhuri S, Gregersen PK. Recent advances in defining the genetic basis of rheumatoid arthritis. *Annu Rev Genomics Hum Genet* 2016;17:273–301.
3 Terao C, Ikari K, Nakayamada S, *et al*. A twin study of rheumatoid arthritis in the Japanese population. *Mod Rheumatol* 2016;26:685–9.
4 Bukhari M, Lunt M, Harrison BJ, *et al*. Rheumatoid factor is the major predictor of increasing severity of radiographic erosions in rheumatoid arthritis: results from the Norfolk arthritis register study, a large inception cohort. *Arthritis Rheum* 2002;46:906–12.
5 Terao C, Yamakawa N, Yano K, *et al*. Rheumatoid factor is associated with the distribution of hand joint destruction in rheumatoid arthritis. *Arthritis Rheumatol* 2015;67:3113–23.
6 Syversen SW, Goll GL, van der Heijde D, *et al*. Prediction of radiographic progression in rheumatoid arthritis and the role of antibodies against mutated citrullinated vimentin: results from a 10-year prospective study. *Ann Rheum Dis* 2010;69:345–51.
7 Hecht C, Englbrecht M, Rech J, *et al*. Additive effect of anti-citrullinated protein antibodies and rheumatoid factor on bone erosions in patients with RA. *Ann Rheum Dis* 2015;74:2151–6.
8 Akdemir G, Verheul MK, Heimans L, *et al*. Predictive factors of radiological progression after 2 years of remission-steered treatment in early arthritis patients: a post hoc analysis of the IMPROVED study. *RMD Open* 2016;2:e000172.
9 Orsolini G, Caimmi C, Viapiana O, *et al*. Titer-Dependent effect of Anti-Citrullinated protein antibodies on systemic bone mass in rheumatoid arthritis patients. *Calcif Tissue Int* 2017;101:17–23.
10 Bugatti S, Bogliolo L, Vitolo B, *et al*. Anti-citrullinated protein antibodies and high levels of rheumatoid factor are associated with systemic bone loss in patients with early untreated rheumatoid arthritis. *Arthritis Res Ther* 2016;18.
11 Vittecoq O, Richard L, Banse C, *et al*. The impact of smoking on rheumatoid arthritis outcomes. *Joint Bone Spine* 2018;85:135–8.
12 Hedström AK, Stawiarz L, Klareskog L, *et al*. Smoking and susceptibility to rheumatoid arthritis in a Swedish population-based case-control study. *Eur J Epidemiol* 2018;33:415–23.
13 van Wesemael TJ, Ajeganova S, Humphreys J, *et al*. Smoking is associated with the concurrent presence of multiple autoantibodies in rheumatoid arthritis rather than with anti-citrullinated protein antibodies per se: a multicenter cohort study. *Arthritis Res Ther* 2016;18.
14 Okada Y, Eyre S, Suzuki A, *et al*. Genetics of rheumatoid arthritis: 2018 status. *Ann Rheum Dis* 2019;78:446–53.
15 Gregersen PK, Silver J, Winchester RJ. The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. *Arthritis Rheum* 1987;30:1205–13.
16 Raychaudhuri S, Sandor C, Stahl EA, *et al*. Five amino acids in three HLA proteins explain most of the association between MHC and seropositive rheumatoid arthritis. *Nat Genet* 2012;44:291–6.
17 Han B, Diogo D, Eyre S, *et al*. Fine mapping seronegative and seropositive rheumatoid arthritis to shared and distinct HLA alleles by adjusting for the effects of heterogeneity. *Am J Hum Genet* 2014;94:522–32.
18 Ohmura K, Terao C, Maruya E, *et al*. Anti-citrullinated peptide antibody-negative RA is a genetically distinct subset: a definitive study using only bone-erosive ACPA-negative rheumatoid arthritis. *Rheumatology* 2010;49:2298–304.
19 Terao C, Ikari K, Ohmura K, *et al*. Quantitative effect of HLA-DRB1 alleles to AcpA levels in Japanese rheumatoid arthritis: no strong genetic impact of shared epitope to AcpA levels after stratification of HLA-DRB1*09:01. *Ann Rheum Dis* 2012;71:1095–7.

20 Terao C, Suzuki A, Ikari K, *et al*. An association between amino acid position 74 of HLA-DRB1 and anti-citrullinated protein antibody levels in Japanese patients with anti-citrullinated protein antibody-positive rheumatoid arthritis. *Arthritis Rheumatol* 2015;67:2038–45.
21 Hiwa R, Ikari K, Ohmura K, *et al*. *HLA-DRB1* Analysis Identified a Genetically Unique Subset within Rheumatoid Arthritis and Distinct Genetic Background of Rheumatoid Factor Levels from Anticyclic Citrullinated Peptide Antibodies. *J Rheumatol* 2018;45:470–80.
22 Klareskog L, Stolt P, Lundberg K, *et al*. A new model for an etiology of rheumatoid arthritis: smoking may trigger HLA-DR (shared epitope)-restricted immune reactions to autoantigens modified by citrullination. *Arthritis Rheum* 2006;54:38–46.
23 Too CL, Yahya A, Murad S, *et al*. Smoking interacts with HLA-DRB1 shared epitope in the development of anti-citrullinated protein antibody-positive rheumatoid arthritis: results from the Malaysian epidemiological investigation of rheumatoid arthritis (MyEIRA). *Arthritis Res Ther* 2012;14.
24 Lee H-S, Irigoyen P, Kern M, *et al*. Interaction between smoking, the shared epitope, and anti-cyclic citrullinated peptide: a mixed picture in three large North American rheumatoid arthritis cohorts. *Arthritis Rheum* 2007;56:1745–53.
25 Bang S-Y, Lee K-H, Cho S-K, *et al*. Smoking increases rheumatoid arthritis susceptibility in individuals carrying the HLA-DRB1 shared epitope, regardless of rheumatoid factor or anti-cyclic citrullinated peptide antibody status. *Arthritis Rheum* 2010;62:369–77.
26 Murphy D, Mattey D, Hutchinson D. Anti-citrullinated protein antibody positive rheumatoid arthritis is primarily determined by rheumatoid factor titre and the shared epitope rather than smoking per se. *PLoS One* 2017;12:e0180655.
27 van der Helm-van Mil AHM, Verpoort KN, le Cessie S, *et al*. The HLA-DRB1 shared epitope alleles differ in the interaction with smoking and predisposition to antibodies to cyclic citrullinated peptide. *Arthritis Rheum* 2007;56:425–32.
28 Terao C, Ohmura K, Ikari K, *et al*. ACPA-negative RA consists of two genetically distinct subsets based on rf positivity in Japanese. *PLoS One* 2012;7:e40067.
29 Yamanaka H, Tohma S. Potential impact of observational cohort studies in Japan on rheumatoid arthritis research and practice. *Mod Rheumatol* 2006;16:75–6.
30 Aletaha D, Neogi T, Silman AJ, *et al*. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League against rheumatism collaborative initiative. *Arthritis Rheum* 2010;62:2569–81.
31 Arnett FC, Edworthy SM, Bloch DA, *et al*. The American rheumatism association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;31:315–24.
32 Terao C, Ohmura K, Kochi Y, *et al*. A large-scale association study identified multiple HLA-DRB1 alleles associated with ACPA-negative rheumatoid arthritis in Japanese subjects. *Ann Rheum Dis* 2011;70:2134–9.
33 Okada Y, Suzuki A, Yamada R, *et al*. HLA-DRB1*0901 lowers anti-cyclic citrullinated peptide antibody levels in Japanese patients with rheumatoid arthritis. *Ann Rheum Dis* 2010;69:1569–70.
34 Okada Y, Kim K, Han B, *et al*. Risk for ACPA-positive rheumatoid arthritis is driven by shared HLA amino acid polymorphisms in Asian and European populations. *Hum Mol Genet* 2014;23:6916–26.
35 Tsuji H, Ikari K, Ohmura K, *et al*. Significant joint-destructive association of HLA-DRB1*04:05 independent of DAS28 in rheumatoid arthritis. *Ann Rheum Dis* 2019;78:284–6.
36 Albandar JM, Streckfus CF, Adesanya MR, *et al*. Cigar, pipe, and cigarette smoking as risk factors for periodontal disease and tooth loss. *J Periodontol* 2000;71:1874–81.
37 Tan EM, Smolen JS. Historical observations contributing insights on etiopathogenesis of rheumatoid arthritis and role of rheumatoid factor. *J Exp Med* 2016;213:1937–50.
38 Costenbader KH, Feskanich D, Mandl LA, *et al*. Smoking intensity, duration, and cessation, and the risk of rheumatoid arthritis in women. *Am J Med* 2006;119:e1-9:503–503.e9.
39 Pratesi F, Petit Teixeira E, Sidney J, *et al*. Hla shared epitope and AcpA: just a marker or an active player? *Autoimmun Rev* 2013;12:1182–7.
40 Mattey DL, Dawes PT, Clarke S, *et al*. Relationship among the HLA-DRB1 shared epitope, smoking, and rheumatoid factor production in rheumatoid arthritis. *Arthritis Rheum* 2002;47:403–7.
41 Krol A, Garred P, Heegaard NHH, *et al*. Interactions between smoking, increased serum levels of anti-CCP antibodies, rheumatoid factors, and erosive joint disease in patients with early, untreated rheumatoid arthritis. *Scand J Rheumatol* 2015;44:8–12.

TRANSLATIONAL SCIENCE

Potential involvement of OX40 in the regulation of autoantibody sialylation in arthritis

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ABSTRACT

Objective An increased proportion of circulating follicular helper T (Tfh) cells was reported in rheumatoid arthritis (RA), but it remains uncertain how Tfh cells affect antibody hyposialylation. We investigated the regulation of autoantibody hyposialylation by Tfh cells in RA using murine model.

Methods Behaviours of Tfh cells and their function on B cell promotion were analysed. Change of arthritogenicity and sialylation of autoantibodies during the course of arthritis was examined by mass spectrometry. Tfh-mediated regulation of hyposialylation was investigated, and the responsible cell surface molecule was specified both in vitro and in vivo. The relation between circulating Tfh cells and hyposialylation was analysed in patients with RA.

Results An increase in Tfh, particularly interleukin-17 producing Tfh (Tfh17) cells, at the onset of arthritis and their enhancement of autoantibody production were found. Autoantibodies at the onset phase demonstrated stronger inflammatory properties than those at the resolution phase, and mass spectrometric analysis revealed their difference in sialylation. In vitro coculture showed enhanced hyposialylation by the Tfh cells via OX40, which was highly expressed in the Tfh and Tfh17 cells. Blockade of OX40 prevented the development of arthritis with reduction in Tfh17 cells and recovery of autoantibody sialylation. Analysis of patients with RA showed abundance of OX40-overexpressing Tfh17 cells, and their proportion correlated negatively with the expression of α2,6-sialyltransferase 1, an enzyme responsible for sialylation.

Conclusions OX40 expressed on Tfh cells can regulate autoantibody sialylation and play a crucial role in the development of autoimmune arthritis.

Key messages

What is already known about this subject?
- Glycosylation change, particularly hyposialylation of autoantibodies, contributes to the pathogenesis of rheumatoid arthritis (RA).
- The proportion of circulating follicular helper T (Tfh) cells is increased and correlates with disease severity in patients with RA.

What does this study add?
- OX40-overexpressing Tfh cells are increased in RA and the murine model.
- Tfh cells play a role in the pathogenesis of RA by regulation of autoantibody hyposialylation via OX40.

How might this impact on clinical practice or future developments?
- Our study will impact on the development of novel therapy for RA which targets glycosylation of autoantibody, which could preserve immune surveillance for malignancy and infection.

INTRODUCTION

Rheumatoid arthritis (RA) is a progressive autoimmune disease characterised by multijoint synovitis.[1] Despite the immunopathogenesis of RA not being fully understood, it is well established that the production of autoantibodies is crucially involved in the disease development.[2] Anticitrullinated protein antibodies (ACPA) are among the autoantibodies which can be detected in patients with RA and have high disease specificity.[3 4] They can appear before the arthritic symptoms[2 5] and have a prognostic role in RA.[2–4] Thus, B cell-mediated autoimmunity and autoantibody development are crucial for the onset of RA.

Besides their role in antigen recognition, antibodies regulate effector cell activation through their constant fragment crystallizable (Fc) regions.[6] Antibodies bear one or several carbohydrate chains or glycans. Glycans at the Asn297 position in the Fc part of IgG regulate binding capability to Fcγ receptors (FcγRs).[7 8] The composition of the IgG-Fc glycosylation, in particular terminal sialic acids, determines effector cell activation and hence the inflammatory properties of antibodies. Low sialylation, or hyposialylation, of Asn297 enhances the proinflammatory activity.[9–12] Patients with RA were reported to have hyposialylated ACPAs,[13] and the existence and importance of hyposialylated antigen-specific antibodies were reported in some mouse models.[13–15] Among these, Pfeifle and his colleagues[14] reported the regulation of sialylation by Th17 cells which had follicular helper T (Tfh) cell-like characteristics in collagen-induced arthritis.

We focused on the detailed mechanisms of when and how Tfh cells affect autoantibody production and sialylation and their reversibility. In a patient with RA, an increased frequency of circulating Tfh cells in the peripheral blood that correlate with serum ACPA levels and disease severity was

reported.[16] In RA mouse models, there are several reports that show the increase of Tfh cells in secondary lymphoid organs.[17 18] From these reports, Tfh cells could have an essential role in RA pathogenesis; however, it is not completely elucidated.

We chose to explore the mechanisms underlying autoantibody hyposialylation using glucose-6-phosphate isomerase (GPI)-induced arthritis (GIA). GPI is also the target autoantigen in the transgenic K/BxN mice, which develop high titres of anti-GPI-specific autoantibodies.[19 20] These autoantibodies are arthritogenic in the K/BxN serum transfer model.[21] In contrast, GIA cannot be transferred into syngeneic recipients by adoptive transfer of anti-GPI antibodies from diseased animals in our previous experiments, whereas B cell depletion and FcγR common gamma chain knock out ameliorated GIA.[22 23] Thus, detailed feature on antibody-transferring obtained from GIA mice in the arthritis resolution phase is still a mystery.

In the present study we revealed a contribution of the OX40-OX40 ligand (OX40L) pathway of Tfh cells and antibody-producing cells in a temporal change of autoantibody sialylation in GIA and RA.

MATERIALS AND METHODS

Mice preparation

Male DBA/1 mice were purchased from Charles River Japan (Tokyo, Japan) and used at 5–9 weeks of age. K/BxN mice were generated by crossing KRN-transgenic mice with non obese diabetes (NOD) mice. Detailed information on all the methods in mice experiments can be found in the online supplementary methods.

Human sample preparation

Peripheral blood mononuclear cells (PBMCs) were collected from Japanese patients with RA (n=31) and patients with osteoarthritis (OA) (n=12). Patients with RA fulfilled either the 1987 revised criteria of the American College of Rheumatology (ACR) for the classification of RA[25] or the 2010 ACR/European League Against Rheumatism classification criteria.[26] All patients were recruited at the University of Tsukuba Hospital from December 2014 to March 2017. All enrolled patients with RA were not being treated at the time of sampling with conventional synthetic disease-modifying antirheumatic drugs (DMARDs), prednisolone or biological DMARDs.

Detailed information on all the methods in human experiments is described in the online supplementary methods.

Statistical analysis

All data were expressed as mean±SEM unless otherwise specified. Differences between two groups were evaluated for statistical significance using the Student's t-test. The Kruskal-Wallis test and Dunn's multiple comparison post-hoc test were used to evaluate differences among three or more groups. In a set of human experiments, the Fisher's exact test was used for comparison of categorical value, and correlations were evaluated using Spearman's correlation test. P values less than 0.05 were considered to denote the presence of statistically significant difference. Statistical analyses were performed using IBM SPSS Statistics V.25 software.

RESULTS

Increase of Tfh17 cells expressing OX40 at the onset of GIA

First, we determined the presence of Tfh cells in the inguinal lymph nodes of GIA. DBA/1 mice were immunised with GPI and induced symmetrical polyarthritis. GIA was induced 7 days after GPI immunisation, reaching peak severity around day 14 and gradually subsided until day 28. Analysis of the proportion of CD4+C-X-C chemokine receptor 5 (CXCR5)+inducible costimulator (ICOS)+ Tfh cells and their subsets showed robust increase in Tfh and interleukin (IL)-17 producing Tfh (Tfh17) cells at day 7 (figure 1A), the onset of GIA (figure 1B,C), and Tfh cells were also increased at day 14. To further determine the accumulation and localisation of Tfh17 cells, we performed histological evaluation of inguinal lymph nodes of GIA. Accumulation of CD4+CXCR5+ Tfh cells in the germinal centres was noted at day 7 but not at day 28 (figure 1D). Immunohistological staining of the serial slides also showed these Tfh cells preferentially produced IL-17 compared with interferon gamma (figure 1E). Based on the accumulation of Tfh17 cells in germinal centres at day 7, we searched for cell surface molecules and found overexpression of OX40 in Tfh, especially Tfh17 cells (figure 1F,G and online supplementary figure 1a, b). In control non-arthritic mice, specific OX40 expression in Tfh17 cells was not observed. Therefore, this overexpression could be connected to arthritis induction (online supplementary figure 1c).

Differentiation of plasmablasts promoted by CD4+CXCR5+ICOS+ Tfh cells

We also checked the fluctuated numbers of plasmablasts, and it was also noted at day 7 and increased at day 14 (figure 2A). This tendency was similar to the fluctuation of Tfh cells (figure 1B). To evaluate B cell differentiation to plasmablasts, we established coculture system with T cells. Tfh cells (obtained from day 7 GIA) and naïve splenic B cells were cocultured (figure 2B). A higher proportion of naïve B cells differentiated into plasmablasts when cocultured with Tfh cells (figure 2C). We also measured antibody concentration in supernatants in cocultures of Tfh cells and plasmablasts (both obtained from day 7 GIA). High levels of anti-GPI antibody, the corresponding autoantibody in GIA, were noted in plasmablasts-Tfh cell cocultures in the presence of GPI (figure 2D). B cell differentiation and autoantibody production were also observed with CD4+CXCR5-ICOS+ cells. ICOS was reported to express highly in Th17 cells; therefore, they could indicate Th17 cell function of B cell promotion. These findings suggest that Tfh cells contribute to GIA by promoting B cell differentiation and autoantibody production. However, Tfh cell-mediated antibody production was not specific in autoimmune arthritis (online supplementary figure 2a), and anti-GPI antibody titres in GIA sera conflicted with this hypothesis. They remained high even after resolution of GIA and normalisation of the proportion of Tfh cells and plasmablasts (figure 2E).

Arthritogenicity and sialylation change of autoantibody during the course of arthritis

To understand the contradiction between the course of arthritis and anti-GPI antibody behaviour, we investigated the inflammatory activity of the antibody. Affinity chromatography-purified anti-GPI antibodies obtained from sera of day 7 (onset phase), day 14 (peak phase) and day 28 (resolution phase) GIA and from sera of K/BxN were used to generate immunocomplexes (ICs) (online supplementary figure 2b, c). The obtained ICs were then incubated with CD11c+ dendritic cells (DCs), and the production of cytokines and chemokines in the supernatants was measured by ELISA (figure 3A,B). Incubation of DCs with GPI-specific ICs from day 7 GIA triggered robust production of tumour necrosis factor-alpha (TNFα) and CXCL1, C-X-C chemokine ligand 1 (CXCL1), whereas those from day 28 GIA showed low inflammatory activity. Notably, the potential of

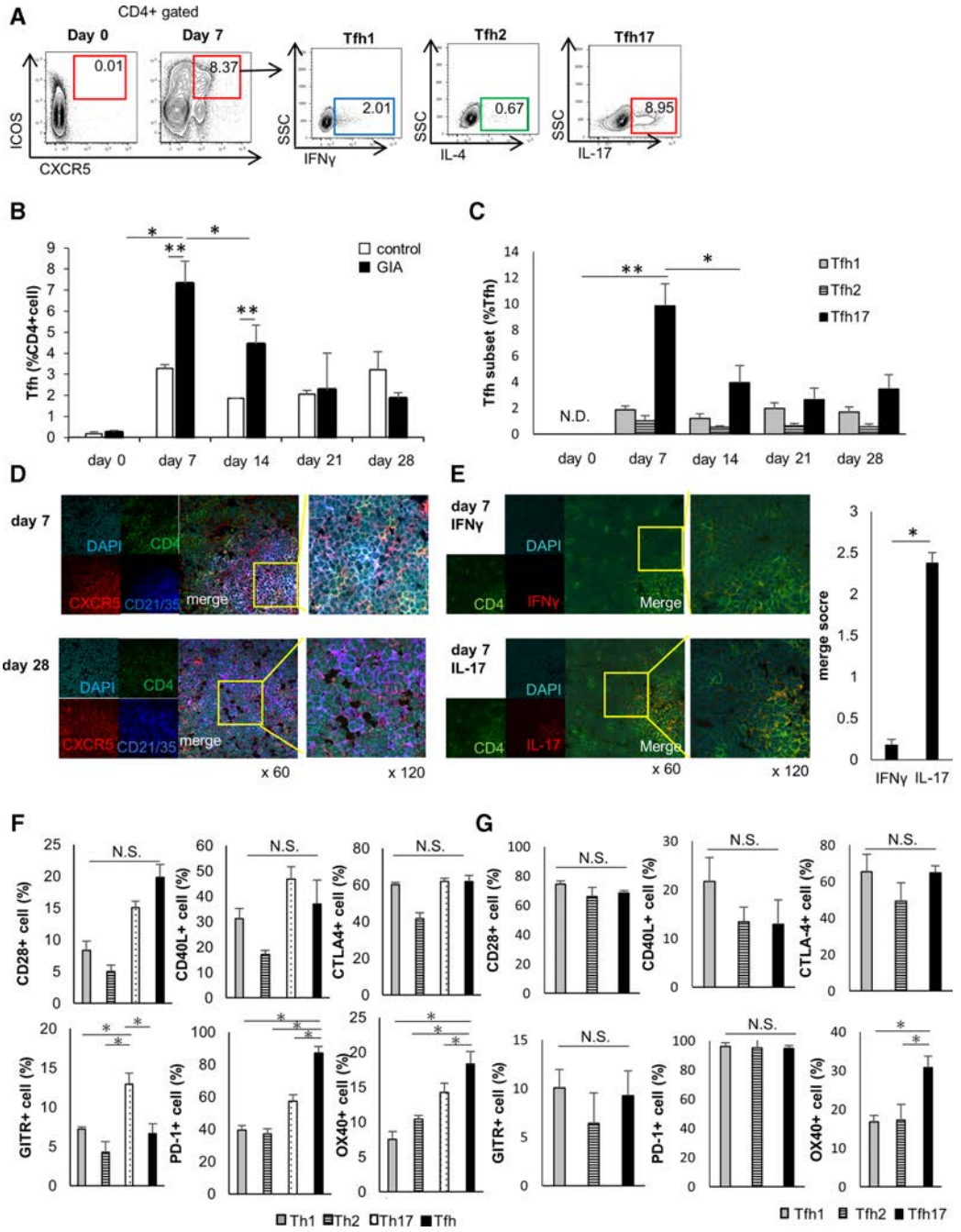

Figure 1 Increased proportions of Tfh and Tfh17 expressing OX40. Arthritis was induced by intradermal injection of 300 µg of GPI/GST fusion protein in emulsified Freund's complete adjuvant on day 0. For control mice, 300 µg of GST was injected intradermally in each DBA/1 mouse on day 0. (A–C) Fluctuations in the proportions of Tfh and Tfh subsets during the study as evaluated by flow cytometry (n=6, each). CD4+ cells were collected from GIA inguinal lymph nodes by MACS technology. The cells were stimulated for 5 hours with 50 ng/mL of PMA and 1 µg/mL of ionomycin. In the control group, 300 µg of GST was injected intradermally in each DBA/1 mouse on day 0 (n=4). (D) Immunofluorescent staining for accumulated Tfh cells in the germinal centres of inguinal lymph nodes of GIA. The frozen sections of the lymph nodes were used. Tfh cells were detected as CD4+CXCR5+ cells (n=4, each). Germinal centres were detected as CD21−CD35+ areas. Original magnification ×60 and ×120 as marked. The merge score of CD4 and CXCR5 was quantified. (E) Immunofluorescent staining of cytokines in the inguinal lymph nodes of day 7 GIA. Images represent the serial slides shown in D. Original magnification ×60 and ×120 as marked. The merge score of CD4 and each cytokine was quantified according to colocalisation ratio of fluorescence using FV10-ASW V.04.01 (n=3, each). (F,G) Cell surface molecule expression in Th and Tfh cell subsets. CD4+ cells were collected from day 7 GIA inguinal lymph nodes by MACS technology and then stimulated for 5 hours with 50 ng/mL of PMA and 1 µg/mL of ionomycin. After stimulation, the expression of cell surface molecules was assessed by flow cytometry in each subset. Th1 cells represented CD4+IFNγ+ cells, while Th2 represented CD4+IL-4+ cells, and Th17 represented CD4+IL-17+ cells (n=8, each). In A–E, the experiments were performed at two times. In F and G, flow cytometric analysis was replicated three times. Data are mean±SEM; *p< 0.05, **p< 0.01. CD40L, CD40 ligand; CTLA4, cytotoxic T-lymphocyte antigen 4; CXCR5, C-X-C chemokine receptor 5; DAPI, 4′,6-diamidino-2-phenylindole; GIA, GPI-induced arthritis; GITR, glucocorticoid-induced tumour necrosis factor receptor; GPI, glucose-6-phosphateisomerase; GST, gluthathione S-transferase; ICOS, inducible costimulator; IFNγ, interferon gamma; IL, interleukin; PD-1, programmed cell death-1; PMA, phorbol 12-myristate13-acetate; SSC, side scatter; Tfh, follicular helper T cells; Tfh17, interleukin-17producing Tfh cells.

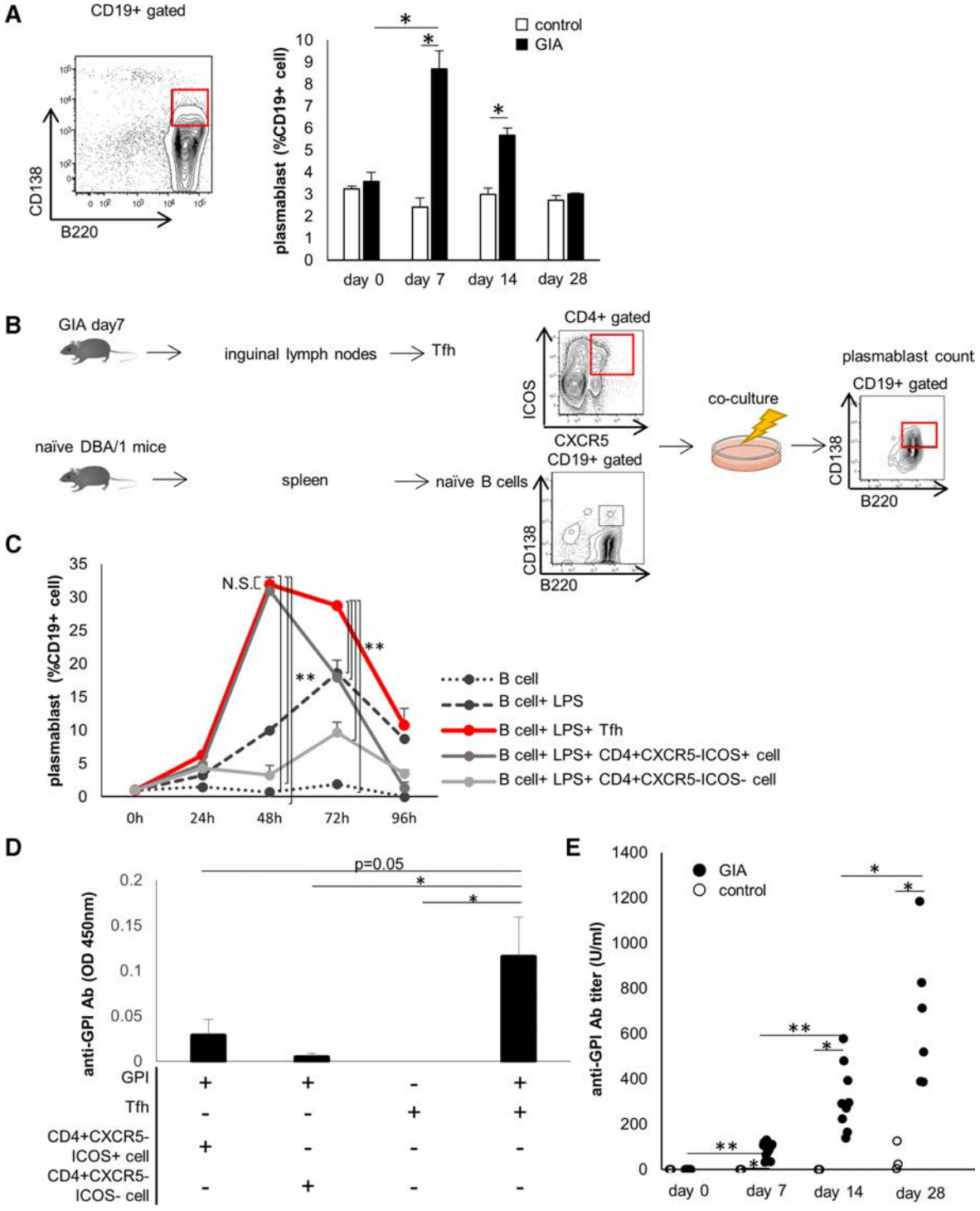

Figure 2 B cell promotion by Tfh cells. (A) Changes in plasmablasts in inguinal lymph nodes during the course of arthritis as evaluated by flow cytometry. CD19+B220+CD138 low cells were defined as plasmablasts. Representative image of plasmablasts is shown in the left panel. DBA/1 mice of the control group were injected intradermally with 300 µg of GST on day 0 (n=6, each). (B) Experimental scheme of T-naïve B cell coculture. Tfh or control CD4+ cells were obtained from inguinal lymph nodes of day 7 GIA by flow cytometric cell sorting. CD43-naïve splenic B cells were collected by MACS technology. Naïve B cells (5×10^4 cells/200 µL) and CD4+ cells including Tfh cells (3×10^4 cells/200 µL) were cocultured with 5 µg/mL of LPS and 2 µg/mL of anti-CD3 Ab at 37°C in 5% CO_2 for 0–96 hours. (C) Proportion of differentiated plasmablast was detected by flow cytometry after T-naïve B cell coculture (n=5). (D) Antigen-specific Ab production from cocultured plasmablasts. Tfh, control CD4+ cells (3×10^4 cells/200 µL) and plasmablasts (5×10^4 cells/200 µL) were sorted from day 7 GIA inguinal lymph nodes using flow cytometry. They were cocultured with or without 10 µM GPI. The culture supernatant was collected after 7 days and the amount of anti-GPI Ab was measured by ELISA (n=6, each). (E) Anti-GPI Ab titres of mice sera. Sera were obtained from GIA and control mice at days 0, 7, 14 and 28 (GIA, n=6–12; control, n=4). The titres were measured by ELISA. Pooled sera of K/BxN mice were used as standard, and the titre was defined as 1600 U/mL. In A–C, the experiments were performed at two times. In D, coculture was replicated three times. Data from four experiment times were combined for E. Data are mean±SEM; *p< 0.05, **p< 0.01. Ab, antibody; CO_2, carbon dioxide; CXCR5, C-X-C chemokine receptor 5; GIA, GPI-induced arthritis; GPI, glucose-6-phosphateisomerase; GST, gluthathione S-transferase; ICOS, inducible costimulator; LPS, lipopolysaccharide; MACS, magnetic-activated cell sorting; OD, optical density; Tfh, follicular helper T cells.

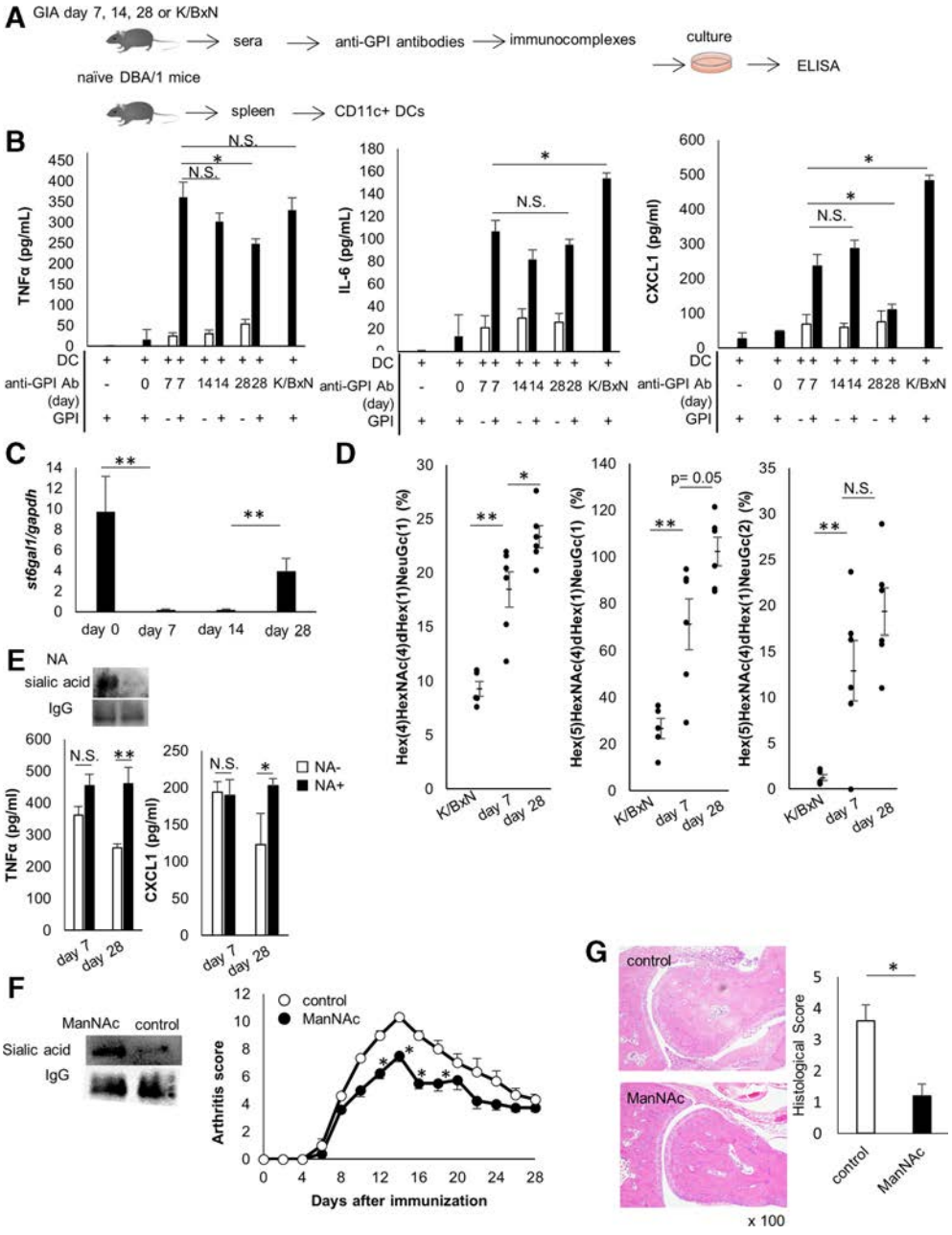

Figure 3 Arthritogenicity and changes in sialylation of anti-GPI antibody. (A) Experimental scheme of DC stimulation. Anti-GPI antibodies were purified from arthritic mice by affinity chromatography. They were incubated with GPI (antibody:GPI=1:2) at 37°C for 1 hour to generate immunocomplexes. CD11c+ DCs were obtained from spleens of naïve DBA/1 mice and stimulated with 2 µg of monometric anti-GPI antibody or immunocomplexes. After 24 hours of stimulation, the culture supernatant was collected for measurement of cytokine and chemokine contents by ELISA. (B) Concentration of TNFα, IL-6 and CXCL1 in the supernatant of stimulated DCs (GIA, n=5, each; K/BxN, n=4). Total IgGs were used for GIA day 0 instead of anti-GPI antibodies because the antibody level was 0 on day 0. (C) The expression of St6gal1 in plasmablasts of GIA. Plasmablasts were sorted by flow cytometry from GIA inguinal lymph nodes, and St6gal1 expression was measured by quantitative PCR (n=6, each). (D) The amount of sialic acid in anti-GPI antibodies was quantified with mass spectrometry. Three types of sialylated glycans were detected. Core peptides of the glycans were analysed with Glyco-RIDGE method and identified as the same sequence with Asn297 of the Fc part of IgGs. Each glycan was compared with the area ratio (sialylated-glycan/asialo-glycan) of extracted ion chromatograms (K/BxN, n=5; GIA: n=6, each). (E) Upregulation of cytokine production from DCs with asialo-antibodies. Sialic acid of antibodies was removed artificially with α2–3,6,8 neuraminidase in vitro. DCs were stimulated and ELISA was performed by the method described in (A). (F,G) Arthritis in ManNAc-fed mice. DBA/1 mice were provided with 5% ManNAc-containing water or distilled water as control from 3 weeks before the induction of arthritis to the end of the experiment. Changes in the amount of sialic acid in anti-GPI antibodies were confirmed with lectin blotting with *Sambucus nigra* lectin. The arthritis score (F, n=8, each) and the histological score of H&E-stained sections of the ankle joints (G, n=5, each) were evaluated. Representative images of H&E-stained sections of control mice (G, top panel) and ManNAc-treated mice (G, bottom panel). We replicated the experiments two times in A–E. F and G were made with a combination of three experiment times. Data are mean±SEM arthritis scores calculated in three independent experiments; *p< 0.05, **p< 0.01. Ab, antibody; CSCL1, C-X-C chemokine ligand 1; DC, dendritic cell; Fc, fragment crystallizable; gadph, glyceraldehyde-3-phosphate dehydrogenase; GIA, GPI-induced arthritis; GPI, glucose-6-phosphateisomerase; IL, interleukin; ManNAc, N-acetyl-D-mannosamine; NA, neuraminidase; St6gal1, α2,6-sialyltransferase 1; TNFα, tumour necrosis factor-alpha.

TNFα production by ICs from day 7 GIA was almost comparable with that from K/BxN mice, which has been confirmed to be arthritogenic.[20 21] CXCL1 and IL-6 production by ICs was clearly increased from K/BxN comparing with GIA. The cytokine and chemokine production was significantly reduced by FcγR blockade with blocking antibody or addition of mouse IgGs instead of GPI, which indicated that it was facilitated by FcγR triggering and anti-IgG antibody did not affect the result (online supplementary figure 2d, e). These data indicated that the inflammatory properties of anti-GPI antibody were changed during the course of arthritis in GIA.

We investigated the mechanism underlying this change. Since the properties of IgG subclasses in the two phases were not different (online supplementary figure 2f), we examined sialylation of anti-GPI antibody. β-galactoside α2,6-sialyltransferase 1 (St6gal1) is the rate-limiting enzyme during the post-translational transfer of sialic acid to the IgG-linked N-glycans in antibody-producing cells. The expression of St6gal1 was markedly decreased in plasmablasts at days 7 and 14 (figure 3C), despite the amounts of sialic acid of the total IgGs and IgGs other than anti-GPI antibody (flow-through IgGs) at days 7 and 28 not being different (online supplementary figure 2g). Notably, mass spectrometric analysis confirmed hyposialylation of anti-GPI antibody at day 7. We found three types of glycans with sialic acid at Asn297 of IgGs from GIA and K/BxN (online supplementary figures 2h, 3a-g). The proportions of anti-GPI antibodies in glycopeptides with the Hex(4)HexNAc(4)dHex(1)NeuGc(1) (the most common fraction) and the Hex(5)HexNAc(4)dHex(1)NeuGc(1) (the second common fraction) were significant and tended to be lower at day 7 GIA, respectively, compared with those at day 28 (figure 3D, online supplementary figure 3a). As expected, the lowest sialylated content was found in anti-GPI antibodies of K/BxN mice (figure 3D).

Having implicated hyposialylation of autoantibody in the onset of arthritis, we next investigated how the promotion of sialylation potentially impacts arthritis. The sialic acid was cleaved from the antibodies using neuraminidase, followed by in vitro DC stimulation. Removal of sialic acid rescued the inflammatory potential of day 28 ICs to a level similar to that of day 7 ICs (figure 3E). In a related in vivo experiment, mice were supplied with water supplemented with the sialic acid precursor N-acetyl-D-mannosamine (ManNAc) and immunised with GPI. We observed an increase of sialic acid amount in anti-GPI antibody isolated from the ManNAc-treated mice (figure 3F). These mice exhibited amelioration of limb swelling and improvement of histological findings (figure 3F,G), suggesting the importance of sialic content in suppressing arthritis.

Regulation of autoantibody hyposialylation by Tfh cells via the OX40-OX40L pathway

We subsequently explored the contribution of Tfh cells to antibody hyposialylation by in vitro coculture system (figure 4A). The stimulation of B cells with sole lipopolysaccharide or coculture with CD4+CXCR5 cells did not result in suppression of the St6gal1 expression in the differentiated plasmablasts. Nevertheless, naïve B cells cocultured with Tfh cells obtained from day 7 GIA showed significant suppression of St6gal1 in differentiated plasmablasts (figure 4B).

Next, we investigated the underlying mechanism of this regulation. Having significant upregulation of OX40 and programmed cell death-1 (PD-1) on Tfh cells (figure 1F), we next focus on OX40-OX40L as well as PD-1-PD-L1 pathway. OX40L expression was noted in germinal-centre B cells and plasmablasts obtained from day 7 GIA mice (online supplementary figure 4a, b). Blockade of OX40-OX40L pathway with monoclonal antibodies resulted in recovery of St6gal1 expression in plasmablasts in the coculture system. This blockade was also associated with a decrease in the proportion of differentiated plasmablasts and survived Tfh cells (figure 4C,D). This recovery was not observed when the pathway was blocked among B cells and control CD4+ cells (online supplementary figure 5a). On the other hand, PD-1-PD-L1 blockade did not affect St6gal1 expression or plasmablast differentiation (figure 4E). Furthermore, treatment of GIA mice with OX40L blocking antibody resulted in the amelioration of GIA (figure 4F,G), associated with recovery of St6gal1 expression in plasmablasts and increase in sialic acid with anti-GPI antibodies (figure 4H). The antibodies were weaker stimulants for cytokine and chemokine production, where artificial desialylation with neuraminidase cancelled this weakening (figure 4I). These changes were accompanied by suppression of Tfh cell expansion, especially that of Tfh17 cells (figure 4J) and decreased anti-GPI antibody production and germinal centre formation (online supplementary figure 5b, c). These changes might contribute to the amelioration as well. To eliminate non-specific effects of Fc portion of antibodies, we performed in vitro and in vivo experiments using anti-OX40L F(ab')2 and obtained similar results with anti-OX40L antibody (online supplementary figure 5d-g). In addition, these phenomena were also observed with IL-23 neutralisation (online supplementary figure 6a-c). It also decreased OX40 expression of Tfh17 cells (online supplementary figure 6d). The results of these experiments highlight the importance of OX40-OX40L pathway expressed on Tfh cells, particularly Tfh17 cells, in the regulation of autoantibody hyposialylation.

Tfh17 cell existence and negative correlation with St6gal1 in patients with RA

To evaluate the above changes in patients with RA, we measured the proportion of Tfh17 cells in them. PBMCs were obtained from treatment-naïve patients with active RA and from patients with OA (their clinical features are summarised in online supplementary table 1). The proportions of Tfh and Tfh17 cells were higher in patients with RA compared with those with OA (figure 5A,B). In addition, the proportion of Tfh17 cells, but not Tfh cells, correlated significantly with the titres of ACPAs (figure 5C). To clarify our hypothesis with mouse experiments, we analysed the expression of St6gal1 in circulating plasmablasts (figure 5D). The proportion of Tfh17 cells correlated strongly and negatively with St6gal1 expression in plasmablasts (figure 5E). In addition, the expression level of OX40 was significantly highest in Tfh17 cells among the Tfh subsets (figure 5F). These data suggested that Tfh or Tfh17 cells could contribute RA-specific antibody production and hyposialylation also via OX40.

DISCUSSION

Here we described the potential role of accumulated Tfh cells, including Tfh17 cells, which promote autoantibody production and regulate their hyposialylation via the OX40-OX40L pathway. This glycosylation change has occurred at the onset of arthritis and recovered at the resolution phase. Blockade of T cell–B cell interaction through the pathway rescued the sialylation and prevented the development of arthritis with reduction of Tfh17 cells. The study also examined patients with RA and showed a negative correlation between the proportion of

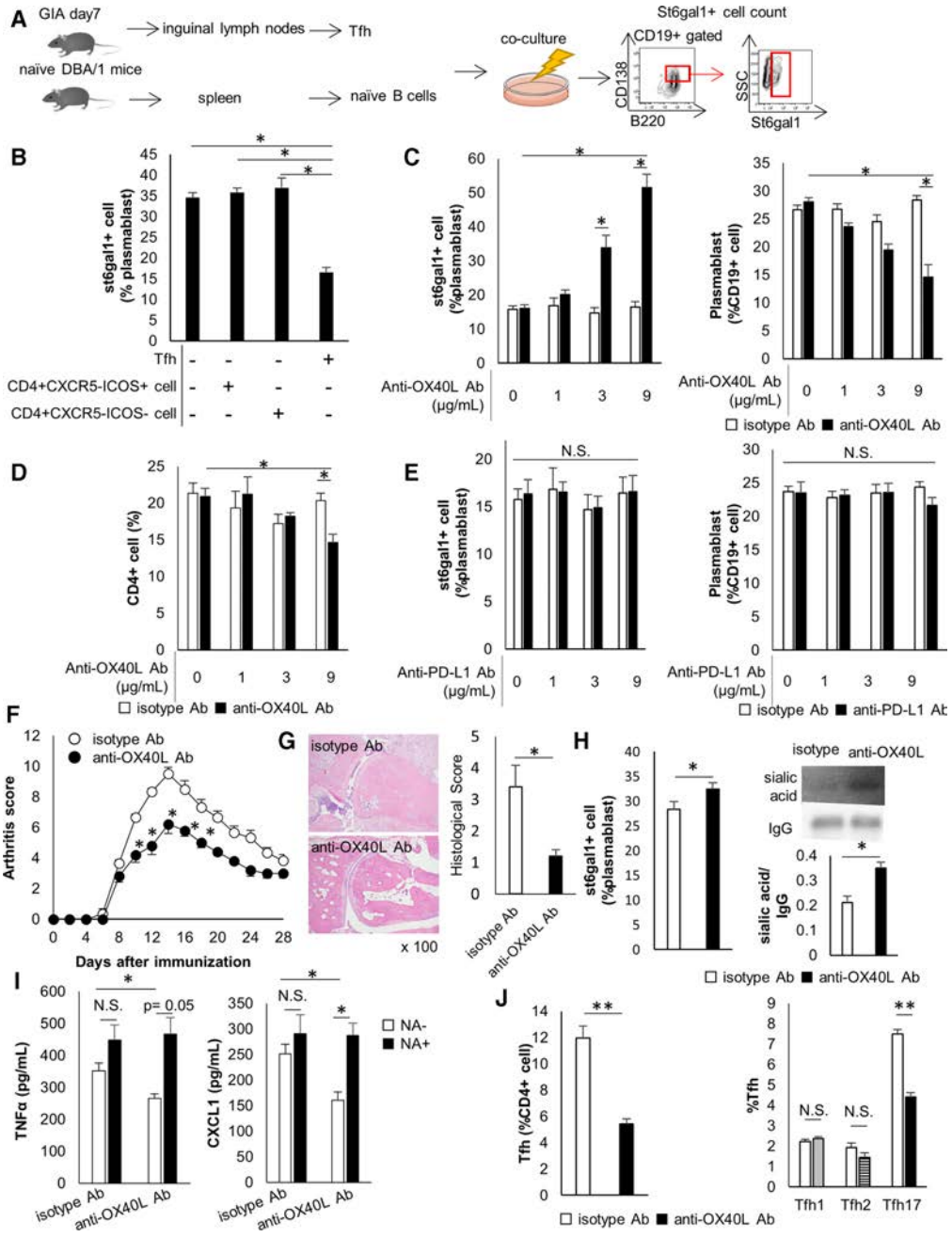

Figure 4 Regulation of autoantibody hyposialylation by Tfh17 via the OX40-OX40L pathway. (A) Experimental scheme of T-naïve B cell coculture. Tfh or control CD4+ cells were obtained from inguinal lymph nodes of day 7 GIA. They were cocultured with CD43-naïve splenic B cells under LPS and anti-CD3 antibody stimulation for 72 hours, and expression of St6gal1 in differentiated plasmablasts was detected. (B) Proportion of St6gal1+ cells in differentiated plasmablasts (n=6, each). (C) Recovery of St6gal1 expression (left) and decrease of plasmablasts (right) by in vitro OX40L blocking. Anti-OX40L antibody or isotype control was added to the culture wells at 0–9 μg/mL to block the OX40-OX40L pathway (n=8 for anti-OX40L antibody group, n=4 for the isotype antibody group). (D) Decrease of CD4+ cells by in vitro OX40L blocking. Anti-OX40L antibody or isotype control was added to the culture wells at 0–9 μg/mL to block the OX40-OX40L pathway (n=6 for anti-OX40L antibody group, n=4 for control group). (E) The proportions of st6gal1+ cells (left) and differentiated plasmablasts (right) were not affected by blocking of the PD-1-PD-L1 pathway with PD-L1 monoclonal antibody. Anti-PD-L1 antibody or isotype control was added to the culture wells at 0–9 μg/mL (n=5 for anti-PD-L1 antibody group, n=4 for the isotype antibody group). (F–J) Anti-OX40L antibody (100 μg) or isotype control was injected intraperitoneally in GIA mice every 2 days from days 0 to 14. Arthritis score (F, n=6, each) and histological score of H&E-stained sections of the ankle joints (G, n=5, each) were evaluated. The proportions of St6gal1+ cells among plasmablasts were analysed at day 7 (H, left, n=5). Changes in the amount of sialic acid in anti-GPI antibodies at day 7 were semiquantified with normalised band intensity of lectin blotting with *Sambucus nigra* lectin (H, right, n=5). DC stimulation with them was performed with or without neuraminidase processing (I, n=5, each; experiments were performed with the same protocol with B and E). The proportion of Tfh and its subset was analysed at day 7 (J, n=5, each). Experiments in the figure were replicated two times except F. In F, arthritis scores were assessed in three independent experiments. Data are mean±SEM; *p< 0.05, **p< 0.01. Ab, antibody; CXCL1, C-X-C chemokine ligand 1; CXCR5, C-X-C chemokine receptor 5; DC, dendritic cell; GIA, GPI-induced arthritis; GPI, GPI-induced arthritis; ICOS, inducible costimulator; LPS, lipopolysaccharide; OX40L, OX40 ligand; PD-1, programmed celldeath-1; PD-L1, programmed death-ligand 1; St6gal1, α2,6-sialyltransferase 1; Tfh, follicular helper T cells; Tfh17, interleukin-17producing Tfh cells; TNFα, tumour necrosis factor-alpha.

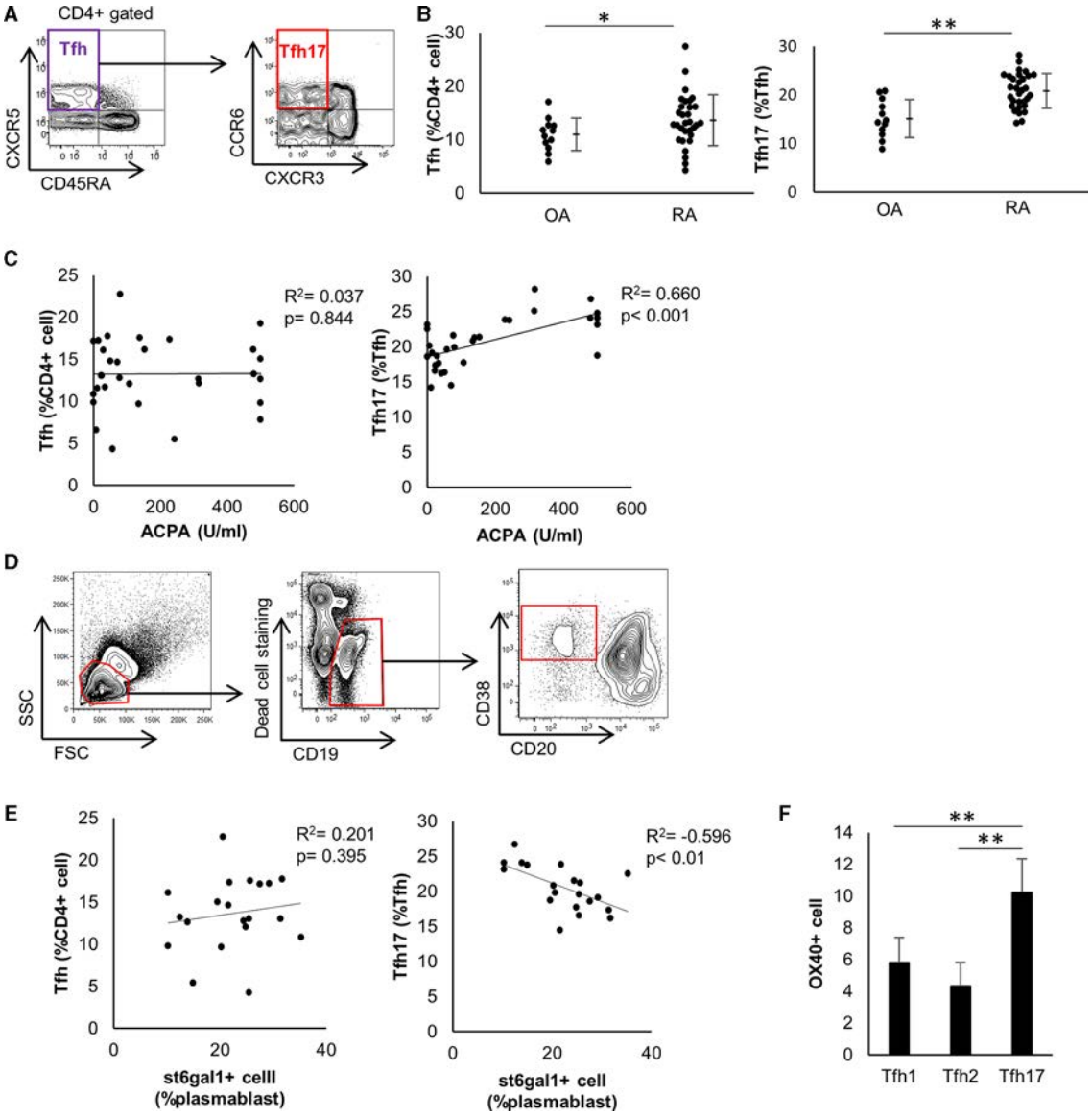

Figure 5 Patients with RA show accumulation of Tfh17 cells, which correlates with St6gal1 expression. (A) Representative image of Tfh and Tfh17 cells in flow cytometry. (B) Proportions of Tfh and Tfh17 cells in peripheral blood of patients with OA (n=12) and patients with RA (n=31). (C) Correlation between the proportion of Tfh17 cells and ACPA titre in patients with RA (n=31). ACPA titres were obtained from medical records of the patients. (D) Gating strategy for circulating plasmablasts. (E) Correlation between the proportion of Tfh and Tfh17 cells and St6gal1 expression in plasmablasts in patients with RA (n=20). (F) Expression of OX40 in different Tfh subsets (n=10). Data are mean±SD; *p<0.05, **p<0.01. ACPA, anticitrullinated protein antibody; CXCR3, C-X-C chemokine receptor 3; CXCR5, C-X-C chemokine receptor 5; FSC, forward scatter; OA, osteoarthritis; RA, rheumatoid arthritis; SSC, side scatter; St6gal1, α2,6-sialyltransferase 1; Tfh, follicular helper T cells; Tfh17, interleukin-17 producing Tfh cells.

circulating Tfh17 cells and St6gal1 expression in plasmablasts and high OX40 expression in Tfh17 cells.

Previous reports showed T cell-dependent versus T cell-independent immunisation protocols against model antigens have been shown to result in distinct glycosylation patterns of the antigen-specific IgG response.[10 28] In addition, the IL-23-Th17 cells axis was found to be responsible for antibody hyposialylation in collagen-induced arthritis.[14] The same study also described the accumulation of Th17 cells in the germinal centres and overexpression of PD-1, which characterises Tfh cells, similar to our findings with Tfh17 cells. Moreover in RA and experimental models, autoantibodies exhibited a specific Fc-linked glycan profile that is distinct from that of total serum IgG.[13 14] In our present study the sialic acid amount of total IgG was also unaffected. These findings were in line with the contribution of antigen-specific antibody production by Tfh cells.

Furthermore, data from human and mouse studies suggest that the OX40-OX40L axis has an important role in RA.[29] Consistent with our findings, blockade of OX40L reduced bone and cartilage destruction in other arthritis models.[30–32] Our present study also showed the proportion of newly differentiated plasmablast was decreased with the blockade, which is also consistent with the reported correlation between high plasma levels of soluble OX40 and ACPAs levels in patients with early-stage RA.[33]

Our present study now shows the potential contribution of the OX40-OX40L pathway in the regulation of sialylation. In RA, it is well established that autoantibody formation precedes the symptomatic inflammatory phase of the disease. Factors that shift asymptomatic autoimmunity to inflammation are therefore of key interest in the perspective of treatment or prevention of the disease onset. Our findings suggest a treatment which targets the pathway would cause glycosylation change of autoantibody

and would be effective especially for patients in early stage. This kind of treatment which aims for 'quality change' of autoantibody will be expected to cause less severe infection, as CD28 and ICOS paired signalling is still intact.

Important questions remain. In this study we did not find the reason why plasmablasts which produced hyposialylated antibodies have diminished in the arthritis resolving phase. It is well known that plasmablasts drain away from germinal centres after maturation from germinal-centre B cells. Therefore, another mechanism will underlie the conversion from 'aggressive' plasmablasts because they are no longer affected by Tfh17 cells. In addition, Engdahl *et al*[34] reported oestrogen-induced St6gal1 expression in patients with RA, and this could be one of the reasons for preferential affection of RA to women in postmenopause or post partum.[35] Although there are some reports which described the relation between oestrogen and CD4+ T cells,[36 37] its effect on Tfh17 cells remains unclear. Further studies are needed to answer these questions.

In summary, we have found a novel role of OX40 expressing Tfh cell which regulated autoantibody hyposialylation in an experimental arthritis. We also showed blockade of the OX40-OX40L pathway led to a reduction of Tfh17 cells and has a preventive effect. Furthermore, the same mechanisms could exist in patients with RA and might pave the way for the development of new therapies, especially in the early induction phase of autoimmune arthritis.

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Contributors IK, IM and TS designed the study. IK, AOs and SK performed the experiments and collected the data. YK supervised the tissue analysis. AT and HKaj performed the mass spectrometric analysis. IK, AOh, AOs, HE, HKaw, YK and HT participated in the discussion. IK, IM and TS analysed the data and wrote the manuscript. All authors have read and approved the manuscript for publication.

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REFERENCES

1 Smolen JS, Aletaha D, McInnes IB. Rheumatoid arthritis. *The Lancet* 2016;388:2023–38.
2 Wegner N, Lundberg K, Kinloch A, *et al*. Autoimmunity to specific citrullinated proteins gives the first clues to the etiology of rheumatoid arthritis. *Immunol Rev* 2010;233:34–54.
3 Avouac J, Gossec L, Dougados M. Diagnostic and predictive value of anti-cyclic citrullinated protein antibodies in rheumatoid arthritis: a systematic literature review. *Ann Rheum Dis* 2006;65:845–51.
4 Whiting PF, Smidt N, Sterne JAC, *et al*. Systematic review: accuracy of anti-citrullinated peptide antibodies for diagnosing rheumatoid arthritis. *Ann Intern Med* 2010;152:456–64.
5 Kroot EJ, de Jong BA, van Leeuwen MA, *et al*. The prognostic value of anti-cyclic citrullinated peptide antibody in patients with recent-onset rheumatoid arthritis. *Arthritis Rheum* 2000;43:1831–5. 2-6.
6 Nimmerjahn F, Ravetch JV. Antibody-Mediated modulation of immune responses. *Immunol Rev* 2010;236:265–75.
7 Yamaguchi Y, Nishimura M, Nagano M, *et al*. Glycoform-dependent conformational alteration of the Fc region of human immunoglobulin G1 as revealed by NMR spectroscopy. *Biochim Biophys Acta* 2006;1760:693–700.
8 Dekkers G, Treffers L, Plomp R, *et al*. Decoding the human immunoglobulin G-glycan repertoire reveals a spectrum of Fc-receptor- and complement-mediated-effector activities. *Front Immunol* 2017;8:877.
9 Rademacher TW, Williams P, Dwek RA. Agalactosyl glycoforms of IgG autoantibodies are pathogenic. *Proc Natl Acad Sci U S A* 1994;91:6123–7.
10 Hess C, Winkler A, Lorenz AK, *et al*. T cell-independent B cell activation induces immunosuppressive sialylated IgG antibodies. *J Clin Invest* 2013;123:3788–96.
11 Ito K, Furukawa J-ichi, Yamada K, *et al*. Lack of galactosylation enhances the pathogenic activity of IgG1 but not IgG2a anti-erythrocyte autoantibodies. *J Immunol* 2014;192:581–8.
12 Böhm S, Schwab I, Lux A, *et al*. The role of sialic acid as a modulator of the anti-inflammatory activity of IgG. *Semin Immunopathol* 2012;34:443–53.
13 Ohmi Y, Ise W, Harazono A, *et al*. Sialylation converts arthritogenic IgG into inhibitors of collagen-induced arthritis. *Nat Commun* 2016;7:1–12.
14 Pfeifle R, Rothe T, Ipseiz N, *et al*. Regulation of autoantibody activity by the IL-23-T_H17 axis determines the onset of autoimmune disease. *Nat Immunol* 2017;18:104–13.
15 Pagan JD, Kitaoka M, Anthony RM. Engineered Sialylation of Pathogenic Antibodies In Vivo Attenuates Autoimmune Disease. *Cell* 2018;172:e13:564–77.
16 Ma J, Zhu C, Ma B, *et al*. Increased frequency of circulating follicular helper T cells in patients with rheumatoid arthritis. *Clinical and Developmental Immunology* 2012;2012:1–7.
17 Chevalier N, Macia L, Tan JK, *et al*. The role of follicular helper T cell molecules and environmental influences in autoantibody production and progression to inflammatory arthritis in mice. *Arthritis Rheumatol* 2016;68:1026–38.
18 Moschovakis GL, Bubke A, Friedrichsen M, *et al*. T cell specific Cxcr5 deficiency prevents rheumatoid arthritis. *Sci Rep* 2017;7:1–13.
19 Matsumoto I, Staub A, Benoist C, *et al*. Arthritis provoked by linked T and B cell recognition of a glycolytic enzyme. *Science* 1999;286:1732–5.
20 Kouskoff V, Korganow AS, Duchatelle V, *et al*. Organ-Specific disease provoked by systemic autoimmunity. *Cell* 1996;87:811–22.
21 Korganow AS, Ji H, Mangialaio S, *et al*. From systemic T cell self-reactivity to organ-specific autoimmune disease via immunoglobulins. *Immunity* 1999;10:451–61.
22 Frey O, Bruns L, Morawietz L, *et al*. B cell depletion reduces the number of autoreactive T helper cells and prevents glucose-6-phosphate isomerase-induced arthritis. *PLoS One* 2011;6:e24718–11.
23 Tanaka-Watanabe Y, Matsumoto I, Iwanami K, *et al*. B cells play a crucial role as antigen-presenting cells and collaborate with inflammatory cytokines in glucose-6-phosphate isomerase-induced arthritis. *Clin Exp Immunol* 2009;155:285–94.
25 Arnett FC, Edworthy SM, Bloch DA. Revised criteria for the classification of rheumatoid arthritis. *Orthop Nurs* 1990;9:58–64.
26 Aletaha D, Neogi T, Silman AJ, *et al*. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League against rheumatism collaborative initiative. *Ann Rheum Dis* 2010;69:1580–8.
27 Togayachi A, Tomioka A, Fujita M, *et al*. Identification of Poly-N-Acetyllactosamine-Carrying glycoproteins from HL-60 human promyelocytic leukemia cells using a site-specific glycome analysis method, Glyco-RIDGE. *J Am Soc Mass Spectrom* 2018;29:1138–52.
28 Oefner CM, Winkler A, Hess C, *et al*. Tolerance induction with T cell-dependent protein antigens induces regulatory sialylated IgGs. *J Allergy Clin Immunol* 2012;129:1647–55.
29 Croft M, Siegel RM. Beyond TNF: TNF superfamily cytokines as targets for the treatment of rheumatic diseases. *Nat Rev Rheumatol* 2017;13:217–33.
30 Yoshioka T, Nakajima A, Akiba H, *et al*. Contribution of OX40/OX40 ligand interaction to the pathogenesis of rheumatoid arthritis. *Eur J Immunol* 2000;30:2815–23.
31 Horai R, Nakajima A, Habiro K, *et al*. TNF-α is crucial for the development of autoimmune arthritis in IL-1 receptor antagonist–deficient mice. *J Clin Invest* 2004;114:1603–11.
32 Gwyer Findlay E, Danks L, Madden J, *et al*. Ox40L blockade is therapeutic in arthritis, despite promoting osteoclastogenesis. *Proc Natl Acad Sci U S A* 2014;111:2289–94.
33 Laustsen JK, Rasmussen TK, Stengaard-Pedersen K, *et al*. Soluble OX40L is associated with presence of autoantibodies in early rheumatoid arthritis. *Arthritis Res Ther* 2014;16:1–9.
34 Engdahl C, Bondt A, Harre U, *et al*. Estrogen induces ST6GAL1 expression and increases IgG sialylation in mice and patients with rheumatoid arthritis: a potential explanation for the increased risk of rheumatoid arthritis in postmenopausal women. *Arthritis Res Ther* 2018;20:1–11.
35 Goemaere S, Ackerman C, Goethals K, *et al*. Onset of symptoms of rheumatoid arthritis in relation to age, sex and menopausal transition. *J Rheumatol* 1990;17:1620–2.
36 Park H-J, Park H-S, Lee J-U, *et al*. Gender-Specific differences in PPARγ regulation of follicular helper T cell responses with estrogen. *Sci Rep* 2016;6:28495.
37 Monteiro C, Kasahara TM, Castro JR, *et al*. Pregnancy favors the expansion of circulating functional follicular helper T cells. *J Reprod Immunol* 2017;121:1–10.

TRANSLATIONAL SCIENCE

Does immunological remission, defined as disappearance of autoantibodies, occur with current treatment strategies? A long-term follow-up study in rheumatoid arthritis patients who achieved sustained DMARD-free status

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ABSTRACT

Objectives Sustained disease-modifying antirheumatic drug (DMARD)-free status, the sustained absence of synovitis after cessation of DMARD therapy, is infrequent in autoantibody-positive rheumatoid arthritis (RA), but approximates cure (ie, disappearance of signs and symptoms). It was recently suggested that immunological remission, defined as disappearance of anti-citrullinated protein antibodies (ACPA) and rheumatoid factor (RF), underlies this outcome. Therefore, this long-term observational study determined if autoantibodies disappear in RA patients who achieved sustained DMARD-free remission.

Methods We studied 95 ACPA-positive and/or RF-positive RA patients who achieved DMARD-free remission after median 4.8 years and kept this status for the remaining follow-up (median 4.2 years). Additionally, 21 autoantibody-positive RA patients with a late flare, defined as recurrence of clinical synovitis after a DMARD-free status of ≥1 year, and 45 autoantibody-positive RA patients who were unable to stop DMARD therapy (during median 10 years) were studied. Anti-cyclic citrullinated peptide 2 (anti-CCP2) IgG, IgM and RF IgM levels were measured in 587 samples obtained at diagnosis, before and after achieving DMARD-free remission.

Results 13% of anti-CCP2 IgG-positive RA patients had seroreverted when achieving remission. In RA patients with a flare and persistent disease this was 8% and 6%, respectively (p=0.63). For anti-CCP2 IgM and RF IgM, similar results were observed. Evaluating the estimated slope of serially measured levels revealed that RF levels decreased more in patients with than without remission (p<0.001); the course of anti-CCP2 levels was not different (p=0.66).

Conclusions Sustained DMARD-free status in autoantibody-positive RA was not paralleled by an increased frequency of reversion to autoantibody negativity. This form of immunological remission may therefore not be a treatment target in patients with classified RA.

Key messages

What is already known about this subject?

- Sustained disease-modifying antirheumatic drug (DMARD)-free remission is the best possible clinical outcome and is increasingly achievable, also in autoantibody-positive rheumatoid arthritis (RA).
- It is unknown if autoantibody-positive patients who achieve this outcome have disappearance of autoantibodies at this point in time.

What does this study add?

- Seroreversion from anti-citrullinated protein antibodies and/or rheumatoid factor positivity to negativity is infrequently observed and in similar frequencies in RA patients who did or did not achieve sustained DMARD-free remission.

How might this impact on clinical practice or future developments?

- Disappearance of autoantibodies is not a feasible long-term treatment target in (established) RA.

INTRODUCTION

Sustained disease-modifying antirheumatic drug (DMARD)-free status is defined as sustained absence of synovitis after cessation of all DMARD therapy and is increasingly achievable by patients with rheumatoid arthritis (RA).[1] This status is also characterised by normalisation of functional status and lower levels of fatigue, pain and morning stiffness and is currently considered the best possible outcome of RA.[1] Absence of anti-citrullinated protein antibodies (ACPA) at disease presentation is an important predictor of achievement of sustained DMARD-free remission; however, with current treatment strategies this outcome is also observed in 10% of ACPA-positive RA.[1–5]

The pathophysiological role of ACPA in RA development or progression is not exactly known. ACPA can be present years before the onset of joint symptoms and disease, indicating that the mere presence of ACPA is not enough to develop disease.[6 7] Studies in the preclinical phase have shown that the ACPA immune response matures once disease onset is approached, as characterised by an increase in ACPA-level, isotype-usage,

avidity and the number of citrullinated epitopes recognised by ACPA.[8–11] In addition, there are changes in Fc glycosylation before RA onset.[12] Once RA is established the ACPA immune response does not mature any further.[13] Besides ACPA, also rheumatoid factor (RF) can be present years before disease onset.[6 7] Since autoantibodies are considered to have a prominent role in seropositive RA and precede symptom development, it is tempting to hypothesise that changes in the autoantibody response occur before or at the time when clinical disease has been extinguished, as is the case when sustained DMARD-free remission is reached. In this light, it was recently suggested that disappearance of autoantibodies is a hallmark of immunological remission and might characterise patients who are able to achieve drug-free remission.[14]

However, so far this hypothesis has not been thoroughly investigated. In a few studies, seroconversion and seroreversion during follow-up of early arthritis and RA patients were investigated. The observations described indicate that both are infrequent and not associated with relevant outcomes such as radiographic damage, functional status or the disease activity score.[15–18] In only one study, the association between seroreversion and drug-free remission was analysed and no association was observed.[19] However, autoantibody levels were only determined at disease presentation and at 1 year of follow-up, thus generally years before achievement of drug-free remission. In addition, follow-up of patients after the achievement of drug-free remission was limited.[19]

We aimed to increase the understanding of the long-term course of RA-related autoantibodies in patients who had achieved the closest available proxy of cure of RA. Therefore, we investigated the association between ACPA and RF seroreversion and achievement of sustained DMARD-free remission in a unique population of RA patients with available serum samples at the time of remission and with a long follow-up duration after achievement of DMARD-free status.

METHODS

Patients

Patients were retrieved from the Leiden Early Arthritis Clinic cohort, which is an inception cohort that includes patients with clinically confirmed arthritis and symptom duration <2 years. At baseline, patients and rheumatologists completed questionnaires, joint counts were performed and blood samples were collected. Follow-up visits were scheduled and blood samples were taken at 3–4 months, 6–8 months, 12 months, 18 months, 24 months and yearly thereafter. Between 1993 and 2014, 3473 patients were consecutively included, of which 1586 patients had a clinical diagnosis of RA and also fulfilled 1987 or 2010 RA classification criteria during the first year of follow-up.[20 21] Of these, 941 patients were ACPA-positive and/or RF-positive at baseline.

Treatment strategies changed over time. In general, patients included in 1993–1995 were initially treated with non-steroidal anti-inflammatory drugs, patients included in 1996–1998 with mild DMARDs (hydroxychloroquine or sulphasalazine) and patients included ≥1999 were initially treated with methotrexate. When this treatment failed, another conventional DMARD was initiated or added. A biological DMARD was allowed in patients who failed on ≥2 conventional DMARDs. Medication used by all studied patients during the observed follow-up period is shown table 1. Disease activity score (DAS44)-guided treatment became common from 2005 onwards with tapering and eventually stopping of treatment if DAS44 remained <2.4 and synovitis was absent at clinical joint examination, and intensifying treatment in case of DAS44 ≥2.4.

Table 1 Medication use during the total follow-up period; stratified by patient group

	Sustained DMARD-free remission (n=95)	Flare after DMARD-free remission (n=21)	Persistent RA (n=45)
Methotrexate, n (%)	76 (80)	21 (100)	44 (98)
Other conventional DMARDs, n (%)	46 (48)	11 (52)	37 (82)
Sulfasalazine, n (%)	27 (28)	6 (29)	28 (62)
Hydroxychloroquine, n (%)	26 (27)	8 (38)	32 (71)
Leflunomide, n (%)	9 (9)	5 (24)	17 (38)
Azathioprine, n (%)	1 (1)	0 (0)	3 (7)
Cyclosporine, n (%)	0 (0)	1 (5)	0 (0)
Gold, n (%)	2 (2)	0 (0)	1 (2)
Biological DMARD, n (%)	14 (15)	6 (29)	19 (42)
TNF-inhibitor, n (%)	11 (12)	4 (19)	17 (38)
Rituximab, n (%)	1 (1)	1 (5)	2 (4)
Abatacept, n (%)	0 (0)	0 (0)	3 (7)
Tocilizumab, n (%)	2 (2)	1 (5)	3 (7)
Omalizumab, n (%)	0 (0)	0 (0)	1 (2)

Numbers indicate the number of patients who used the indicated medication at any time during follow-up; therefore, the indicated percentages for the different groups do not add up to 100%. The duration that patients used the medication and the number of patients using combination therapy is not indicated here.

DMARD, disease-modifying antirheumatic drug; RA, rheumatoid arthritis; TNF, tumor necrosis factor.

Of the 1586 RA patients, medical files were studied on occurrence of sustained DMARD-free remission until April 2017. This outcome was defined as absence of synovitis (by physical examination) after cessation of all DMARD therapy (including biologics and systemic and intra-articular corticosteroids) for at least 1 year *and* for the remainder of follow-up. Patients who experienced a flare of clinical synovitis early or late after DMARD cessation were considered as not in sustained DMARD-free remission. The date of sustained DMARD-free remission was the date 1 year after DMARD cessation. Patients who did not achieve remission were censored at the date when medical files were explored or at an earlier date when they were lost to follow-up or had died. Ninety-five of 941 ACPA-positive and/or RF-positive RA patients achieved sustained DMARD-free remission after a median follow-up of 4.8 years (figure 1). After achievement of sustained DMARD-free remission, patients were additionally followed for median 4.2 years. Except for 1 patient, all patients were included from 1999 onwards.

In addition, 21 autoantibody-positive RA patients who experienced a late flare were studied. These patients had absence of clinical synovitis for ≥1 year after DMARD cessation, thus were initially in DMARD-free remission. However, this remission was not sustained since these patients had recurrence of synovitis and needed to restart DMARDs during the remainder of follow-up. The median duration of being in DMARD-free remission before a flare occurred was 2.2 years. As control, 45 autoantibody-positive RA patients who were unable to stop DMARD therapy because of persistent swollen joints during follow-up, were evaluated. These patients were selected from the group of autoantibody-positive RA patients who never achieved DMARD-free remission based on comparable inclusion period and on available serum samples at baseline, 1 year and at 7–8 years follow-up or earlier in case patients had a shorter follow-up duration.

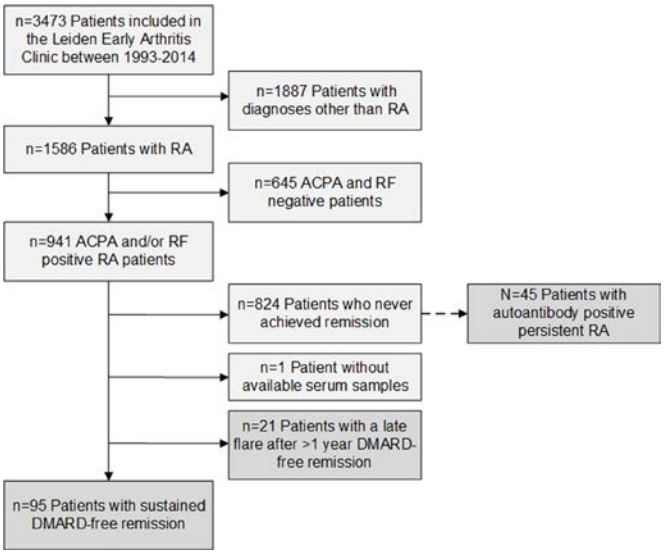

Figure 1 Flowchart of patient selection. DMARD-free remission was defined as the absence of clinical synovitis for ≥1 year after DMARD cessation. Flares were defined as recurrence of synovitis after having achieved DMARD-free remission, thus recurrence of synovitis >1 year after DMARD cessation. Sustained DMARD-free remission was defined as absence of synovitis after DMARD cessation during the complete follow-up, but at least for 1 year. As control, 45 autoantibody-positive patients with persistent RA were selected from the group of autoantibody-positive RA patients who never achieved DMARD-free remission based on comparable inclusion period as patients who achieved sustained remission and based on available serum samples. ACPA,anti-citrullinated protein antibodies; DMARD, disease-modifying antirheumatic drug; RA, rheumatoid arthritis; RF, rheumatoid factor.

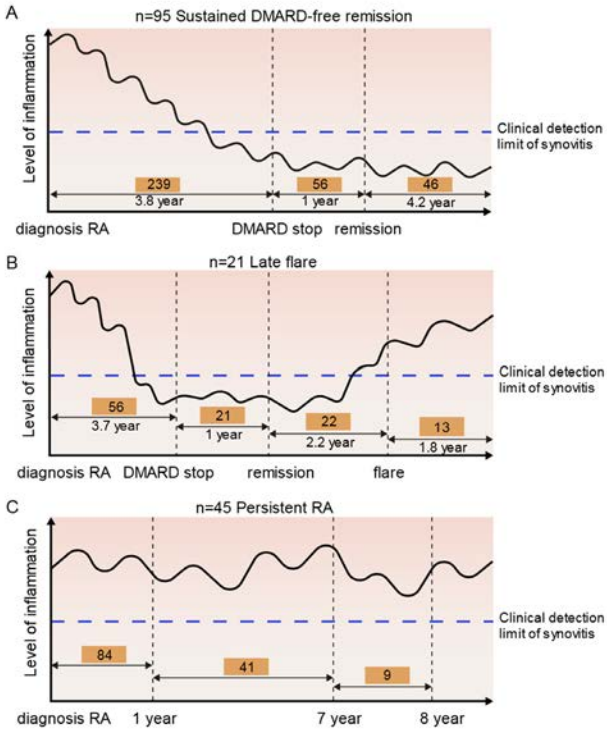

Figure 2 Overview of samples measured during follow-up of patients who achieved sustained DMARD-free remission (A), patients with a late flare (B) and patients with persistent RA (C). Numbers indicate the number of samples measured within the indicated time periods. In total, 587 samples were measured. Of patients who achieved sustained DMARD-free remission, samples were obtained at diagnosis, before and at or after achieving remission. DMARD,disease-modifying antirheumatic drug; RA, rheumatoid arthritis.

Median total follow-up of the studied patients was 10 years and was comparable between the studied groups.

Serological measurements

Anti-cyclic citrullinated peptide 2 (anti-CCP2) IgG and RF IgM were measured in 587 serum samples obtained at diagnosis, before and after achieving DMARD-free remission, using enzyme-linked immunosorbent assays as described previously.[22 23] In all cases, <5% of controls were autoantibody positive with the cut-offs used. In short, for IgM-RF, human IgG1 was used as the capture antigen and bound antibodies were detected with F(ab')2 fragments of peroxidase-conjugated antihuman IgM. The cut-off for positivity was 8 IU/mL. For anti-CCP2 IgG, the anti-CCP2 test (Immunoscan RA Mark 2; Euro-Diagnostica, Arnhem, The Netherlands) was used with a cut-off value of 25 units/mL, as described in the manufacturer's instructions. An overview of the measured samples during follow-up for the different groups is depicted in figure 2. Of each patient, a median of three samples was measured. In addition, anti-CCP2 IgM was measured in the first and last available serum sample of patients who were positive for anti-CCP2 IgG at disease presentation. In brief, microtiter plates were coated with citrullinated CCP2. An arginine control was used as control for citrulline specificity of the anti-CCP antibodies. Plates were incubated for 1 hour at 37°C with serum samples, 50 µL/well, at a dilution of 1:50. To detect anti-CCP2 IgM, plates were incubated for 1 hour at 37°C, 50 µL/well, with peroxidase-conjugated anti-human IgM. Pooled serum samples of highly positive patients were used in all plates to generate standard curves. Autoantibody levels were estimated by interpolation from these standard curves and were expressed in arbitrary units per millilitre. Samples were considered positive when the signal was higher than the mean +2 SD of serum samples of 64 healthy control subjects in total. This resulted in a cut-off value of 12 AU/mL. In addition, to ascertain that the obtained signal within the anti-CCP2 IgM ELISA was citrulline-specific, the difference between the signal against the citrullinated peptide and the unmodified arginine peptide had to be >0.1 (OD >0.1)

Statistical analyses

The difference between the first and last available serum sample was used to calculate the proportion of patients with seroreversion. This was performed separately for patients positive for anti-CCP2 IgG, anti-CCP2 IgM and RF IgM. Differences in seroreversion between the three patient groups were compared with the Fisher exact test. To test whether changes in ACPA and/or RF levels during follow-up were associated with achievement of sustained DMARD-free remission, Cox proportional hazards regression analyses were performed with time till achievement of remission as outcome. For these analyses, patients with a late flare were combined with patients with persistent RA as one group. Changes in anti-CCP2 IgG and RF IgM level per year were estimated with linear regression analyses for each patient individually. These changes in levels over time were used as predictor in Cox proportional hazards regression analyses. Antibody levels below the detection limit were imputed with a value of 0.

Table 2 Baseline characteristics of RA patients who achieved sustained DMARD-free remission, who flared after being in DMARD-free remission and of patients with persistent RA

	Sustained DMARD-free remission (n=95)	Late Flare after ≥1 year of DMARD-free remission (n=21)	Persistent RA (n=45)	P value
Age in years, mean (SD)	54 (17)	52 (13)	55 (12)	0.63
Female, n (%)	65 (68)	13 (62)	30 (67)	0.85
Symptom duration in weeks, median (IQR)	17 (10–35)	20 (8–37)	20 (13–38)	0.56
66-SJC, median (IQR)	5 (3–9)	5 (2–13)	5 (3–8)	0.81
68-TJC, median (IQR)	8 (3–13)	12 (4–15)	5 (4–11)	0.32
Autoantibody status				0.047
Anti-CCP2 IgG+ RF IgM-, n(%)	9 (9)	0 (0)	4 (9)	
Anti-CCP2 IgG- RF IgM+, n(%)	41 (43)	6 (29)	10 (22)	
Anti-CCP2 IgG+ RF IgM+, n(%)	45 (47)	15 (71)	31 (69)	
CRP (mg/L), median (IQR)	11 (3–28)	11 (4–23)	14 (5–40)	0.39
ESR (mm/hour), median (IQR)	26 (14–49)	29 (13–44)	29 (20–41)	0.75

Patients with persistent RA were selected from the group of autoantibody RA patients who never achieved DMARD-free remission based on comparable inclusion period as patients who achieved sustained remission and based on available serum samples. Symptom duration, time between symptom onset and inclusion in cohort.
CRP, C reactive protein; DMARD, disease-modifying antirheumatic drug; RA, rheumatoid arthritis; RF, rheumatoid factor; SJC, 66-swollen joint count; TJC, 68-tender joint count; anti-CCP2, anti-cyclic citrullinated peptide 2.

Several subanalyses were performed to study whether results on seroreverion could be ascribed to variation around the cut-off level, whether results were different when groups were stratified for autoantibody combinations, whether results were different in patients who were treated early, or whether results were dependent on the follow-up duration. Finally, for RF a second cut-off for RF positivity was used. This was done as the cut-off that is used in clinical practice has a specificity of 95% when compared with healthy controls, but a reduced specificity when patients with other arthritides were used as reference.[24] A cut-off of 33 allowed a specificity of 98% relative to patients with other early arthritides in our cohort (data not shown) which was then equal to the specificity of ACPA.

SPSS V.23.0 was used and p values <0.05 were considered significant.

RESULTS

Patient characteristics

Baseline characteristics of the studied autoantibody-positive patients are presented in table 2 and are similar between the different groups, with the exception that patients who achieved sustained DMARD-free remission were less frequently ACPA positive.

Anti-CCP2 IgG and RF IgM seroreversion were not associated with achievement of sustained DMARD-free remission

First, anti-CCP2 IgG levels were serially measured in the three different patient groups (figure 3A). Of anti-CCP2 IgG-positive RA patients who achieved sustained DMARD-free remission, 13% had reverted to anti-CCP2 IgG negativity around the time of remission (figure 4A). However, for RA patients with a late flare or with persistent disease, seroreversion was observed in 8% and 6%, respectively, which was not significantly different from patients who achieved sustained DMARD-free remission (p=0.63).

Patients with seroreversion had lower median anti-CCP2 IgG levels at disease presentation than patients without seroreversion (42 and 420 AU/mL, respectively, p<0.001). Ever use of biological DMARDs was comparable in patients with and without seroreversion (33% and 32%, respectively, p=1.00). To further analyse the effect of differences in medication use between patients, analyses were stratified for medication ever used during follow-up. This revealed similar results as in the whole group of patients (online supplementary table 1).

Similar results were observed for RF IgM (figures 3B and 4B). RF-positive patients who achieved sustained DMARD-free remission had seroreversion in 20%, whereas this occurred in 6% and 15% of patients with a late flare and with persistent disease, respectively (p=0.44, figure 4B). RF IgM levels were lower in patients with seroreversion than in patients who remained positive for RF IgM (19 and 53 IU/mL, respectively, p=0.003). Thus, ACPA or RF seropositive RA patients who achieved sustained DMARD-free remission did not become more frequently seronegative than patients who did not achieve remission.

Changes in RF IgM levels were larger in patients with sustained DMARD-free remission than in patients with persistent RA

Next, it was evaluated whether changes in autoantibody levels during the total follow-up period differed between patients. The change in anti-CCP2 IgG level per year was not associated with achievement of sustained DMARD-free remission (p=0.66). For RF IgM-positive patients, the change in RF level was associated with achievement of sustained DMARD-free remission; for every 10-unit decrease in RF IgM level per year the rate of sustained DMARD-free remission increased by 16% (p<0.001). Thus, seropositive RA patients who achieved sustained DMARD-free remission did not have disappearance of autoantibodies; however, there was a significant decrease of RF IgM levels in patients who achieved sustained DMARD-free remission compared with patients who did not.

Anti-CCP2 IgM seroreversion was not associated with sustained DMARD-free remission

Finally, the proportion of patients seroreverting from anti-CCP2 IgM positive to negative was studied as we hypothesised that if the ACPA immune response had changed in patients who achieved remission, this could be reflected by a decreased presence of anti-CCP2 IgM, since IgM is an indication of an ongoing immune response. Of anti-CCP2 IgG-positive patients, 25%–29% were also positive for anti-CCP2 IgM at disease presentation within the different groups . During follow-up, 31% (4/13) of the anti-CCP2 IgG and IgM-positive patients

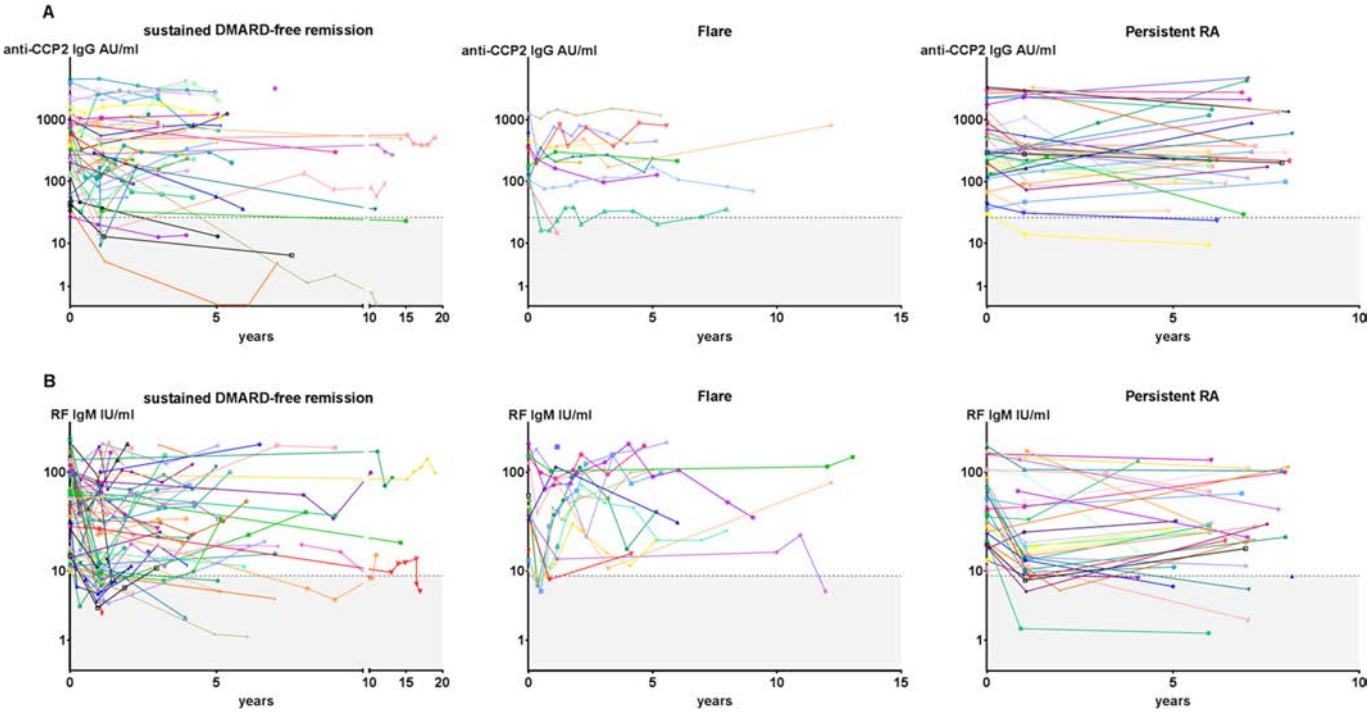

Figure 3 Anti-CCP2 IgG levels in ACPA-positive RA patients (A) and RF IgM levels in RF-positive patients (B) during follow-up, stratified for clinical outcome. Dotted lines indicate the cut-off values (25 AU/mL for anti-CCP2 IgG and 8 IU/mL for RF IgM). Values below the detection limit were imputed with the value of 0. Number of ACPA and RF-positive patients in each group: DMARD-free sustained remission: ACPA+ n=54, RF+ n=86, flare: ACPA+ n=15, RF+ n=21, persistent RA: ACPA+ n=35, RF+ n=41. ACPA, anti-citrullinated protein antibodies; anti-CCP2, anti-cyclic citrullinated peptide 2; DMARD, disease-modifying antirheumatic drug; RA, rheumatoid arthritis; RF, rheumatoid factor.

who achieved DMARD-free remission seroreverted from positive to negative anti-CCP2 IgM (figure 5). For patients with a late flare and patients with persistent RA, this occurred in 100% (3/3) and 60% (6/10), respectively. Thus, patients who achieved sustained DMARD-free remission and who were seropositive for anti-CCP2 IgM at disease presentation did not serorevert more frequently than patients who did not achieve remission.

Sensitivity analyses

To investigate whether our results were driven by patients with autoantibody levels fluctuating around the cut-off, analyses were performed in patients with baseline autoantibody levels above the median which showed that none of the anti-CCP2 IgG-positive patients had seroreverted. For RF IgM, 8% of patients who achieved sustained DMARD-free remission, none of the patients with a late flare and 10% of patients with persistent disease had seroreverted.

To study the hypothesis that early treatment might be associated with higher rates of seroreversion, analyses were repeated in patients with short symptom duration before treatment start (<12 weeks) and with long symptom duration (≥12 weeks). Of anti-CCP2 IgG-positive patients who achieved remission, 10% of the patients with a short symptom duration had reverted to seronegativity; this was 15% of patients with a long symptom duration (p=1.00). For RF IgM, these percentages were 25% and 18%, respectively (p=0.54). Thus, seroreversion rates were not higher in patients with earlier treatment initiation.

Next, it was assessed whether single autoantibody positivity was associated with higher seroreversion rates. Of ACPA-positive patients, 7% of ACPA+ RF+ and 23% of ACPA+ RF- patients had reverted from anti-CCP2 IgG positivity to negativity (p=0.12). Of RF-positive patients, 6% of ACPA+ RF+ and 34% of ACPA- RF+ patients had reverted to RF IgM negativity (p<0.001). In addition, analyses were stratified for different autoantibody combinations, since patients with sustained DMARD-free remission were less frequently positive for both ACPA and RF than the other studied groups. Similar to our main analysis, seroreversion rates for ACPA and RF were not significantly different between patients with and without sustained remission (online supplementary table 2). Thus, single RF-positive patients seroreverted more often (when cut-off of 8 was used) than ACPA+ RF+ patients, but the frequency of seroreversion was not higher in the group that achieved sustained remission group compared with the group that did not.

Analyses were also repeated in patients who had a follow-up duration of ≥4.2 years after achievement of sustained DMARD-free remission (ie, within 50% of patients with the longest follow-up after DMARD cessation). This analysis was performed to verify if patients with shorter follow-up after DMARD cessation influenced the results, as these patients could be at risk of developing a late flare. Of anti-CCP2 IgG-positive RA patients who achieved sustained DMARD-free remission, 6% had reverted to anti-CCP2 negativity around the time of remission, which was not different from patients with a late flare and with persistent disease, who had seroreversion in 8% and 6%, respectively (p=1.00). Of RF IgM-positive RA patients who achieved sustained DMARD-free remission, 16% had reverted to RF IgM negativity. Of patients with a late flare and with persistent disease this occurred in 6% and 15%, respectively (p=0.78).

Finally, seroreversion for RF was also studied when the cut-off for positivity was set at 33. Also then no differences in seroreversion rates were observed between the three different groups (p=0.86, figure 4C). When the different autoantibody combinations were studied with this cut-off, 6% of ACPA+ RF+ and 16% of ACPA+ RF- patients had reverted to anti-CCP2 negativity (p=0.27). Of RF-positive patients, 37% of ACPA+ RF+

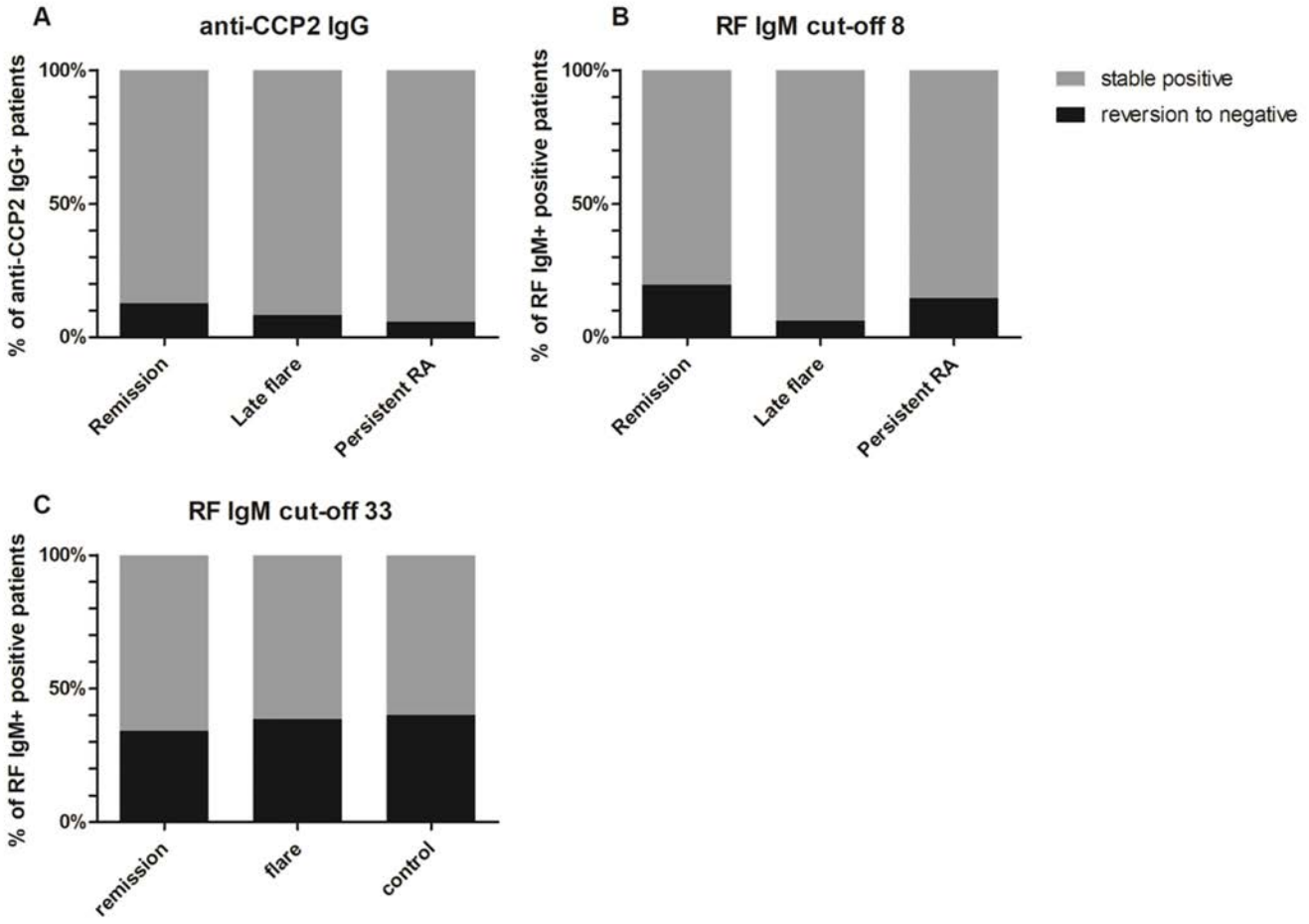

Figure 4 Reversion to anti-CCP2 IgG (A) and RF IgM (B, C) seronegativity in autoantibody-positive RA patients who achieved sustained DMARD-free remission, who had a late flare and in patients with persistent RA. Analyses were performed in patients positive for anti-CCP2 IgG (A) or positive for RF IgM (B, C). For the analyses presented in (A) and (B) the cut-offs used were determined by the manufacturer and are similar to those used in clinical practice. For RF, also a cut-off of 33 was used to define positivity. This cut-off resulted in a specificity of 98% relative to patients with other early arthritides and was then comparable to the ACPA test (C). Seroreversion was defined as shifting from seropositive at baseline to seronegative in the last available serum sample; for patients who achieved sustained DMARD-free remission the last sample was measured at the time of remission. Number of patients in each group: sustained DMARD-free remission: ACPA+ n=47, RF+ cut-off 8 n=71, RF+ cut-off 33 n=44, late flare: ACPA+ n=12, RF+ cut-off 8 n=16, RF+ cut-off 33 n=13, persistent RA: ACPA+ n=35, RF+ cut-off 8 n=41, RF+ cut-off 33 n=25. ACPA, anti-citrullinated protein antibodies; anti-CCP2, anti-cyclic citrullinated peptide 2; DMARD, disease-modifying antirheumatic drug; RA, rheumatoid arthritis; RF, rheumatoid factor.

and 35% of ACPA- RF+ patients had reverted to RF IgM negativity (p=1.00). Thus, also when a different cut-off for RF positivity was used, patients who achieved sustained DMARD-free remission did not serorevert more often than patients with a late flare or with persistent disease.

DISCUSSION

Currently, a sustained DMARD-free status is the best possible clinical outcome as, per definition, clinically apparent synovitis is persistently absent and patients in this status also have resolution of symptoms and normalised functional status.[1] This outcome is achievable in autoantibody-positive RA, although with a lower frequency than in autoantibody-negative RA. The biological nature underlying this type of persistent remission is unknown. It was recently suggested that it is characterised by disappearance of autoantibodies.[14] The present large observational study with a unique, long follow-up period and with samples measured at the time of remission, explored this hypothesis. No association between remission and reversion to autoantibody negativity was demonstrated. Hence, although it has been suggested that immunological remission is characterised by disappearance of autoantibodies,[14] we studied patients in the deepest form of clinical remission and observed no increased frequency of reversion to seronegativity in this group.

To our knowledge, this is the first study in which seroreversion rates in patients with long-standing DMARD-free status were investigated. Importantly, for both ACPA and RF seroreversion was infrequent and not related to clinical outcome. In previous studies in patients with established RA who were treated with DMARDs and thus had persistent disease, similar seroreversion rates were observed.[18 25–27] Thus, seroreversion rates observed here are in line with previous findings in RA and are not increased in patients with sustained DMARD-free status.

When analyses were repeated in patients with high autoantibody levels (above the median) at baseline, seroreversion for anti-CCP2 IgG was not observed anymore, and seroreversion for RF IgM was less frequent than in the whole group of autoantibody-positive patients. This suggests that the observed

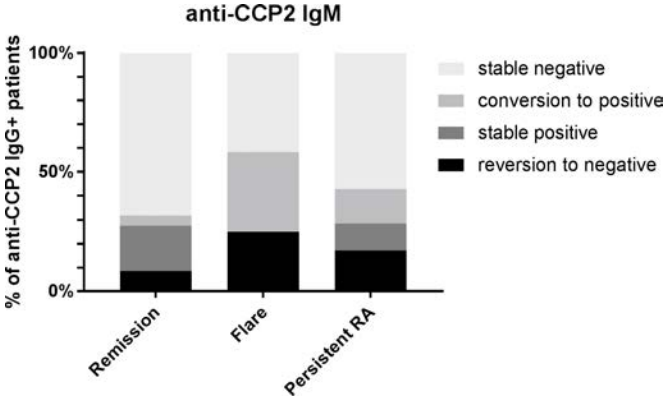

Figure 5 Change in anti-CCP2 IgM status during follow-up in patients who achieved DMARD-free sustained remission, who had a flare and in patients with persistent RA. Data are shown for the subgroup of anti-CCP2 IgG+ patients at baseline. Number of patients in each group: DMARD-free sustained remission: n=47, flare: n=12, persistent RA: n=35. Anti-CCP2, anti-cyclic citrullinated peptide 2; DMARD, disease-modifying antirheumatic drug; RA, rheumatoid arthritis.

seroreversion rates were mainly the result of patients who fluctuated around the cut-off level, and therefore that true seroreversion of ACPA and RF is only sporadically observed.

Although no association between remission and seroreversion was observed, patients who achieved a DMARD-free status had a larger decrease in RF levels during follow-up than patients who did not achieve this outcome. The slopes of the levels along follow-up are relevant to appreciate the immunological evolution, which was different for RF and ACPA. Several studies have shown that improvement in disease activity is accompanied by decrease in RF levels,[27–31] although some other studies did not observe this.[18 19 32] A unique feature of this study is that a prolonged period of absence of clinical synovitis is observed, which is a much more stringent outcome than improvement in disease activity scores. Previous studies showed no relation between ACPA levels and disease activity and our data also demonstrated no relation with sustained DMARD-free status.[27–31]

Previously, it was shown that anti-CCP2 IgM remains present in RA patients during treatment in a persistent disease phase, suggesting that the anti-CCP immune response is continuously reactivated.[33] Interestingly, we have shown here that anti-CCP2 IgM also remained persistently present in patients who achieved sustained DMARD-free remission. This suggests that even in patients who are clinically cured, the anti-CCP response is persistently activated. ACPA characteristics other than level and IgM and IgG isotypes were not investigated. However, it is known that ACPA-level is highly associated with ACPA fine specificity and the number of ACPA isotypes.[34] Based on this, it can be presumed that these characteristics were also not different between patients who did or did not achieve a sustained DMARD-free status. Nonetheless, we do not rule out that these, or other characteristics of the autoantibody response in RA correlate with the induction of sustained DMARD-free remission. Altogether, disappearance of clinical disease is not accompanied by changes in the humoral ACPA response in serum. Thus, whereas for RA development it is not yet elucidated whether ACPA play a role in the pathophysiology or act as bystander, the present data suggest that the ACPA response does not explain the maintained resolution of clinical disease. Whether other immunological markers, for instance, changes in characteristics of autoantibody expressing B-cells, associate with this phenotypic outcome, and therefore would be a better definition of immunological remission, is subject of further research.

A strength of this study is that patients had a long follow-up period also after DMARD cessation (median 4.2 years after achievement of sustained DMARD-free remission, thus median 5.2 years after DMARD stop). This follow-up time supports the validity of the outcome, as patients with an early flare after DMARD stop were never considered to be in remission, and also patients with a late flare were identified and excluded from the group of patients who achieved sustained DMARD-free remission. Late flares generally occurred 2.2 years after DMARD cessation and the large majority of patients were followed for a longer period of time after DMARD stop. A subanalysis in the patients who were followed for >4 years after achieving remission showed similar results, showing the robustness of the data in this respect. Of course, we do not know if the autoantibody-positive patients will get a flare of disease after an even longer follow-up period; this is subject of future studies. Some of the patients have been discharged from the outpatient clinic because of prolonged absence of synovitis and symptoms. Importantly, it is plausible that if symptoms will recur patients will return to our clinic since the Leiden University Medical Center is the only referral centre in a healthcare region of ~400 000 inhabitants and has very easy access services, allowing that patients with symptoms suspicious of RA are seen within 1 week.[35]

Treatment was not protocolised and the applied treatments changed over time. This resulted in heterogeneity of treatments received by patients. Nonetheless, when stratifying patients in groups according to medications ever used during follow-up, results remained similar.

Another limitation might be that not of all patients with sustained DMARD-free remission serum samples were available after medication was stopped. However, when analyses were repeated in this subgroup of patients, similar results were obtained (data not shown).

Previously, it was suggested that remission can be defined according to different conditions and presence of immunological remission, defined as the disappearance of autoantibodies, was suggested to be the deepest form of remission. In this long-term study, we were able to analyse a large number of ACPA-positive patients who achieved sustained DMARD-free remission. In this unique dataset, we observed that disappearance of autoantibodies rarely occurred, and that patients who achieved the best possible outcome of RA did not become more often seronegative than patients with persistent disease. Therefore, in our view, this definition of immunological remission should not be a long-term treatment target.

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Contributors DMB, LEB, RT and AvdH-vM contributed to the conception and study design. DMB analysed the data. DMB, RT and AvdH-vM contributed to interpretation of the data. DMB and AvdH-vM wrote the first version of the manuscript and LEB and RT revised it critically. DMB, LEB, RT and AvdH-vM read and approved the final manuscript.

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REFERENCES

1 Ajeganova S, van Steenbergen HW, van Nies JAB, *et al*. Disease-Modifying antirheumatic drug-free sustained remission in rheumatoid arthritis: an increasingly achievable outcome with subsidence of disease symptoms. *Ann Rheum Dis* 2016;75:867–73.
2 van der Woude D, Young A, Jayakumar K, *et al*. Prevalence of and predictive factors for sustained disease-modifying antirheumatic drug-free remission in rheumatoid arthritis: results from two large early arthritis cohorts. *Arthritis Rheum* 2009;60:2262–71.
3 de Rooy DPC, van der Linden MPM, Knevel R, *et al*. Predicting arthritis outcomes--what can be learned from the Leiden early arthritis clinic? *Rheumatology* 2011;50:93–100.
4 van der Kooij SM, Goekoop-Ruiterman YPM, de Vries-Bouwstra JK, *et al*. Drug-Free remission, functioning and radiographic damage after 4 years of response-driven treatment in patients with recent-onset rheumatoid arthritis. *Ann Rheum Dis* 2009;68:914–21.
5 Haschka J, Englbrecht M, Hueber AJ, *et al*. Relapse rates in patients with rheumatoid arthritis in stable remission tapering or stopping antirheumatic therapy: interim results from the prospective randomised controlled retro study. *Ann Rheum Dis* 2016;75:45–51.
6 Rantapää-Dahlqvist S, de Jong BAW, Berglin E, *et al*. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis & Rheumatism* 2003;48:2741–9.
7 Nielen MMJ, van Schaardenburg D, Reesink HW, *et al*. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. *Arthritis & Rheumatism* 2004;50:380–6.
8 van der Woude D, Rantapää-Dahlqvist S, Ioan-Facsinay A, *et al*. Epitope spreading of the anti-citrullinated protein antibody response occurs before disease onset and is associated with the disease course of early arthritis. *Ann Rheum Dis* 2010;69:1554–61.
9 van de Stadt LA, de Koning MHMT, van de Stadt RJ, *et al*. Development of the anti-citrullinated protein antibody repertoire prior to the onset of rheumatoid arthritis. *Arthritis & Rheumatism* 2011;63:3226–33.
10 Sokolove J, Bromberg R, Deane KD, *et al*. Correction: autoantibody epitope spreading in the pre-clinical phase predicts progression to rheumatoid arthritis. *PLoS One* 2012;7.
11 Suwannalai P, van de Stadt LA, Radner H, *et al*. Avidity maturation of anti-citrullinated protein antibodies in rheumatoid arthritis. *Arthritis & Rheumatism* 2012;64:1323–8.
12 Rombouts Y, Ewing E, van de Stadt LA, *et al*. Anti-citrullinated protein antibodies acquire a pro-inflammatory Fc glycosylation phenotype prior to the onset of rheumatoid arthritis. *Ann Rheum Dis* 2015;74:234–41.
13 Willemze A, Trouw LA, Toes REM, *et al*. The influence of AcpA status and characteristics on the course of RA. *Nat Rev Rheumatol* 2012;8:144–52.
14 Schett G, Emery P, Tanaka Y, *et al*. Tapering biologic and conventional DMARD therapy in rheumatoid arthritis: current evidence and future directions. *Ann Rheum Dis* 2016;75:1428–37.
15 Ursum J, Bos WH, van Dillen N, *et al*. Levels of anti-citrullinated protein antibodies and IgM rheumatoid factor are not associated with outcome in early arthritis patients: a cohort study. *Arthritis Res Ther* 2010;12.
16 Burr ML, Viatte S, Bukhari M, *et al*. Long-Term stability of anti-cyclic citrullinated peptide antibody status in patients with early inflammatory polyarthritis. *Arthritis Res Ther* 2012;14.
17 Barra L, Bykerk V, Pope JE, *et al*. Anticitrullinated protein antibodies and rheumatoid factor fluctuate in early inflammatory arthritis and do not predict clinical outcomes. *J Rheumatol* 2013;40:1259–67.
18 Rönnelid J, Wick MC, Lampa J, *et al*. Longitudinal analysis of citrullinated protein/peptide antibodies (anti-CP) during 5 year follow up in early rheumatoid arthritis: anti-CP status predicts worse disease activity and greater radiological progression. *Ann Rheum Dis* 2005;64:1744–9.
19 de Moel EC, Derksen V, Trouw LA, *et al*. In RA, becoming seronegative over the first year of treatment does not translate to better chances of drug-free remission. *Ann Rheum Dis* 2019;77:1836–8.
20 Arnett FC, Edworthy SM, Bloch DA, *et al*. The American rheumatism association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis & Rheumatism* 1988;31:315–24.
21 Aletaha D, Neogi T, Silman AJ, *et al*. Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League against rheumatism collaborative initiative. *Ann Rheum Dis* 2010;2010:1580–8.
22 Verpoort KN, Cheung K, Ioan-Facsinay A, *et al*. Fine specificity of the anti-citrullinated protein antibody response is influenced by the shared epitope alleles. *Arthritis Rheum* 2007;56:3949–52.
23 Ioan-Facsinay A, Willemze A, Robinson DB, *et al*. Marked differences in fine specificity and isotype usage of the anti-citrullinated protein antibody in health and disease. *Arthritis Rheum* 2008;58:3000–8.
24 De Rycke L, Peene I, Hoffman IEA, *et al*. Rheumatoid factor and anticitrullinated protein antibodies in rheumatoid arthritis: diagnostic value, associations with radiological progression rate, and extra-articular manifestations. *Ann Rheum Dis* 2004;63:1587–93.
25 Ursum J, Bos WH, van de Stadt RJ, *et al*. Different properties of AcpA and IgM-RF derived from a large dataset: further evidence of two distinct autoantibody systems. *Arthritis Res Ther* 2009;11.
26 Kastbom A, Strandberg G, Lindroos A, *et al*. Anti-CCP antibody test predicts the disease course during 3 years in early rheumatoid arthritis (the Swedish TIRA project). *Ann Rheum Dis* 2004;63:1085–9.
27 Bos WH, Bartelds GM, Wolbink GJ, *et al*. Differential response of the rheumatoid factor and anticitrullinated protein antibodies during adalimumab treatment in patients with rheumatoid arthritis. *J Rheumatol* 2008;35:1972–7.
28 Bobbio-Pallavicini F, Alpini C, Caporali R, *et al*. Autoantibody profile in rheumatoid arthritis during long-term infliximab treatment. *Arthritis Res Ther* 2004;6:R264–72.
29 Alessandri C, Bombardieri M, Papa N, *et al*. Decrease of anti-cyclic citrullinated peptide antibodies and rheumatoid factor following anti-TNFalpha therapy (infliximab) in rheumatoid arthritis is associated with clinical improvement. *Ann Rheum Dis* 2004;63:1218–21.
30 Bobbio-Pallavicini F, Caporali R, Alpini C, *et al*. High IgA rheumatoid factor levels are associated with poor clinical response to tumour necrosis factor alpha inhibitors in rheumatoid arthritis. *Ann Rheum Dis* 2007;66:302–7.
31 Böhler C, Radner H, Smolen JS, *et al*. Serological changes in the course of traditional and biological disease modifying therapy of rheumatoid arthritis. *Ann Rheum Dis* 2013;72:241–4.
32 Vis M, Bos WH, Wolbink G, *et al*. Igm-Rheumatoid factor, anti-cyclic citrullinated peptide, and anti-citrullinated human fibrinogen antibodies decrease during treatment with the tumor necrosis factor blocker infliximab in patients with rheumatoid arthritis. *J Rheumatol* 2008;35:425–8.
33 Verpoort KN, Jol-van der Zijde CM, Papendrecht-van der Voort EAM, *et al*. Isotype distribution of anti-cyclic citrullinated peptide antibodies in undifferentiated arthritis and rheumatoid arthritis reflects an ongoing immune response. *Arthritis Rheum* 2006;54:3799–808.
34 Toes REM, Huizinga TJW. Update on autoantibodies to modified proteins. *Curr Opin Rheumatol* 2015;27:262–7.
35 van Nies JAB, Brouwer E, van Gaalen FA, *et al*. Improved early identification of arthritis: evaluating the efficacy of Early Arthritis *Recognition* Clinics. *Ann Rheum Dis* 2013;72:1295–301.

TRANSLATIONAL SCIENCE

Inflammatory cytokines shape a changing DNA methylome in monocytes mirroring disease activity in rheumatoid arthritis

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ABSTRACT

Objective Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease that mainly targets joints. Monocytes and macrophages are critical in RA pathogenesis and contribute to inflammatory lesions. These extremely plastic cells respond to extracellular signals which cause epigenomic changes that define their pathogenic phenotype. Here, we interrogated how DNA methylation alterations in RA monocytes are determined by extracellular signals.

Methods High-throughput DNA methylation analyses of patients with RA and controls and in vitro cytokine stimulation were used to investigate the underlying mechanisms behind DNA methylation alterations in RA as well as their relationship with clinical parameters, including RA disease activity.

Results The DNA methylomes of peripheral blood monocytes displayed significant changes and increased variability in patients with RA with respect to healthy controls. Changes in the monocyte methylome correlate with DAS28, in which high-activity patients are divergent from healthy controls in contrast to remission patients whose methylome is virtually identical to healthy controls. Indeed, the notion of a changing monocyte methylome is supported after comparing the profiles of same individuals at different stages of activity. We show how these changes are mediated by an increase in disease activity-associated cytokines, such as tumour necrosis factor alpha and interferons, as they recapitulate the DNA methylation changes observed in patients in vitro.

Conclusion We demonstrate a direct link between RA disease activity and the monocyte methylome through the action of inflammation-associated cytokines. Finally, we have obtained a DNA methylation-based mathematical formula that predicts inflammation-mediated disease activity for RA and other chronic immune-mediated inflammatory diseases.

Key messages

What is already known about this subject?

- Monocytes/macrophages are a crucial cell population involved in rheumatoid arthritis (RA) pathogenesis and inflammatory lesions.
- Patients with RA display DNA methylation alterations in both immune cells and synoviocytes.

What does this study add?

- We demonstrate for the first time that the methylome of monocytes from peripheral blood acts as an epigenetic sensor of the inflammatory milieu in patients with RA.
- Our results show that the DNA methylome of monocytes changes in a dynamic fashion according to the DAS28 in patients with RA.

How might this impact on clinical practice or future developments?

- We have obtained a mathematical formula that can predict inflammation-mediated disease activity for RA and could be potentially used for other chronic autoimmune diseases with undefined activity scores.

INTRODUCTION

Monocytes and macrophages are essential players in the pathology of a variety of inflammatory diseases, such as rheumatoid arthritis (RA). In RA, these cells are major contributors to the damage observed in joint synovial tissues, although their actions can also be extended to the peripheral blood, where they spread inflammation at a systemic level. Intense research in the past few years has emphasised the role of environmental factors in the functional specialisation of monocytes and macrophages. This occurs through a wide range of cytokine receptors which activate downstream signalling cascades that ultimately modulate the activity and recruitment of transcription factors, orchestrating both epigenome and transcriptome remodelling. Monocyte plasticity is achieved by a highly responsive epigenome, whose dynamic has not been fully characterised yet. A major example of the epigenomic plasticity of monocytes/macrophages is represented by the occurrence of relevant DNA methylation changes. Different studies have revealed the relevance of the de novo DNA methyltransferase 3A (DNMT3A) and the methylcytosine dioxygenase Ten-eleven translocation (TET) 2 in monocytes due to their high expression levels and owing to their essential

role in determining functionality, including differentiation and activation during inflammatory responses.[1–3] In this regard, DNA methylation stands out as a major epigenetic mechanism, which potentially could reflect the influence of disease-associated inflammation in monocytes.

One of the main features of RA is the infiltration of the synovial membrane by mononuclear cells, including monocytes, which become activated by interacting with the synovial microenvironment. In the synovium, monocytes differentiate into macrophages, which are major drivers of RA-associated inflammation. These cells secrete proinflammatory cytokines, growth factors and metalloproteases, leading to joint inflammation. In fact, their presence in the synovium correlates with disease activity and radiographic progression.[4] These cytokines are released into the synovial fluid and also reach the blood stream. Evidence linking systemic inflammation and local inflammation in the synovium is reinforced by the observation of alterations occurring simultaneously in both joints and peripheral blood of patients with RA.[5 6]

During the course of the disease, patients with RA undergo periods of high disease activity followed by remission after appropriate treatment. The Disease Activity Score of 28 joints (DAS28), developed to standardise and compare results in clinical trials of new drugs for treating RA, is the most reliable and widely accepted method to measure disease activity in RA.[7] The DAS28 is a clinical index of RA disease activity that combines information from swollen joints, tender joints, the acute phase response and general health, and it has become a routine clinical marker.[8] Despite the general link between DAS28 and overall inflammation, the biological significance of this score has not been fully clarified. The general consensus is that the DAS28 correlates with serum levels of inflammatory cytokines[9]; however, DAS28 does not allow prediction of the response to treatment nor define aberrant changes at the molecular level in patients with RA.

In this study, the analysis of DNA methylation of peripheral blood monocytes from patients with RA unveils the plasticity of their methylomes as an excellent reader of systemic inflammation and disease activity. These findings provide multiple possibilities for monocyte DNA methylation as a marker for RA and other inflammatory conditions.

METHODS

Patient involvement

Patients were involved to conduct this research. They were informed that blood samples would be taken during their scheduled visits to their doctors and would not involve additional intervention. Patients who meet the American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) criteria for [10]RA were selected by disease activity, namely high disease activity and remission, defined by DAS28. The clinical data of the patients included in the study are summarised in the online supplementary table 1. Samples were obtained from two hospitals: the main cohort was recruited in Hospital Clinic in Barcelona (n=33 different patients with RA, n=10 second visit samples) and the Centre for Experimental Medicine and Rheumatology, William Harvey Research Institute, Barts and The London School of Medicine and Dentistry in London (n=4). Blood samples from healthy controls (n=17) were matched in age and gender with both the remission and activity patient groups. Healthy controls were collected through the Catalan Blood and Tissue Bank which follows the principles of the World Medical Association Declaration of Helsinki. The Committee for Human Subjects of the two local hospitals approved the study, which was conducted in accordance with the ethical guidelines of the 1975 Declaration of Helsinki. All samples were in compliance with the guidelines approved by the local ethics committee, and all donors received oral and written information about the possibility that their blood would be used for research purposes.

Purification of human monocytes from patients and healthy controls

For monocyte isolation, peripheral blood mononuclear cells (PBMCs) from patients with RA and different DAS28 and healthy controls were isolated using Ficoll-Paque gradient centrifugation. Monocytic populations were sorted from mononuclear fraction using anti-CD33, anti-CD11b and anti-CD15 (CD33 +CD11b+CD15- monocytes) antibodies and anti-CD14 and anti-CD16 antibodies for the different monocyte subsets.

DNA methylation profiling

Infinium HumanMethylationEPIC BeadChips (Illumina) were used to analyse DNA methylation. DNA samples were bisulphite-converted using an EZ DNA methylation kit (Zymo Research, Orange, CA). After bisulphite treatment, the remaining assay steps were performed using the specifications and reagents supplied by the manufacturer. The array was hybridised using a temperature-gradient programme, and arrays were imaged using a BeadArray Reader (Illumina). Image processing and intensity data extraction software and procedures were as previously described. Each methylation data point was obtained from a combination of the Cy3 and Cy5 fluorescent intensities from the methylated and unmethylated alleles. Background intensity computed from a set of negative controls was subtracted from each data point. For representation and further analysis, we used beta and M values. The beta value was calculated as the ratio of the methylated probe intensity to the overall intensity (the sum of the methylated and unmethylated probe intensities). The M value was calculated as the $\log_2$ ratio of the intensities of the methylated and unmethylated probe. Beta values ranging from 0 to 1 were used for visual methylation representations as indicated. For statistical purposes, the use of M values is more appropriate since beta values have severe heteroskedasticity for highly methylated or unmethylated CpG sites.[11] The methylation data reported in this paper was deposited in the Gene Expression Omnibus (GEO) database (accession numbers GSE134429 and GSE134425).

Quality control, data normalisation and detection of differentially methylated and variable CpGs

Methylation array data were processed in the statistical language R using methods from the bioconductor libraries *minfi*, *lumi* and *limma*. Data quality was assessed using the standard pipeline from the minfi package.[12] Raw data were normalised by the Illumina method before the calculation of beta and M values. To exclude technical and biological bias, we removed CpGs from X and Y chromosomes and eliminated the probes containing either a single nucleotide polymorphism (SNP) at the CpG interrogation site or at the single nucleotide extension. For that purpose, we used the function *dropLociWithSnps* from minfi package that removes the corresponding SNP-containing probes. Additionally, batch effect correction was performed using *ComBat* function from sva package[13] with disease activity classification of the patients as a covariate.

For the comparison of healthy donors versus the entire RA cohort, we identified differentially methylated CpG sites using t-test and selecting CpGs with a false discovery rate (FDR) <0.05. In addition, we used the iEVORA package[14] to identify differentially variable positions (DVPs). This algorithm identifies

differences in variance using Bartlett's test (FDR<0.001), followed by the comparison of means using t-test (p<0.05) to regularise the variability test which is overly sensitive to single outliers.

We also performed Spearman's correlation, a non-parametric approach to measure the association of two variables to identify CpG sites in which DNA methylation correlated with DAS28 in patients with RA. We selected these CpG sites for which Spearman's correlation coefficient (rho) was higher than 0.5 and had a correlation p value <0.01. Additionally, we verified that monocyte age-related CpG sites[15] did not display any significant enrichment with our identified differentially methylated CpG sites.

For DAS28 prediction using multiple linear regression models, the validation cohort methylation array data were processed simultaneously with the discovery cohort data using the aforementioned bioconductor tools and packages, and batch effect correction was performed using *ComBat* function from sva package.

Identification of dynamic enhancers in LPS-treated human monocytes

We used public ChIP-seq data from the Blueprint Consortium (http://www.blueprint-epigenome.eu/) for different histone modifications (H3K4me1, H3K27ac and H3K27me3), generated from control monocytes and lipopolysaccharide (LPS)-exposed monocytes. These ChIP-seq data allowed us to define several enhancer categories such as poised enhancers, corresponding to regions enriched with H3K4me1 and H3K27me3, primed enhancers, enriched with H3K4me1 and active enhancers, enriched with H3K4me1 and H3K27ac. In addition, we also determined the dynamics of those enhancers when monocytes were exposed to the inflammatory challenge.

Bisulfite pyrosequencing

Bisulfite modification of genomic DNA isolated from monocytes was performed by standard methods. PCR primers (see online supplementary table 7) were designed with the PyroMark Assay Design V.2.0 software (QIAGEN). PCR products were pyrosequenced with the PyromarkTM Q24 system (QIAGEN), according to the manufacturer's protocol.

Cytokine quantification in serum samples

For the analysis of 13 different cytokines associated to inflammation (chemokine (C-C motif) ligand 2 (CCL2), interferon (IFN) α2, IFNγ, interleukin (IL)-1β, IL-6, IL-8, IL-10, IL-12, IL-17A, IL-18, IL-23, IL-33 and tumour necrosis factor alpha (TNFα)) from blood plasma samples of RA and healthy controls individuals, we used the Pre-defined Human Inflammation Panel LEGENDplex (BioLegend) following the manufacturer's instructions.

In vitro stimulation of monocytes with inflammatory cytokines

PBMCs were isolated using Ficoll-Paque gradient centrifugation. PBMCs were then cultured for 4 days in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 20% of heat-inactivated human serum. Cells were stimulated with human recombinant TNFα (10 ng/mL), IFNα (100 ng/mL) or IFNγ (100 ng/mL) from day 0. At day 4, cells were harvested and monocytes were isolated by flow cytometry using the same sorting strategy as RA peripheral blood samples. Finally, genomic DNA was isolated, bisulfite modified and hybrised on Infinium HumanMethylationEPIC BeadChips arrays.

Statistical analysis and multiple linear regression model generation

Cytokine quantification data were analysed with Prism V.6.0 (GraphPad). Statistical analyses were performed using the Mann-Whitney test, except when indicated. The levels of significance were indicated as follows: *p<0.05, **p<0.01, ***p<0.001. For the generation of multiple linear regression models, the normality of the independent variables (identified CpG sites with significant Spearman estimates) was verified using the Kolmogorov-Smirnov test. Subsequently, a stepwise multiple linear regression model was generated to identify the associated variables that explain the greater proportion of the variability of the dependent variable (DAS28), avoiding possible confounding factors. Coefficient estimation was performed with the least-squares method, and the independence of the residual values was verified by the Durbin-Watson test, selecting those models with values between 1.5 and 2.5. In addition, a diagnosis of the collinearity of the independent variables was performed. Statistical calculations were carried out using the statistical package SPSS V.24.

RESULTS

Peripheral blood monocytes from patients with RA display a wide range of changes in their DNA methylome

We sorted CD33 +CD11b+CD15- cells from peripheral blood samples of patients with RA and healthy individuals (figure 1A) for DNA methylation profiling. With this approach, we were able to isolate the entire monocytic population, including classical ($CD14^{high}CD16-$), non-classical ($CD14^{dim}CD16++$) and intermediate ($CD14^{high}CD16+$) monocyte subsets (see online supplementary figure 1A). The cohort of patients with RA (n=33) were well characterised for their gender, age, seropositivity for anticitrullinated peptide antibodies and their DAS28 (see online supplementary table 1). In parallel, we isolated peripheral blood monocytes from healthy controls (n=17) matching in age and gender. We tested the plasma of the patients with RA and healthy controls for a panel of 13 inflammatory cytokines/chemokines and observed significantly increased levels of TNFα, IL-1β, IFNα and MCP1 (p value <0.05) (figure 1B and online supplementary table 2), whereas other cytokines such as IL-10 and IL-6 had increased levels but did not reach statistical significance.

We then performed DNA methylation profiling of isolated monocytes from RA and controls. Using data from the *estimateCellCounts* tool from minfi package,[12] we confirmed that our purification protocol resulted in the isolation of *bona fide* monocytes, as they cluster together to those isolated using anti-CD14 antibody in a multidimensional scaling plot (figure 1C). We used bisulfite pyrosequencing, a different approach to measure DNA methylation, to validate the reliability of our analysis. We observed a significant and substantial high correlation (R=0.89, $p<2.2e^{-16}$) between values obtained from microarray and pyrosequencing (see online supplementary figure 1B).

Comparison of the DNA methylation profiles between RA and healthy controls revealed the existence of significant alterations. Specifically, we found 89 hypermethylated and 44 hypomethylated CpG sites in monocytes from patients with RA compared with healthy controls (FDR<0.05) (figure 1D and online supplementary table 3). Genomic Regions Enrichment of Annotations Tool analysis[16] using the genomic locations of those differentially methylated CpG positions (DMPs) revealed enrichment for categories including abnormal circulating IFNα and TNF levels and autoimmune disease for hypermethylated CpGs and regulation of myeloid cells differentiation and positive regulation of IL-1β for hypomethylated CpGs (figure 1E). These results suggest a potential implication of these inflammatory cytokines and their downstream signalling pathways in the acquisition of an aberrant DNA methylation signature in patients with RA. On further inspection of the most proximal individual genes to the DMPs,

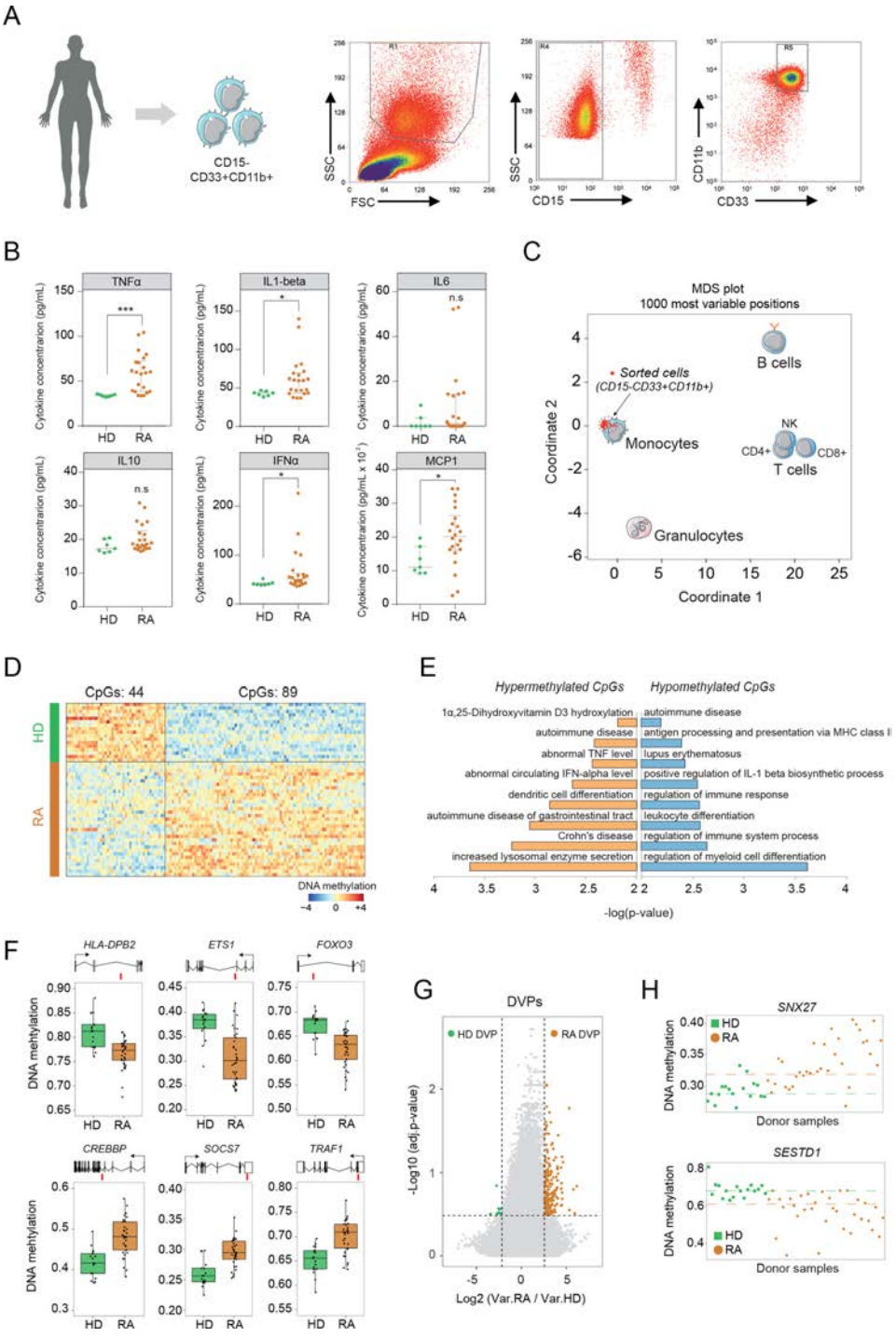

Figure 1 Characterisation and DNA methylation analysis of CD15-CD33+CD11b+ cells isolated from RAand HDs samples. (A) Scheme depicting the monocytic population (CD15-CD33+CD11b+) isolated from peripheral blood of patients with RA and HDs and the sorter strategy used for that isolation. (B) Plasma levels of different inflammatory cytokines measured in RA and HD peripheral blood samples. (C) MDS plot generated from the 1000 most variable methylated CpG sites of monocytes, T cells (CD4+, CD8+ and NK cells), B cells and granulocytes, using the mdsPlot function from minfi package. The 1000 most variable methylated CpG sites of CD15-CD33+CD11b+ cells isolated from RA and HD samples are depicted as red dots. (D) DNA methylation heatmap of CD15-CD33+CD11b+ cells isolated from RA and HD peripheral blood. The heatmap includes all CpG-containing probes displaying significant methylation changes (FDR<0.05). A scale is shown at the bottom ranging from −4 (lower DNA methylation levels, blue) to +4 (higher methylation levels, red). (E) Representation of selected gene ontology categories obtained from the analysis of differentialy methylated CpG sites comparing HD and RA samples using Genomic Regions Enrichment of Annotations Tool. (F) Beta values showing methylation of individual CpGs in both the hypermethylated and the hypomethylated CpG sets. A schematic representation of each gene is depicted. Arrows refer to TSS and transcription direction (in red the analysed CpGs location). (G) Analysis of differentially variable positions identified with iEVORA algorithm. Significant DVPs are those with a t-test value <0.01 and an adjusted Bartlett test value <0.01. (H) DNA methylation plot of selected genes displaying DNA methylation variability in HD and RA group of samples. Mann-Whitney tests were used to determine significance (*p<0.05, **p<0.01 and ***p<0.001; n.s., not significant). DVP, differentially variable position; FDR, false discovery rate; HD, healthy donor; n.s., not significant; RA, rheumatoid arthritis; TSS, transcriptional start site.

we identified several genes with essential functions in relation with monocyte/macrophage/dendritic cell biology (see online supplementary table 4). Hypomethylated CpGs mapped to genes including *HLA-DPB2*, *ETS1* and *FOXO3* and hypermethylated CpGs mapped to genes including *CREBBP*, *SOCS7* and *TRAF1* (figure 1F). We performed a chromatin states enrichment analysis of the two differentially methylated sets using public monocyte data from Roadmap Epigenomics Project generated with ChromHMM.[17] This analysis revealed a significant enrichment at enhancers and also at transcription start sites flanking regions (see online supplementary figure 1C). Furthermore, analysis of transcription factor (TF) binding motifs showed enrichment for interferon regulatory factor (IRF) and PU.1 motifs (see online supplementary figure 1D).

Recently, it has been described that patients with RA are characterised by expansion of the intermediate subset of monocytes.[18 19] As indicated previously, our purification procedure allowed the recovery of these three subsets of monocytes. Fluorescence-activated cell sorting (FACS) analysis of the three subsets revealed that the expansion of the intermediate monocyte subset in RA is also observed in our cohort (see online supplementary figure 1E). To discard the possibility that expansion of the intermediate subset could be influencing the detection of altered DNA methylation patterns in our isolated monocytes, we analysed a selection of CpG sites in the three monocytic subsets by pyrosequencing comparing patients with RA to healthy controls. We observed similar changes in DNA methylation for all three monocytic subsets in patients with RA (see online supplementary figure 1F), indicating that these changes in DNA methylation were not the result of the expansion of a specific subset but occurred in parallel in all subpopulations.

Given the intrinsic heterogeneity of the RA cohort regarding several clinical features, we hypothesised that the RA DNA methylation profiles may also display higher heterogeneity than healthy controls. The potential importance of increased DNA methylation variability in disease has been noted recently.[14 20] We observed a substantial and significant enrichment of DVPs in the RA group when compared with healthy controls (figure 1G). Figure 1H exemplifies two genes displaying increased variability in DNA methylation in patients with RA compared with healthy donors (figure 1H).

DNA methylome of monocytes reflects disease activity in patients with RA

The increased DNA methylation variability in RA monocytes in comparison with healthy controls suggests that diverse factors, including drug treatment as well as the specific systemic inflammation at the moment the samples were obtained, could influence the DNA methylation profile of monocytes, which are indeed highly sensitive to environmental changes.[21 22]

In this regard, when we evaluated the different therapeutic regimes of the patients in our cohort (namely anti-IL6R, anti-TNF, methotrexate and glucocorticoids among others), we did not find any significant correlation between patient treatments and DNA methylation (data not shown). However, we detected a substantial number of CpG sites whose methylation is associated with the erythrocyte sedimentation rate (ESR) and the C-reactive protein (CRP) levels, two biochemical parameters reflecting the global inflammation that are used to calculate the DAS28 in patients with RA (see online supplementary figures 2A,B). These data set the notion that the inflammatory status of the patients, reflected by the associated disease activity score, could be one of the major sources of epigenetic variability within the patient cohort with RA. Following this line of reasoning, we performed a Spearman correlation to determine the occurrence of significant DNA methylation changes in relation to DAS28. We identified 2327 CpG sites (hyper.HA) whose methylation levels positively correlated with DAS28 (rho <0.5 and p value <0.01) and 2591 CpG sites (hypo.HA) with an inverse correlation with DAS28 (rho >-0.5 and p value <0.01) (figure 2A and online supplementary table 5). Interestingly, the methylation profiles of both hypo.HA and hyper.HA CpG sites were highly similar between remission patients and healthy controls in an unsupervised representation (figure 2B and online supplementary figure 2C). We also inspected a DNA methylation dataset of monocytes of a cohort of patients with RA from a previously published study.[23] By comparing their data and ours, we observed the same trend of changes in the methylomes in connexion with disease activity (see online supplementary figure 2D), despite that the patients with RA from Mok *et al* were classified using a different marker of disease activity, Clinical Disease Activity Index (CDAI). Furthermore, using our data, we were able to identify the same differentially methylated region influenced by disease activity found in their study (see online supplementary figure 2E).

In our analysis, some of the CpGs correlating with DAS28 were situated in or in close proximity to genes that were associated with relevant signalling pathways in inflammatory conditions, such as IFN and TNF pathways. Some of those genes include *STAT3*[24–26], *FPR2*,[27] *TNFAIP8*[28] *and IL19*[29] among others (figure 2C and online supplementary table 6). Moreover, we used bisulfite pyrosequencing to analyse additional CpG sites nearby the ones interrogated in the microarray. The inspection of those CpG sites showed a similar trend in DNA methylation changes (see online supplementary figure 2F).

To understand how DAS28 is linked to specific DNA methylation changes, we studied several features of the aforementioned CpG sites. Specifically, the analysis of TF binding motifs revealed that in the case of the hyper.HA set, we observed significant enrichment for PU.1 and other ETS family TFs, together with several IRFs (figure 2D). For the hypo.HA set, we observed enrichment for binding motifs of C/EBP family members (figure 2D). Interestingly, during macrophage differentiation C/EBPα has been shown to be responsible for upregulation of TET2,[30] an enzyme that catalyses 5-methylcytosine oxidation, leading to its demethylation.

To reinforce the biological relevance of the identified CpGs from Hyper.HA and Hypo.HA clusters, we performed enrichment analysis of chromatin states, which revealed a significant enrichment at different enhancer categories (figure 2E). This observation is in accordance with what was observed by other studies that focuses on DNA methylation changes during terminal myeloid differentiation, which provides biological relevance to the relationship between DNA methylation and chromatin states.[3 31 32] In this regard, we further investigated whether the DNA methylation changes associated to DAS28 could influence enhancer activity in the context of inflammation. For this analysis, we used public data from a model of LPS-induced inflammation. In fact, LPS administration has been successfully used as a model of systemic inflammation and promotes the induction of autoimmune arthritis in mice.[33–38] Interestingly, we observed a significant enrichment for hyper.HA CpGs in dynamic LPS-responsive enhancers (active-to-primed enhancers and primed-to-active enhancers) and also at de novo LPS-responsive enhancers (figure 2F and G). These results suggest that the identified DNA methylation changes in high-activity patients with RA influence the activity of regulatory elements, such as enhancers under inflammatory conditions.

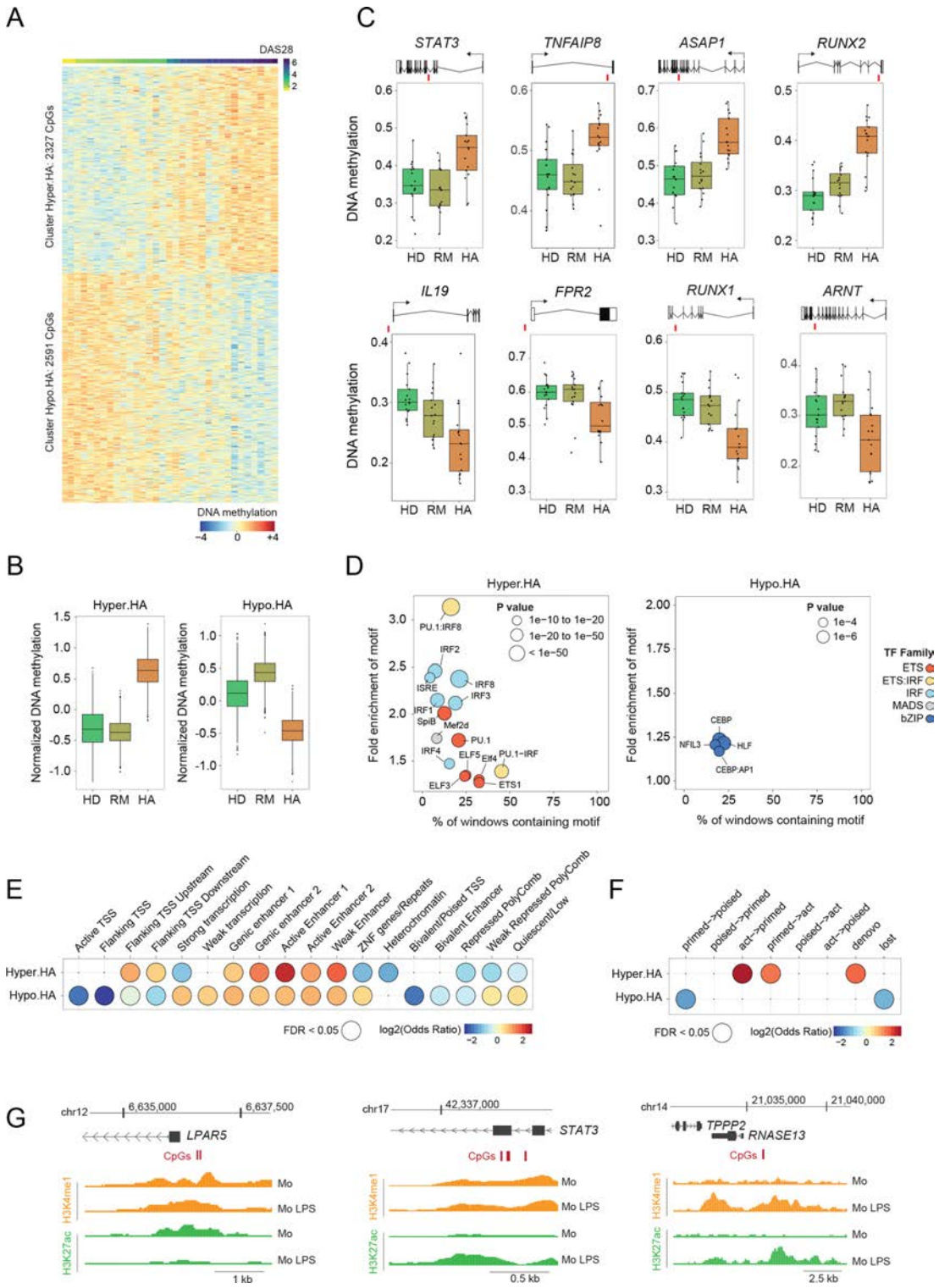

Figure 2 Correlation of disease activity score and DNA methylation in patient with RA. (A) Heatmap of patients with RA DNA methylation ordered by DAS28. The heatmap includes all CpG-containing probes displaying a significant Spearman correlation with DAS28 score (p value < 0.01, Spearman correlation coefficient ρ>0.5). (B) Normalised methylation values from heatmap showing overall group methylation of HD, RM patients (DAS28 <2.6) and HA patients (including both DAS28 >3.2 for moderate and DAS28 >5.1 for high activity). (C) Beta values showing methylation of selected individual CpGs in both the hyper.HA and the hypo.HA sets. A schematic representation of each gene is depicted. Arrows refer to TSS and transcription direction (in red the analysed CpGs location). (D) Significantly enriched TFs in motif enrichment analysis with Hypergeometric Optimization of Motif EnRichment (HOMER) on hyper.HA/hypo.HA CpGs. A 500 bp region centred around the CpG sites was used in the analysis. Relative fold enrichment, FDR and TF family are shown. (E) Chromatin states enrichment analysis for both hyper.HA and hypo.HA sets based on ChromHMM monocyte published data from Roadmap Epigenomics Project. (F) Enrichment analysis of DAS28-associated differentially methylated CpG sites at different enhancers categories in control monocytes and LPS-treated monocytes. (G) Graphs depicting H3K4me1 and H3K27ac profiles at genomic regions surrounding selected DAS28-associated differentially methylated CpG sites. DAS28, Disease Activity Score 28; FDR, false discovery rate; HA, higher activity; HD, healthy donor; RA, rheumatoid arthritis; RM, remission; TF, transcription factor; TSS, transcriptional start site.

Finally, we investigated whether the connection between monocyte methylation and disease activity is also applicable to non-RA chronic inflammatory diseases. Hence, we examined a previously published monocyte methylation dataset of multiple sclerosis patients.[39] Interestingly, on the inspection of the 4918 CpGs identified in our study in the multiple sclerosis DNA methylation dataset, we observed the same trend of methylation changes in connection with disease activity (see online supplementary figure 2G).

DNA methylation profiles switch during the transition from high disease activity to remission and vice versa

Our results indicate that disease activity in RA is highly related to the DNA methylome of peripheral blood monocytes. To confirm the plasticity of the monocyte methylome in relation to the activity course of the disease, we obtained a second blood sample for a selection of 10 patients with RA included in the first analysis and performed DNA methylation profiling. The comparison of the average levels of the hyper.HA and hypo.HA sets between these paired monocyte samples (corresponding to the same set of individuals but at a different time point in which they displayed a different DAS28 value) confirmed the plasticity of their methylomes and reinforced the concept that the profile in remission is highly similar to healthy donors (figure 3A). Additionally, a broad relationship between the DAS28 variation and the range of DNA methylation change was observed (figure 3B). In fact, comparison of single individuals at different DAS28 levels led to the same conclusion when looking at the average levels of the hyper.HA/hypo.HA sets (figure 3C) and at selected individual CpG sites with higher correlation scores (figure 3D). Interestingly, DAS28 variation is associated with DNA methylation changes in a different degree depending on the patient, indicating that, despite the general trend, additional factors, such as genetic background[40 41], may also influence such relationship.

Altogether, these observations led to the notion that monocyte DNA methylome is linked to the inflammatory environment in the blood and reflects disease activity, estimated by the DAS28.

In vitro stimulation of monocytes with inflammatory cytokines partially recapitulates the methylome of high-activity patients

Our results suggest that the inflammatory environment during high-activity periods is able to induce DNA methylation changes in monocytes. In this regard, serum or plasma levels of cytokines are considered to be indicative of disease severity in patients with RA.[42 43] We performed a correlation analysis between the plasma levels of inflammatory cytokines and the disease activity score of patients with RA and observed that several cytokines, including TNFα, IFNα, IL23, IFNγ and IL-10 showed significant positive correlations, among which TNFα displayed the highest and most significant correlation with DAS28 (figure 4A).

This result suggests that DAS28 can be related to the cytokine levels in the plasma of the patients with RA and reinforces its value as a good indicator of the systemic inflammation. Therefore, the elevated levels of these cytokines may provide a particular environment influencing the monocyte methylome. Thus, we explored the possibility of recapitulating some of the methylation changes observed in patients with high DAS28 RA by exposing monocytes from healthy individuals to several of the cytokines with the most robustly increased levels in individuals with RA at high DAS28, that is, TNFα, IFNα and IFNγ.

We exposed PBMCs isolated from healthy donors to these three cytokines individually and cultured in plates treated with polyhydroxyethylmethacrylate (poly-HEMA). The use of poly-HEMA-coated plates prevents monocyte attachment to plates and the subsequent differentiation of monocytes, which may result in additional differentiation-associated methylation changes.[44] Following 4 days of exposure to the aforementioned cytokines, we isolated the monocytic fraction by sorting CD33+CD11b+CD15- cells and carried out DNA methylation profiling (figure 4B). The three cytokines promoted changes in the DNA methylation status of several hundred CpG sites (see online supplementary figure 3A). IFNγ was able to promote the greatest number of hypermethylation events (346 CpGs), sharing a fraction of them with both TNFα and IFNα. On the other hand, TNFα was able to promote the largest number of hypomethylation events (400 CpGs) with very little overlap with the two IFNs (figure 4C). These three cytokines stimulate several pathways downstream their receptors, including NFκB and AP-1 downstream of TNFα, and STAT1 and STAT2 downstream of IFNs, that ultimately modulate the expression of several IRF TF family members (figure 4D). In fact, the analysis of TF binding motifs indicated that some of the aforementioned pathways can be directly influencing the DNA methylation changes. For instance, we observed a significant enrichment of the subunits of the NF-kB complex and AP-1 in the hypomethylated CpG sites after treatment with TNFα and a significant enrichment for IRFs in the hypomethylated CpG sites of IFN-treated cells (figure 4E). Most importantly, many CpG sites in the in vitro analysis are also common with those undergoing DNA methylation changes in relation to DAS28 (figure 4F). This ability of TNFα, IFNα and IFNγ to recapitulate DNA methylation changes indicates that pathologically elevated levels of these cytokines in patients with high disease activity are participating effectors leading to the acquisition of the observed methylation profiles.

Generation of a regression model using the DNA methylome to estimate the disease activity score of patients with RA

Next, we interrogated whether DAS28-associated DNA methylation profile could be used as a marker to estimate the activity of patients with RA to generate a disease-activity estimation model. This mathematical model could potentially be useful for RA and for other immune-mediated inflammatory disease with less well-defined disease activity scores. As such, we first generated probability distributions with the normalised DNA methylation values of the previously identified CpG sites (hyper.HA and hypo.HA sets) from the discovery cohort, that is, the initial 33 RA samples (figure 5A, left panel). These distributions allowed us to calculate the probability of a sample, with a blind activity score, to fit one of the two distributions (remission or moderate/high activity). Using this strategy, we calculated the probability of each identified CpG site from each patient within the validation cohort (corresponding to the aforementioned second visit paired samples) to fit into high activity or remission distributions. We then assigned the inferred category according to the highest average probability to fit into one of the two distributions. With this approach, we were able to successfully infer the activity category of 7 out of 10 samples in the validation cohort (figure 5A, right panel).

To improve the disease-activity estimation model, aiming to infer the general disease activity category and the specific DAS28 value of the patient based on DNA methylation profile, we performed a multiple linear regression analysis, using the samples from the discovery cohort. In this respect, we generated several models that work with different combinations of CpG sites. We observed that only models 2–5 displayed optimal values of tolerance, used to exclude collinearity and lie within

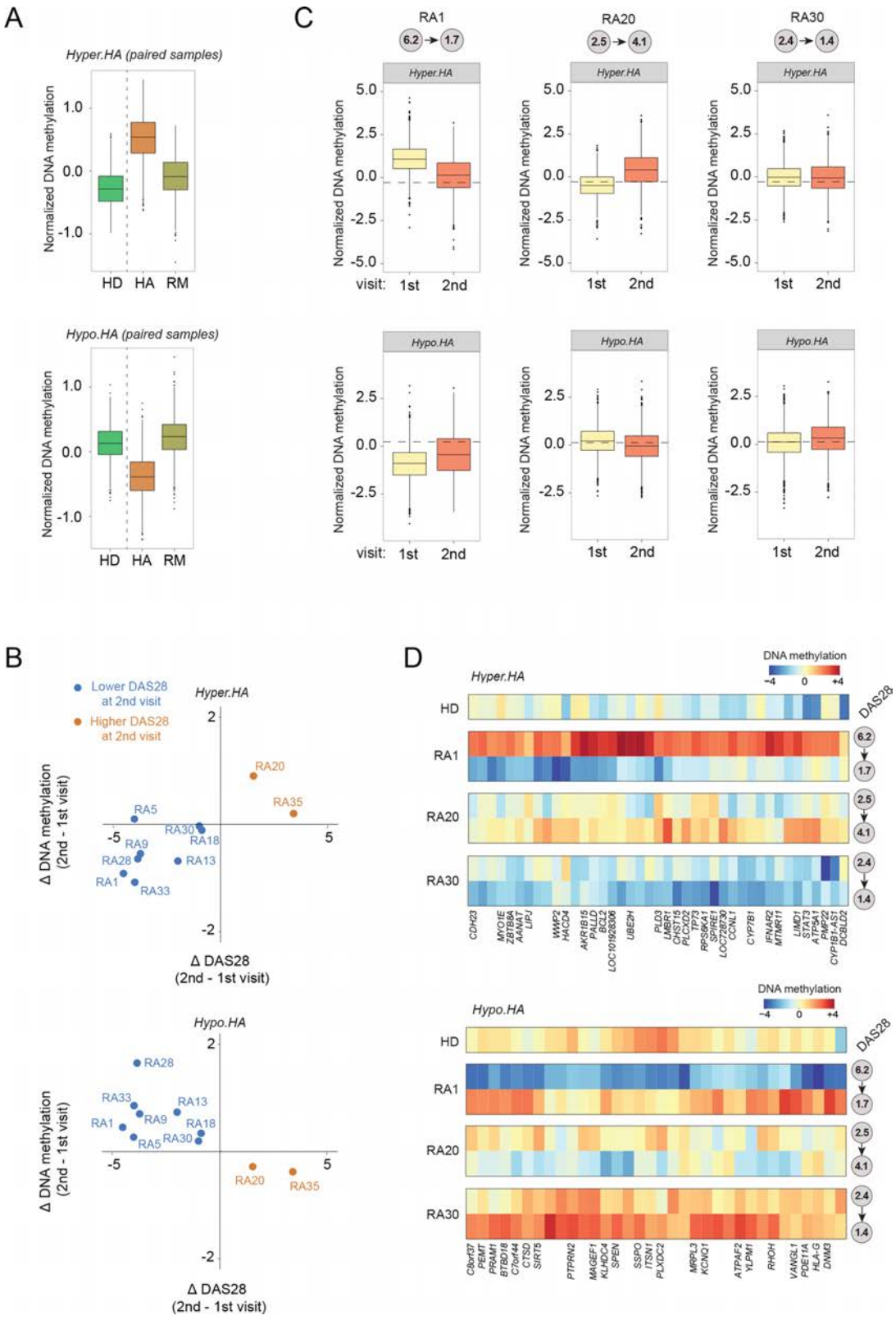

Figure 3 Reversibility of DNA methylation associated to DAS28. (A) Box plots showing normalised average methylation levels of previously identified CpGs sites (CpGs sites which methylation correlates with DAS28) in paired-samples isolated from the same individual but at different time points with different DAS28 scores. The average methylation levels of healthy donors is also shown as a reference (B) Dot plots showing the relationship between the variance of DAS28 and the variance of DNA methylation in paired-samples, for hyper.HA set at the top, and hypo.HA at the bottom (C) Box plots depicting normalised average methylation levels of previously identified CpGs sites in selected patients (RA1, RA20 and RA30), where DAS28 value is decreased, increased or maintained at remission levels, respectively. Dashed line indicating HD median values as a reference. (D) Heatmap depicting normalised methylation levels of selected CpGs sites according with the highest correlation scores in selected patients showing the dynamics of DNA methylation according to DAS28 score. DAS28, Disease Activity Score 28; HA, higher activity; HD, healthy donor; RA, rheumatoid arthritis; RM, remission.

A

Plasma cytokine	TNFα	IFNα	IL23	IFNγ	IL10
Spearman rho	0.56	0.53	0.48	0.45	0.44
95% confidence interval	0.179 to 0.793	0.127 to 0.783	0.064 to 0.757	0.015 to 0.736	0.005 to 0.731
p-value (two-tailed)	0.007	0.011	0.023	0.038	0.042

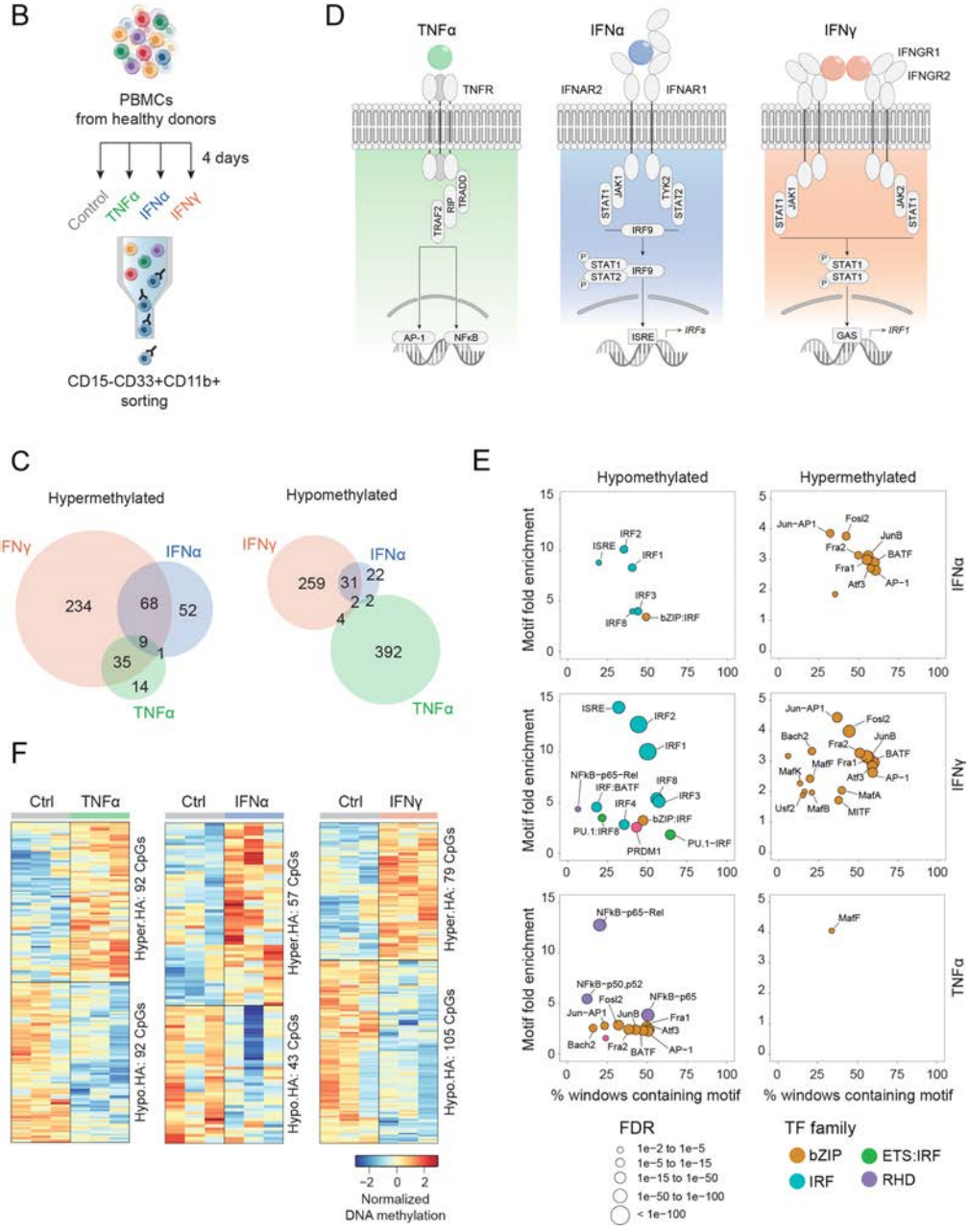

Figure 4 In vitro exposure to TNFα, IFNα or IFNγ partially recapitulates the methylome of high-activity patients with RA. (A) Spearman correlation of cytokine levels quantified in blood plasma and DAS28 score in patients with RA, ordered by correlation coefficient (ρ). P value and 95% CIs are also shown. (B) Schematic diagram depicting the in vitro model for RA monocytes. PBMCs were cultured with or without TNFα, IFNα or IFNγ during 4 days and then, monocytes were sorted as CD15-CD33+CD11b+ cells for subsequent analyses. (C) Venn diagram showing the overlap and the number of differentially methylated CpG sites for each cytokine treatment compared with control cells. For the differentially methylated CpGs, only CpGs with a p value <0.001 and difference beta-value >0.15 were selected. (D) Schematic diagram depicting the signalling pathways of TNFα, IFNα and IFNγ. (E) Significantly enriched transcription factors in motif enrichment analysis with HOMER on hyper or hypomethylated CpGs after treatment with inflammatory cytokines. A 500 bp region centred around the CpG sites was used in the analysis. Relative fold enrichment, FDR and TF family are shown. (F) DNA methylation heatmap of CD15-CD33+CD11b+ cells isolated from PBMCs stimulated in vitro with TNFα, IFNα and IFNγ. The heatmap includes all CpG-containing probes displaying significant methylation changes (p value<0.05) and that recapitulate the dynamic of hyper.HA and hypo.HA CpG sites (CpG-containing probes displaying a significant Spearman correlation with DAS28 score). A scale is shown at the bottom ranging from −2 (lower DNA methylation levels, blue) to +2 (higher methylation levels, red). DAS28, Disease Activity Score 28; FDR, false discovery rate; HA, higher activity; IFN, interferon; IL, interleukin; PBMC, peripheral blood mononuclear cell; TF, transcription factor; TNF, tumour necrosis factor.

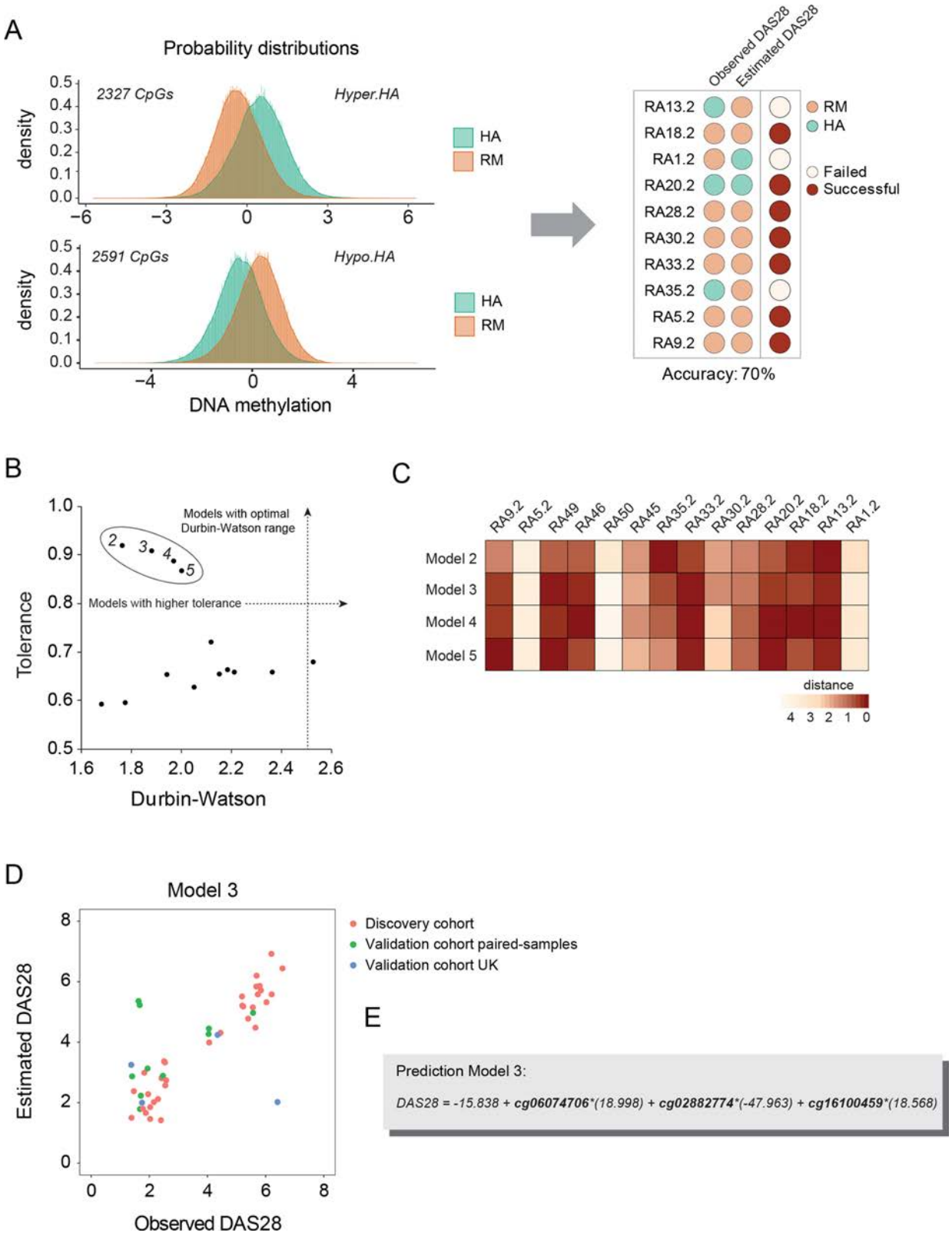

Figure 5 Disease-activity estimation model approaches and performances. (A) Probability distributions of the normalised DNA methylation values of hyper.HA (top left) and hypo.HA (bottom left). On the right, a panel with the corresponding observed and estimated DAS28 category and the success of the estimation is depicted. Normalised methylation data for each patient of the validation cohort are used to generate a probability distribution and test the probability of that patient to fit into HA or RM distributions. We assign the inferred category according to the highest probability to fit into HA or RM distributions. (B) Analysis of the collinearity (tolerance) and autocorrelation (Durbin-Watson statistic) of the first 14 multiple linear regression models. Models with better performance (models 2–5) are outlined. (C) Heatmap showing the performance of the selected models 2–5 in estimating DAS28 values. The accuracy (distance) of the estimate is calculated as difference of estimated minus observed DAS28 values. (D) Dot plot depicting observed DAS28 values versus estimated DAS28 values using model 3. (E) Disease-activity estimation model 3 formula. DAS28, Disease Activity Score 28; HA, higher activity; RA, rheumatoid arthritis; RM, remission.

the appropriate Durbin-Watson range, used to avoid the presence of autocorrelation (figure 5B). Subsequently, we tested the performance of these four models using the validation cohort as well as four additional independent RA samples obtained from a different hospital in London (and therefore changing the clinical team and the geographic origin of the samples). We observed that model 3 was the most precise model to infer DAS28 values based on DNA methylation, in which accuracy was measured as the shortest distance between the observed and the estimated DAS28 values (figure 5C,D). This model consisted of a multiple linear regression containing the methylation values of three different CpG sites, namely cg06074706, cg02882774 and cg16100459, located in the gene bodies of *PALM2*, *NDUFA6* and *LINC01539* genes, respectively. These CpGs were present in all the subsequent models, which included additional CpG sites, although the highest accuracy in DAS28 estimation was achieved with model 3. In all, we conclude that the model 3 formula appeared to be the best estimator in experimentally determining DAS28 score from DNA methylation profiles (figure 5E).

DISCUSSION

In this study, we have established that the inflammatory milieu in the peripheral blood of active patients with RA is associated with an altered DNA methylome of circulating monocytes. We have identified a cluster of several thousand CpG sites whose methylation levels correlate with patient disease activity score, which mainly measures the activity in the joints. We have also demonstrated that TNFα and IFNs are able to induce some of the DNA methylation changes occurring at high disease activity where the levels of these cytokines are increased. Most importantly, we have generated a mathematical model that allows the estimation of the DAS28 score of a given patient with RA from their monocyte DNA methylation dataset. This highlights the relevance of our findings in the clinical setting, and its potential applicability to other immune-mediated inflammatory conditions.

Our findings indicate that the association between the DNA methylome and RA disease activity can be determined in peripheral blood monocytes. The functional categories among the differential methylated CpG sites points at abnormal circulating IFNα and TNFα levels, reinforcing the relevance of these cytokines in peripheral blood to influence DNA methylation during high activity peaks. This was confirmed by proving the ability of TNFα and IFNs to modify the monocyte methylome and recapitulate the observed trend in patients with RA. It is likely that monocytes/macrophages present in the inflamed joints may have more dramatic changes in their DNA methylation profiles where the inflammatory factors are at a higher concentration than in the peripheral blood.[45] However, the finding of a robust and consistent correlation between methylation and DAS28 in those peripheral blood monocytes is of great value since it constitutes a less invasive approach to obtain biological samples from patients with RA. A previous study revealed that the hypomethylation of the *CYP2E1* promoter in monocytes of patients with RA correlated to RA activity.[23] By analysing our own data, we were also able to detect hypomethylation of the *CYP2E1* promoter region. Furthermore, on inspection of the DAS28-associated CpGs identified in our study, we observed the same trend of methylation changes related to disease activity, which confirms the consistency of our data with respect to Mok and colleagues' study. Nevertheless, the small discrepancies between both studies might be explained by the different patient activity distributions, since the Mok *et al* cohort displays a higher number of remission patients. This fact may compromise the identification of disease activity-associated methylation changes in such study. Also, in agreement with another study from the same team,[46] we observed no significant overlap between the methylation signature of monocytes and synoviocytes in patients with RA.

It is likely that additional cytokines, through a direct and/or cellular-mediated complex response, participate in shaping the DNA methylome. The identification of the specific contributions of cytokines in inducing the altered DNA methylation patterns in RA monocytes can constitute a challenge due to the complexity and diversity of the inflammatory milieu of those patients. However, in our study, we have demonstrated that DAS28 significantly correlates with the methylome of monocytes from patients with RA, suggesting that, regardless of the deregulated cytokines in those patients, different inflammatory environments display common mechanisms of action and also impact the myeloid compartment epigenome in a similar manner.

Our analysis was performed in a cell population (CD15-CD33+CD11b+) comprising distinct monocytic subsets. As shown in our study, intermediate monocytes are expanded in patients with RA, in concordance to what has been described by others.[18 19] The analysis of selected CpG sites in the three monocytic subsets indicates the existence of a shared molecular mechanism in the establishment of the aberrant methylation profiles. The acquisition of DNA methylation changes in relation to DAS28 occurs in sites that are enriched for both ETS and IRF TF motifs. Among the ETS TF family, PU.1 has been previously described to interact with elements of the DNA methylation machinery, such as TET2 and DNMT3a and to be involved in both active demethylation and methylation processes in the context of monocyte-to-osteoclast differentiation.[32] It is therefore plausible that, under certain inflammatory signals, the recruitment of PU.1 to specific genomic loci triggers changes in the methylome of monocytes that we have discovered in our analysis.

Human monocytes are highly plastic, both phenotypically and epigenetically, and can act as sensors of the inflammatory environmental changes. Several disease activity scores have been validated in the context of RA, but most are based to a great extent on biomarkers such as CRP and ESR. However, these markers cannot be used as an accurate measurement of inflammation.[47] The use of improved sets of biomarkers, such as epigenetic patterns, specifically DNA methylation profiles, could potentially better stratify patients with RA. Furthermore, the potential use of methylation profiles to estimate the disease activity score of the patients could be extended to other immune-mediated inflammatory clinical entities including multiple sclerosis, psoriatic arthritis, ankylosing spondylitis and others proving a use beyond RA.

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Contributors J.R-U, CC-F, JDC and EB conceived experiments. JR-U, CC-F, TL and LC performed experiments. JR-U, CC-F, TL, FC-M and OM-P performed biocomputing analysis; JRU, CC-F and MLB performed statistical analysis. RC, FH, AN, CP and JDC

performed patient selection, provided samples and analysed the data. JR-U, CC-F, FC-M, AG-G, RC, JM, JDC and EB analysed the data. AG-G illustrated graphical representations. EB, JR-U and CC-F wrote the paper. All authors read and approved the final manuscript.

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REFERENCES

1 Zhang Q, Zhao K, Shen Q, *et al*. Tet2 is required to resolve inflammation by recruiting HDAC2 to specifically repress IL-6. *Nature* 2015;525:389–93.
2 Vento-Tormo R, Álvarez-Errico D, Garcia-Gomez A, *et al*. DNA demethylation of inflammasome-associated genes is enhanced in patients with cryopyrin-associated periodic syndromes. *J Allergy Clin Immunol* 2017;139:202–11.
3 Rodríguez-Ubreva J, Català-Moll F, Obermajer N, *et al*. Prostaglandin E2 leads to the acquisition of DNMT3A-Dependent tolerogenic functions in human myeloid-derived suppressor cells. *Cell Rep* 2017;21:154–67.
4 Haringman JJ, Gerlag DM, Zwinderman AH, *et al*. Synovial tissue macrophages: a sensitive biomarker for response to treatment in patients with rheumatoid arthritis. *Ann Rheum Dis* 2005;64:834–8.
5 Hueber W, Tomooka BH, Zhao X, *et al*. Proteomic analysis of secreted proteins in early rheumatoid arthritis: anti-citrulline autoreactivity is associated with up regulation of proinflammatory cytokines. *Ann Rheum Dis* 2007;66:712–9.
6 Chandra PE, Sokolove J, Hipp BG, *et al*. Novel multiplex technology for diagnostic characterization of rheumatoid arthritis. *Arthritis Res Ther* 2011;13.
7 Aletaha D, Smolen JS. Diagnosis and management of rheumatoid arthritis: a review. *JAMA* 2018;320:1360–72.
8 Fransen J, van Riel PLCM. The disease activity score and the EULAR response criteria. *Rheum Dis Clin North Am* 2009;35:745–57.
9 Zhao P-W, Jiang W-G, Wang L, *et al*. Plasma levels of IL-37 and correlation with TNF-α, IL-17A, and disease activity during DMARD treatment of rheumatoid arthritis. *PLoS One* 2014;9:e95346.
10 Aletaha D, Neogi T, Silman AJ, *et al*. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League against rheumatism collaborative initiative. *Arthritis Rheum Dis* 2010;69:1580–8.
11 Du P, Zhang X, Huang C-C, *et al*. Comparison of Beta-value and M-value methods for quantifying methylation levels by microarray analysis. *BMC Bioinformatics* 2010;11:587.
12 Aryee MJ, Jaffe AE, Corrada-Bravo H, *et al*. Minfi: a flexible and comprehensive Bioconductor package for the analysis of Infinium DNA methylation microarrays. *Bioinformatics* 2014;30:1363–9.
13 Leek JT, Johnson WE, Parker HS, *et al*. The SVA package for removing batch effects and other unwanted variation in high-throughput experiments. *Bioinformatics* 2012;28:882–3.
14 Teschendorff AE, Gao Y, Jones A, *et al*. DNA methylation outliers in normal breast tissue identify field defects that are enriched in cancer. *Nat Commun* 2016;7.
15 Reynolds LM, Taylor JR, Ding J, *et al*. Age-Related variations in the methylome associated with gene expression in human monocytes and T cells. *Nat Commun* 2014;5:5366.
16 McLean CY, Bristor D, Hiller M, *et al*. Great improves functional interpretation of cis-regulatory regions. *Nat Biotechnol* 2010;28:495–501.
17 Ernst J, Kellis M. Chromatin-state discovery and genome annotation with ChromHMM. *Nat Protoc* 2017;12:2478–92.
18 Tsukamoto M, Suzuki K, Seta N, *et al*. Increased circulating CD14brightCD16+ intermediate monocytes are regulated by TNF-α and IL-6 axis in accordance with disease activity in patients with rheumatoid arthritis. *Clin Exp Rheumatol* 2018;36:540–4.
19 Tsukamoto M, Seta N, Yoshimoto K, *et al*. CD14brightCD16+ intermediate monocytes are induced by interleukin-10 and positively correlate with disease activity in rheumatoid arthritis. *Arthritis Res Ther* 2017;19.
20 Teschendorff AE, Widschwendter M. Differential variability improves the identification of cancer risk markers in DNA methylation studies profiling precursor cancer lesions. *Bioinformatics* 2012;28:1487–94.
21 Rodríguez-Ubreva J, Garcia-Gomez A, Ballestar E. Epigenetic mechanisms of myeloid differentiation in the tumor microenvironment. *Curr Opin Pharmacol* 2017;35:20–9.
22 Álvarez-Errico D, Vento-Tormo R, Sieweke M, *et al*. Epigenetic control of myeloid cell differentiation, identity and function. *Nat Rev Immunol* 2015;15:7–17.
23 Mok A, Rhead B, Holingue C, *et al*. Hypomethylation of CYP2E1 and DUSP22 promoters associated with disease activity and erosive disease among rheumatoid arthritis patients. *Arthritis Rheumatol* 2018;70:528–36.
24 Mori T, Miyamoto T, Yoshida H, *et al*. IL-1β and TNFα-initiated IL-6-STAT3 pathway is critical in mediating inflammatory cytokines and RANKL expression in inflammatory arthritis. *Int Immunol* 2011;23:701–12.
25 Degboé Y, Rauwel B, Baron M, *et al*. Polarization of rheumatoid macrophages by TNF targeting through an IL-10/STAT3 mechanism. *Front Immunol* 2019;10:3.
26 Dallagi A, Girouard J, Hamelin-Morrissette J, *et al*. The activating effect of IFN-γ on monocytes/macrophages is regulated by the LIF-trophoblast-IL-10 axis via STAT1 inhibition and STAT3 activation. *Cell Mol Immunol* 2015;12:326–41.
27 Lee HY, Kim SD, Shim JW, *et al*. Activation of human monocytes by a formyl peptide receptor 2-derived pepducin. *FEBS Lett* 2010;584:4102–8.
28 Kumar D, Whiteside TL, Kasid U. Identification of a novel tumor necrosis factor-alpha-inducible gene, *SCC-S2*, containing the consensus sequence of a death effector domain of Fas-associated death domain-like interleukin- 1beta-converting enzyme-inhibitory protein. *J Biol Chem* 2000;275:2973–8.
29 Liao Y-C, Liang W-G, Chen F-W, *et al*. Il-19 induces production of IL-6 and TNF-alpha and results in cell apoptosis through TNF-alpha. *J Immunol* 2002;169:4288–97.
30 Kallin EM, Rodríguez-Ubreva J, Christensen J, *et al*. Tet2 facilitates the derepression of myeloid target genes during CEBPα-induced transdifferentiation of pre-B cells. *Mol Cell* 2012;48:266–76.
31 Vento-Tormo R, Company C, Rodríguez-Ubreva J, *et al*. Il-4 orchestrates STAT6-mediated DNA demethylation leading to dendritic cell differentiation. *Genome Biol* 2016;17:4.
32 de la Rica L, Rodríguez-Ubreva J, García M, *et al*. Pu.1 target genes undergo Tet2-coupled demethylation and DNMT3B-mediated methylation in monocyte-to-osteoclast differentiation. *Genome Biol* 2013;14:R99.
33 Seemann S, Zohles F, Lupp A. Comprehensive comparison of three different animal models for systemic inflammation. *J Biomed Sci* 2017;24:60.
34 Yoshino S, Sasatomi E, Mori Y, *et al*. Oral administration of lipopolysaccharide exacerbates collagen-induced arthritis in mice. *J Immunol* 1999;163:3417–22.
35 Yoshino S, Sasatomi E, Ohsawa M. Bacterial lipopolysaccharide acts as an adjuvant to induce autoimmune arthritis in mice. *Immunology* 2000;99:607–14.
36 de Bont N, Netea MG, Rovers C, *et al*. Lps-Induced release of IL-1 beta, IL-1ra, IL-6, and TNF-alpha in whole blood from patients with familial hypercholesterolemia: no effect of cholesterol-lowering treatment. *J Interferon Cytokine Res* 2006;26:101–7.
37 Dumitru CD, Ceci JD, Tsatsanis C, *et al*. TNF-α Induction by LPS Is Regulated Posttranscriptionally via a Tpl2/ERK-Dependent Pathway. *Cell* 2000;103:1071–83.
38 Netea MG, Nold-Petry CA, Nold MF, *et al*. Differential requirement for the activation of the inflammasome for processing and release of IL-1beta in monocytes and macrophages. *Blood* 2009;113:2324–35.
39 Kular L, Liu Y, Ruhrmann S, *et al*. Dna methylation as a mediator of HLA-DRB1*15:01 and a protective variant in multiple sclerosis. *Nat Commun* 2018;9:2397.
40 Liu Y, Aryee MJ, Padyukov L, *et al*. Epigenome-Wide association data implicate DNA methylation as an intermediary of genetic risk in rheumatoid arthritis. *Nat Biotechnol* 2013;31:142–7.
41 Chen L, Ge B, Casale FP, *et al*. Genetic drivers of epigenetic and transcriptional variation in human immune cells. *Cell* 2016;167:e24:1398–414.
42 McInnes IB, Buckley CD, Isaacs JD. Cytokines in rheumatoid arthritis - shaping the immunological landscape. *Nat Rev Rheumatol* 2016;12:63–8.
43 Mateen S, Zafar A, Moin S, *et al*. Understanding the role of cytokines in the pathogenesis of rheumatoid arthritis. *Clinica Chimica Acta* 2016;455:161–71.
44 Bhattacharya A, Agarwal M, Mukherjee R, *et al*. 3D micro-environment regulates NF-κβ dependent adhesion to induce monocyte differentiation. *Cell Death Dis* 2018;9.
45 Wright HL, Bucknall RC, Moots RJ, *et al*. Analysis of SF and plasma cytokines provides insights into the mechanisms of inflammatory arthritis and may predict response to therapy. *Rheumatology* 2012;51:451–9.
46 Rhead B, Holingue C, Cole M, *et al*. Rheumatoid arthritis naive T cells share hypermethylation sites with synoviocytes. *Arthritis Rheumatol* 2017;69:550–9.
47 Orr CK, Najm A, Young F, *et al*. The utility and limitations of CRP, ESR and DAS28-CRP in appraising disease activity in rheumatoid arthritis. *Front. Med.* 2018;5.

EPIDEMIOLOGICAL SCIENCE

Incidence and time trends of joint surgery in patients with psoriatic arthritis: a register-based time series and cohort study from Denmark

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ABSTRACT

Objective To investigate time-trends and cumulative incidence of joint surgery among patients with psoriatic arthritis (PsA) compared with the general population.

Methods In this nationwide register-based cohort study, The Danish National Patient Registry was used to identify incident PsA patients. The 5-year incidence rates (IR) and incidence rate ratios (IRR) of joint surgery were calculated in four calendar-period defined cohorts. Each patient was matched with ten non-PsA individuals from the general population cohort (GPC). The cumulative incidences of any joint and joint-sacrificing surgery, respectively, were estimated using the Aalen-Johansen method.

Results From 1996 to 2017, 11 960 PsA patients (mean age 50 years; 57% female) were registered. The IRR of any joint surgery was twice as high for PsA patients compared with GPCs across all calendar periods. Among patients with PsA, 2, 10 and 29% required joint surgery at 5, 10 and 15 years after diagnosis. The risk of surgery in PsA patients diagnosed at 18–40 years was higher (22%) than in GPC 60+ year old (20%) after 15 years of follow-up.

Conclusions The use of joint surgery among PsA patients remained around twofold higher from 1996 to 2012 compared with GPC. After 15 years of follow-up, nearly 30% of the PsA patients had received any surgery, and even a person diagnosed with PsA at the age of 18–40 years had a higher risk of surgery than GPCs of 60+ year old. Thus, the high surgical rates represent an unmet need in the current treatment of PsA.

Key messages

What is already known about this subject?

- 40%–60% of patients with psoriatic arthritis (PsA) will develop erosive arthritis and joint-related surgery may ultimately be necessary for pain relief. However, the secular trends in surgery rates must be seen in the context of trends in a general population cohort (GPC).

What does this study add?

- Using data from a large nationwide population-based cohort of patients with PsA compared with a GPC we demonstrated that the 5-year incidence rate of joint surgery in PsA was twice as high as a matched GPC and did not change substantially from 1996 to 2012.
- Our cumulative risk analysis showed that after 15 years of follow-up, 29% of the PsA patients had received surgery.

How might this impact on clinical practice or future developments?

- Clinicians should be aware of high joint related surgical rates in the PsA population and implement a treat-to-target strategy early after diagnosis.
- Future studies will be needed to identify the impact of biological DMARD treatment on the need for surgery using individual-level based information.
- Surgical rates represent a possible treatment outcome to monitor in future studies.

INTRODUCTION

In the 1970s, psoriatic arthritis (PsA) was considered a relatively benign disorder, but during recent decades it has become evident that 40%–60% of PsA patients will develop erosive arthritis, loss of joint architecture and associated loss of function.[1] This positions PsA as a significant health concern.[2–6] X-ray-assessed structural damage in joints of PsA patients appears of similar magnitude and impact as seen in patients with rheumatoid arthritis (RA).[7] For patients with PsA, the mainstay of treatment is pharmacological, but surgery may ultimately be necessary for pain relief and restoration of physical function. Although conventional synthetic DMARDS are commonly prescribed for PsA, studies show marginal if any benefit concerning radiological progression.[8 9]

The introduction of biological DMARDS (bDMARDs) has had dramatic therapeutic effects and have demonstrated an ability to retard the radiological progression of peripheral arthritis; dactylitis, enthesitis and spondylitis.[10–13] It is unclear if the introduction of bDMARDS has translated into a reduced need for joint surgery as observed in RA populations as it was introduced later and more gradually in PsA.[14–16] Lewinson *et al* found a paradoxical increase in surgery following the introduction of bDMARDs in the UK.[16] Nystad *et al* did not detect a reduced need for surgery in Norwegian PsA patients prescribed bDMARDS and the risk of joint surgery was the same regardless if patients were diagnosed in 1985 or 1998.[17]

The sparse data on utilisation of joint surgery in PsA warrants a large-scale population-based study. In this nationwide register-based study, we aimed to study time-trends in the incidence of joint surgery among PsA patients compared with a general population cohort (GPC) in Denmark from 1996 to 2012, and further, we investigated the cumulative incidence of joint surgery up to 15 years after patients were diagnosed with PsA.

METHODS

Study design

We conducted a nationwide register-based study in accordance with the REporting of studies Conducted using Observational Routinely-collected Data (RECORD) - guidelines,[18] investigating the 5-year incidence rate (IR) of a first joint surgery in four calendar-period defined cohorts from 1996 to 2012, and second, we estimated the cumulative incidence of joint surgery from 1996 to 2017 in Danish PsA patients.

Accurate register-linkage is possible on an individual-based level in Denmark by using the unique central personal registry number assigned at birth or on emigration. The study period was from 1 January 1996 to 31 December 2017.

Data sources

Danish National Patient Registry

Established in 1977 and used for registration of diagnoses and surgical interventions at inpatient and outpatient (since 1995) hospital contacts.[19] With every discharge, information is provided on up to 20 discharge diagnoses coded by the International Classification of Diseases (ICD; ICD-8 from 1977 to 1993, ICD-10 from 1994). Since 1996, surgeries have been coded according to the Nordic Medico-Statistical Committee (NOMESCO) system.[20] The NOMESCO constitutes the first common Nordic classification of surgical procedures and is an abbreviated list of surgical procedures for Denmark, Finland, Norway and Sweden published for the first time in 1989.

Civil Registration System

The Civil Registration System (CRS) has been used for registration of deaths and migrations among all Danish citizens since 1968.[19] From CRS dates of birth, emigration and death for all patients were obtained. Further, CRS was used for matching PsA patients with GPCs.

Study populations

PsA patients

All incident patients diagnosed with PsA (ICD-10: M070, M071, M072, M073, M073A, M073B) at a rheumatology or general internal medicine department at private and public hospitals in Denmark from 1996 through 2017. Prevalent PsA patients with a first diagnosis before 1996 were excluded as were patients under the age of 18 years.

General population cohort

Each incident PsA patient was matched with up to 10 non-PsA individuals from the general population of Denmark. Matching criteria were sex, year of birth and municipality. This matching was performed only once at the initial cohort identification stage, and thus no replacement matching was undertaken following subsequent patient exclusions.

Outcomes

Primary outcome

The primary outcome was the occurrence of any first joint surgery registered in Danish National Patient Registry (DNPR) (see online supplementary table S1 for surgeries and associated NOMESCO codes).

Secondary outcomes

We further dissected the primary outcome into joint sacrificing and non-joint sacrificing surgery of upper the upper extremities (shoulder, elbow and wrist/hand) and the lower extremities (hip, knee and ankle/foot). We defined joint sacrificing surgery as arthroplasty and arthrodesis and non-joint sacrificing surgery as soft tissue, synovial surgery or joint surfaces, mainly synovectomies. Finally, we investigated total hip arthroplasty (THA) and total knee arthroplasty (TKA) as individual outcomes due to the high frequency and costs, and classification as major surgery.

Statistics

Time-trends in surgery

Incident PsA patients from 1996 to 2012 were grouped into cohorts according to predefined calendar periods of diagnosis: 1996–2000; 2001–2004; 2005–2008; 2009–2012). Within each calendar cohort, the 5-year age-standardised and sex-standardised IR with 95% CIs of joint surgery was calculated. Patients diagnosed between 2013 and 2017 could not be followed up for a full 5 years and were not included in the time

Table 1 Baseline characteristics and demographics of incident psoriatic arthritis (PsA) patients and a matched general population cohort (GPC) according to calendar period of diagnosis

	1996–2000		2001–2004		2005–2008		2009–2012		2013–2017*	
	PsA	GPC	PsA	GPC	PsA	GPC	PsA	GPC	PsA	GPC
Individuals (n)	1635	16 020	1548	15 180	2466	24 298	3041	29 939	3270	23 859
Age in years, median (IQR)	48.6 (38.9, 56.3)	48.3 (38.7, 55.8)	49.8 (38.8, 58.1)	49.4 (38.6, 57.8)	49.5 (39.7, 58.4)	49.4 (39.7, 58.4)	50.0 (39.8, 59.6)	49.8 (39.7, 59.3)	52.1 (41.4, 62.1)	51.6 (41.2, 61.4)
Female sex, n (%)	918 (56.1)	8967 (56.0)	843 (54.5)	8299 (54.7)	1381 (56.0)	13 623 (56.1)	1796 (59.1)	17 720 (59.2)	1862 (56.9)	13 598 (57.0)
CVD, n (%)	64 (3.9)	608 (3.8)	100 (6.5)	673 (4.4)	147 (6.0)	1126 (4.6)	205 (6.7)	1357 (4.5)	202 (6.2)	1108 (4.6)
COPD, n (%)	21 (1.3)	237 (1.5)	28 (1.8)	295 (1.9)	56 (2.3)	392 (1.6)	74 (2.4)	454 (1.5)	93 (2.8)	382 (1.6)
Depression, n (%)	12 (0.7)	51 (0.3)	22 (1.4)	127 (0.8)	39 (1.6)	246 (1.0)	50 (1.6)	394 (1.3)	78 (2.4)	385 (1.6)
IBD, n (%)	14 (0.9)	52 (0.3)	15 (1.0)	85 (0.6)	20 (0.8)	142 (0.6)	28 (0.9)	177 (0.6)	41 (1.3)	138 (0.6)
Diabetes, n (%)	33 (2.0)	213 (1.3)	59 (3.8)	278 (1.8)	82 (3.3)	509 (2.1)	105 (3.5)	665 (2.2)	157 (4.8)	546 (2.3)
Uveitis, n (%)	8 (0.5)	15 (0.1)	15 (1.0)	40 (0.3)	11 (0.4)	45 (0.2)	24 (0.8)	49 (0.2)	39 (0.2)	33 (1.0)

*Only included in the analysis of the cumulative incidence of surgery as patients diagnosed in this period did not have a full 5 years of follow-up.
COPD, chronic obstructive pulmonary disease; CVD, cardiovascular disease; IBD, inflammatory bowel disease.

Table 2 Incidence of any first joint surgery during 5 years of follow-up in each calendar period and calculated incidence rate ratios

Calendar period	Joint surgeries (n)		Person-years (pyrs)		Incidence rate per 1000 pyrs (95% CI)*		Incidence rate ratio (95% CI) within each cohort using the first calendar cohorts as references		Incidence rate ratio (95% CI) comparing PsA with GPC in each calendar period	
	PsA	GPC	PsA	GPC	PsA	GPC	PsA	GPC	PsA	GPC
1996–2000	145	536	7966	74092	18.2 (15.4 to 21.4)	7.2 (6.6 to 7.9)	Ref	Ref	2.4 (2.0 to 2.9)	Ref
2001–2004	160	643	7573	70850	21.1 (18.0 to 24.7)	9.1 (8.4 to 9.8)	1.2 (0.9 to 1.5)	1.3 (1.1 to 1.4)	2.3 (1.9 to 2.7)	Ref
2005–2008	264	1261	12128	115198	21.7 (19.2 to 24.6)	11.0 (10.4 to 11.6)	1.2 (1.0 to 1.5)	1.5 (1.4 to 1.7)	2.0 (1.7 to 2.3)	Ref
2009–2012	316	1536	14921	143524	21.1 (18.9 to 23.7)	10.7 (10.2 to 11.3)	1.2 (1.0 to 1.4)	1.5 (1.3 to 1.6)	2.0 (1.7 to 2.2)	Ref

*Age and sex adjusted incidence rates.
GPC, general population cohort; PsA, psoriatic arthritis.

trends analysis. We subsequently calculated incidence rate ratios (IRR) internally for the PsA calendar cohorts using the 5-year age and sex-standardised IR with the 1996–2000 PsA cohort as a reference. The same calculations were carried out for the corresponding GPC calendar cohorts. We furthermore compared the IR of surgery among PsA patients and GPC within each calendar period of interest by calculating IRRs with GPC as a reference in each calendar period. In the individual analysis patients and GPCs could only contribute with one event, the first surgery.

Cumulative incidence

The cumulative incidence of any first joint surgery was estimated in all PsA patients diagnosed, and GPCs matched between 1996 and 2017. For this, we used the Aalen-Johansen estimator.[21] In these analyses, patients and GPCs were followed up from date of diagnosis to the time of any first joint surgery, death, emigration, or end of 2017, whichever occurred first.

In a secondary analysis, we estimated the cumulative incidence of a first joint sacrificing surgery. Lastly, the age-specific cumulative incidence proportion of any joint, joint and non-joint sacrificing surgery was estimated stratified by age at diagnosis (18–40, 40–60, 60+ years).

RESULTS

In total, 11 960 PsA patients and 109 296 GPCs (mean age for PsA 50.0 years and 49.6 for GPC; 57% female in both groups) were identified between 1996 and 2017 (table 1).

The number of incidents PsA patients doubled from 2001–2004 to 2013–2017. A higher proportion of patients with PsA suffered from comorbidities compared with GPC across all calendar periods, and the proportion of PsA patients with comorbidities increased from 1996 to 2012.

Time-trends in joint surgery

Table 2 shows the 5-year IR of any joint surgery in patients diagnosed with PsA within each calendar period compared with GPC. The IR of surgery was doubled in the PsA population in all calendar periods ranging from 18.2 (15.4–21.4) surgeries to 21.7 (19.2–24.6) surgeries per 1000 pyrs. The IRR of a first joint surgery was twice as high for PsA patients compared with GPCs across all calendar periods.

Similarly, the overall IR of joint and non-joint sacrificing surgery was 10.7 (9.8–11.7) and 13.0 (12.0–14.2) per 1000 pyrs, respectively in the PsA population compared with an overall IR of 3.7 (3.5–3.9) and 7.0 (6.8 to 7.3) in GPC from 1996 to 2012. The IR of non-joint sacrificing surgery was higher than joint sacrificing surgery within the PsA group except in 2001–2004. The increase in joint sacrificing surgery from 2001 to 2004 is also reflected in higher IR of THA and TKA among PsA patients during that period (online supplementary figure S1).

The IR of surgery to the lower extremities was higher than for upper extremities in both PsA and GPC cohorts for all calendar periods (online supplementary figure S2).

Cumulative incidence of surgery

The cumulative incidence proportion of any joint surgery was 1.7 (1.4–2.0), 10.4 (9.6–11.2) and 28.9% (27.2–30.6) among PsA patients after 5, 10 and 15 years of disease duration, respectively. Among GPCs, the corresponding proportions were 0.5 (0.5–0.6), 4.6 (4.5–4.8) and 14.6% (14.2–15.1) at 5, 10 and 15 years of follow-up (figure 1). A similar increased cumulative incidence of joint sacrificing surgery (figure 1) was observed at all timepoints. The proportion of PsA patients who had THA and

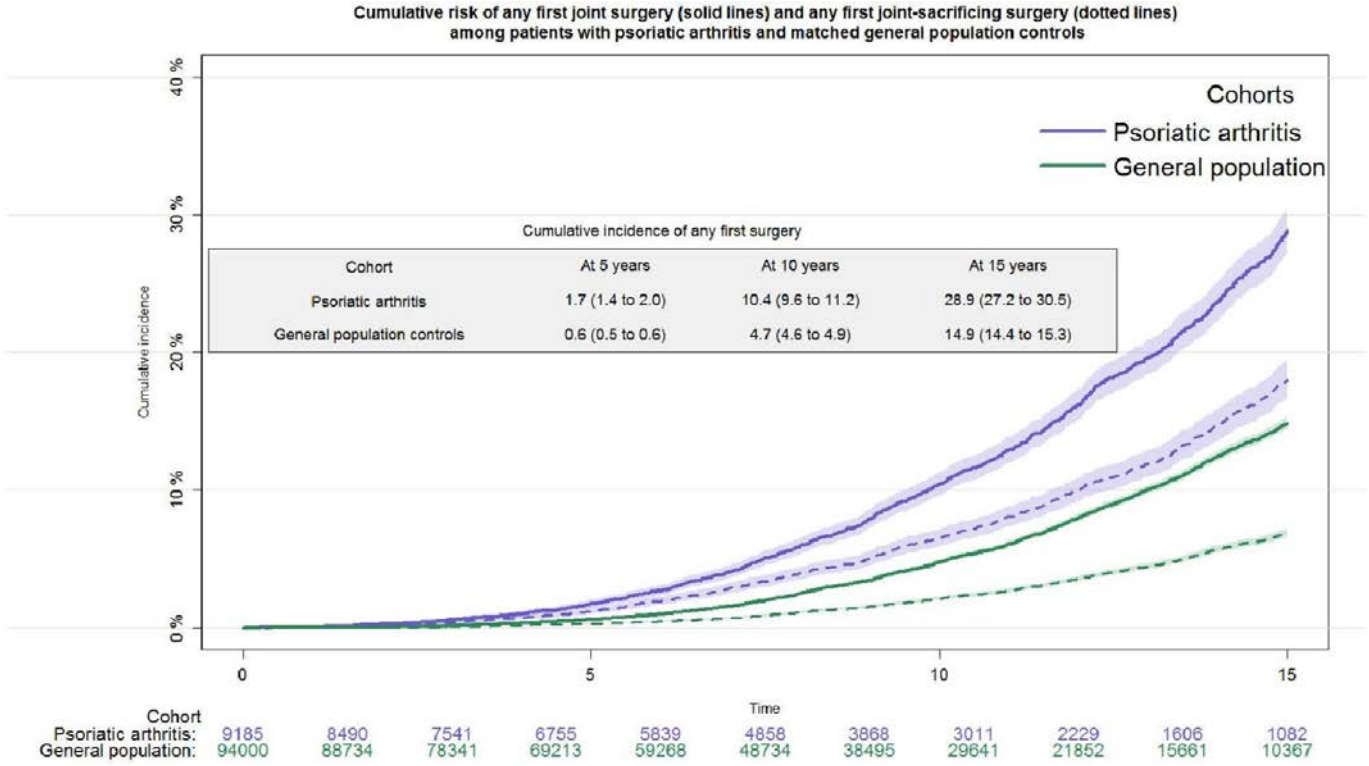

Figure 1 Cumulative incidence proportion of any first joint surgery (solid lines) and first joint-sacrificing surgery (dotted lines) among patients with psoriatic arthritis and matched general population controls using the Aalen-Johansen estimator.

TKA 15 years following diagnosis were 6.7 (5.8–7.6) and 6.9% (6.0–7.8).

The cumulative incidence of any joint related surgery stratified by age categories at PsA diagnosis (figure 2) showed that at 15 years of follow-up all age strata of PsA patients were of higher risk of surgery than any GPC age strata. Notably, the risk of surgery in PsA patients diagnosed at 18–40 years was higher than in GPC 60+ year old after 15 years of follow-up, 22.4 (19.3–25.6) and 19.9% (18.7–20.9), respectively.

Already after 5 years of follow-up, the cumulative incidence of joint sacrificing surgery among PsA patients was almost higher in the age group of 40–60 years 1.2% (0.9–1.6)

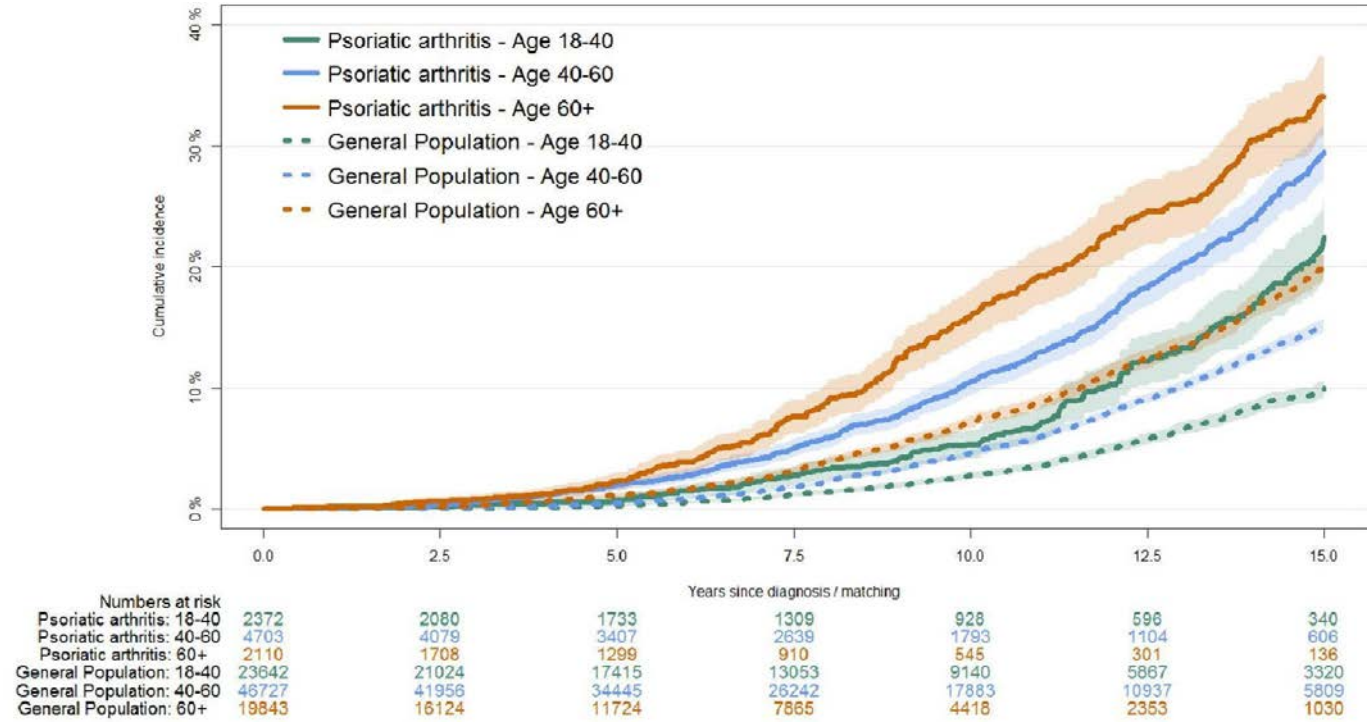

Figure 2 Cumulative incidence of any joint surgery during follow-up according to age strata of 18–40 years, 40–60 years and 60+ years at the time of psoriatic arthritis diagnosis (solid lines) compared with age-matched general population cohort (dotted lines).

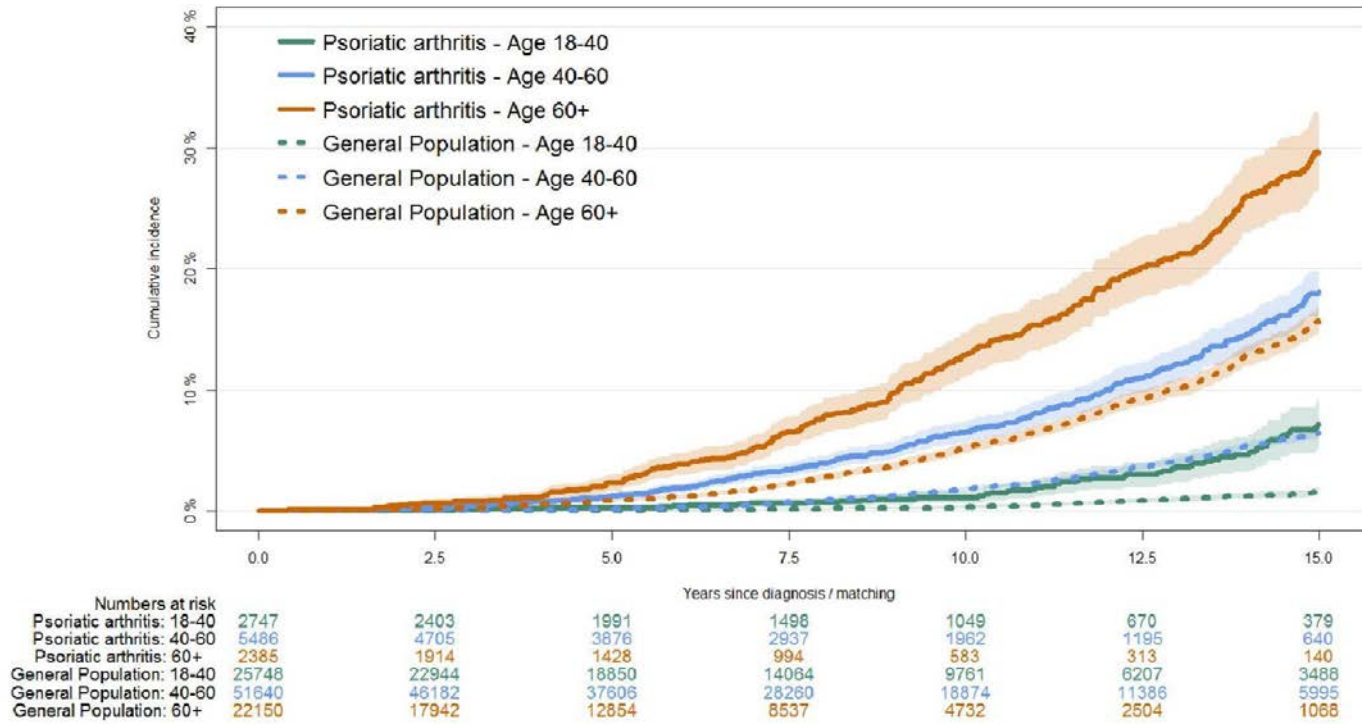

Figure 3 Cumulative incidence of joint sacrificing surgery over time according to age strata of 18–40 years, 40–60 years and 60+ years at the time of psoriatic arthritis diagnosis (solid lines) compared with age-matched general population cohort (dotted lines).

compared with GPC at 60+0.9% (0.7–1.0) (figure 3). For the young age group 18–40 years the relative difference in joint sacrificing surgery was 7.1 (5.2–9.1) in PsA against 1.6% (1.3–2.0) in GPC. The highest cumulative incidence of THA and TKA was found in PsA patients diagnosed at 60+ years compared with all other groups (online supplementary figures S3 and S4). PsA patients diagnosed age 40–60 years the risk of TKA 7.2 (5.9–8.6) was higher than GPC matched at age 60+5.6 (5.0–6.3).

After 15 years of disease duration, all PsA patients age groups were at higher risk of non-joint sacrificing surgery compared with any age group of GPCs (figure 4).

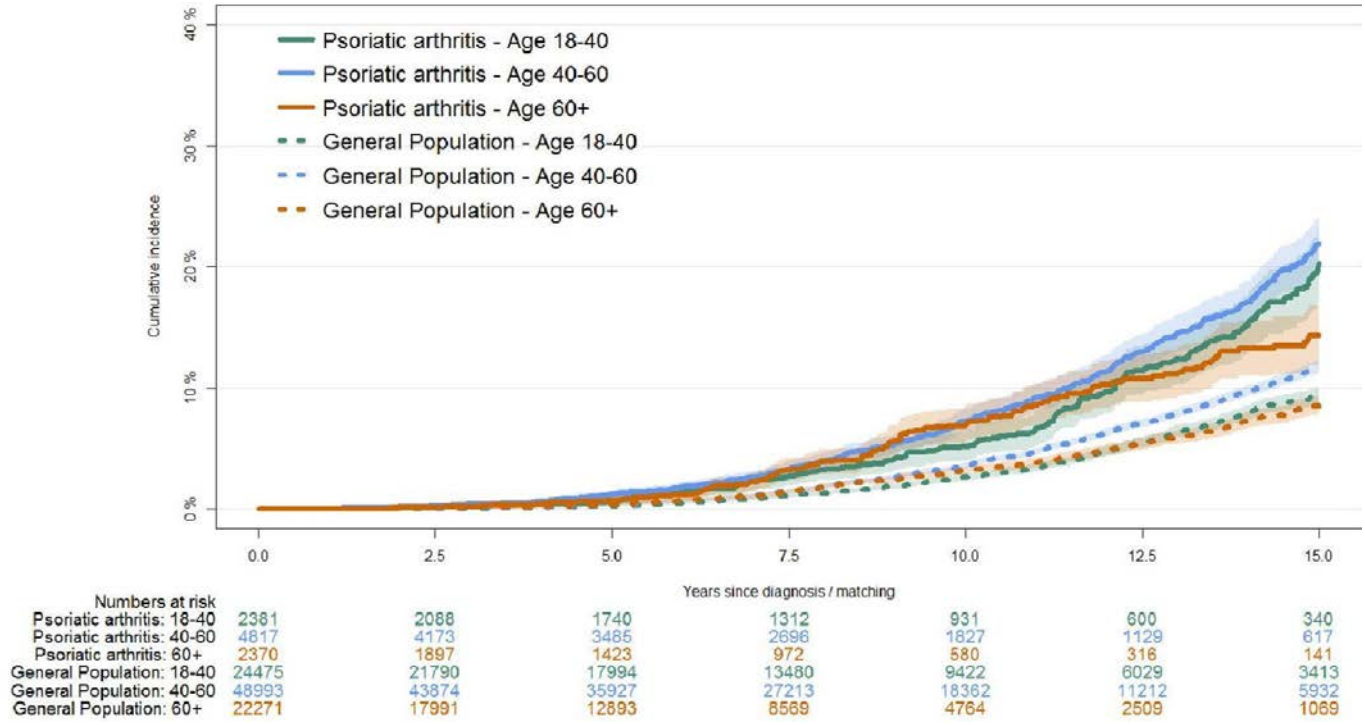

Figure 4 The cumulative incidence of non-joint sacrificing surgery over time according to age strata of 18–40 years, 40–60 years and 60+ years at the time of psoriatic arthritis diagnosis (solid lines) compared with age-matched general population cohort (dotted lines).

DISCUSSION

In this nationwide cohort study of PsA patients, we demonstrated an almost constant 5-year IR in any joint surgery from 1996 to 2012. The trend of surgery was almost similar to that of the GPC but with more than twice as high rates among PsA patients at any time point. The cumulative incidence of surgery was much higher in PsA patients, and almost 30% received joint surgery after 15 years of disease duration compared with 15% among GPCs. Even a person diagnosed with PsA between the age of 18–40 years had a higher risk of surgery than a non-PsA 60+ year old after 15 years of follow-up. The 5-year IR of joint surgery in the Danish PsA population remained high opposed to a decrease in incidence in a Danish RA population in the bDMARD era.[14]

It is a puzzle why surgical rates remain high in PsA. Evidence exists that bDMARDs has been less aggressively implemented in PsA,[22] and an intensive treat-to-target strategy have been much harder to exercise due to the heterogeneous nature of the disease and adherence to different composite measures of disease activity. This lack of strict guidelines may offer some explanation of why an efficacious treatment introduced at a national level in Denmark only show a yearly treatment initiation of 118 patients on average.[16] Hopefully, the benefits of the treatment have yet to come and treat-to-target strategies have emerged,[23] and gradually sees implementation. It can also be speculated that the choice of surgery is decided more on subjective markers of disease such as Visual Analogue Scale-pain and tender joints which in PsA seem less impacted by the current treatment regimes.[24]

Our observed high surgical rates at a population level is in alignment with an observational study from Norway by Nystad *et al*,[17] which do not detect a decrease in joint surgery even after the introduction of bDMARDS in 1999. It was speculated that it was too early to identify a change in surgical prognosis in patients in their cohort diagnosed from 1999 and onward. Lewinson *et al* explained an increase in surgery in the bDMARD era with better disease control in PsA unveiling coexisting osteoarthrosis which perhaps was augmented by higher healthcare surveillance in PsA patients.

Few studies have previously looked at the utilisation of joint surgery in PsA. In a single-centre study, Zangger *et al* found that 7% of a PsA cohort required surgery after a mean disease duration of 13.9 years.[25] In comparison, our study showed that 15% needed surgery after 15 years.

In contrast, a cross-sectional study by Haque *et al* showed that 48% required surgical intervention at a mean disease duration of 1.6±12.1 years.[26] The high proportion of patients who had surgery in that cohort may in part be generated by the inclusion of non-joint sacrificing surgery of more diagnostic than therapeutic impact such as diagnostic arthroscopy or other surgeries due to injuries not necessarily attributed to PsA pathophysiology. These types of surgery were excluded from our data, and in comparison, we found cumulative incidences of non-joint surgery of 3, 12.5% and 20% at 5, 10 and 15 years after diagnosis, respectively. The short mean disease duration before surgery found by Haque could be attributed to the fact that almost 27% of the surgery was done before the PsA diagnosis was established due to the cross-sectional design. A recent study by Lewinson *et al* found an increasing IR of arthroplasty in PsA from 1995 to 2010.[27] Our study also noticed an increase in arthroplasty around 2003 but opposed to Lewinson we then observed a decrease in IR especially in THA from 2003 which dropped slightly from 4.8 to 3.3 in 2012. We have no clear explanation for an increase in joint arthroplasty in 2001–2004, but a change in government in late 2001 and increased political focus on bringing down waiting lists for elective surgeries offer at least one potential explanation.

Limitations

In register-based studies, there is an intrinsic risk of misclassification bias which we tried to minimise by including only patients diagnosed at an inpatient or outpatient facility specialised in rheumatology or general internal medicine according to the DNPR. Using DNPR as a source register for identification of PsA is potentially biased towards a selection of more severe cases while patients with mild disease who are managed entirely at primary care units are not included. However, a previous study in Sweden, a country comparable to Denmark, have proven this to be a minor concern and would only increase the number of cases by <4%, and increase the degree of misclassification.[28] Misclassification may have the potential to influence our findings by an increase in the completeness of diagnostic and surgical coding over time but we find temporal trends in misclassification unlikely to significantly have affected our results. Our design did not allow to adjust for risk factors such as osteoarthritis and fractures, and we did not have access to information on body mass index. Nevertheless, the purpose was also to give a descriptive account of the need for surgery in PsA.

The mean age at onset PsA in our cohort is at the beginning of the fifth decade and there is a risk that surgical rates may be influenced by age-related osteoarthritis but the high rate of surgery in PsA compared with GPC as presented in this study is still staggering even though the indication is osteoarthritis or erosive joint damage. The mean age for TKA and THA surgery with osteoarthritis as the indication is in Denmark 69 years,[29] which is at the end of the sixth decade and in this context the PsA cohort in our study have a higher cumulative risk of surgery at a younger age. Because of the nature of observational studies, we cannot ascertain whether our results reflect changes in referral patterns, a shift in diagnostic focus, for example, the introduction of the CASPAR criteria. To investigate the impact of bDMARDs on the need for joint surgery, further studies using individual-level based information on DMARD treatment are needed.

Strengths

Our study benefits from a large nationwide population-based cohort of patients with PsA with access to complete 15-year follow-up period which encompasses the outcome of the csDMARD era and the beginning of the bDMARD era. We compared our results with age and gender-matched GPC to account for secular trends.

In conclusion, we found that the 5-year IR of joint related surgery in PsA did not change substantially from 1996 to 2012; however, during this period, the incidence remained twice as high as observed in matched GPCs. Thus, representing an important unmet need and treatment outcome to monitor in future studies. After 15 years of follow-up, 29% of the PsA patients had received surgery.

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Contributors JG-M, RLC and LD had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. JG-M, RLC and LD: study concept and design. RLC and LD: acquisition of data. RLC: statistical analysis. All authors: interpretation of data; critical revision of the manuscript for important intellectual content. JG-M: drafting of the manuscript. LD and LEK: study supervision.

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REFERENCES

1 Slobodin G, Rosner I, Rozenbaum M, *et al*. Psoriatic arthropathy: where now? *Isr Med Assoc J* 2009;11:430–4.
2 Gladman DD, Antoni C, Mease P, *et al*. Psoriatic arthritis: epidemiology, clinical features, course, and outcome. *Ann Rheum Dis* 2005;64(suppl_2):ii14–17.
3 Gladman DD, Day MS, Nam D, *et al*. Psoriatic arthritis. *J Am Acad Orthop Surg* 2005;20:118–23.
4 Alonso JCT, Perez AR, Castrillo JMA, *et al*. Psoriatic arthritis (PA): a clinical, immunological and radiological study of 180 patients. *Rheumatology* 1991;30:245–50.
5 Kristensen LE, Englund M, Neovius M, *et al*. Long-Term work disability in patients with psoriatic arthritis treated with anti-tumour necrosis factor: a population-based regional Swedish cohort study. *Ann Rheum Dis* 2013;72:1675–9.
6 Kristensen LE, Jørgensen TS, Christensen R, *et al*. Societal costs and patients' experience of health inequities before and after diagnosis of psoriatic arthritis: a Danish cohort study. *Ann Rheum Dis* 2017;76:1495–501.
7 Rahman P, Nguyen E, Cheung C, *et al*. Comparison of radiological severity in psoriatic arthritis and rheumatoid arthritis. *J Rheumatol* 2001;28:1041–4.
8 Kingsley GH, Kowalczyk A, Taylor H, *et al*. A randomized placebo-controlled trial of methotrexate in psoriatic arthritis. published online first: 10AD.
9 Eder L, Thavaneswaran A, Chandran V, *et al*. Tumour necrosis factor α blockers are more effective than methotrexate in the inhibition of radiographic joint damage progression among patients with psoriatic arthritis. *Ann Rheum Dis* 2014;73:1007–11.
10 Antoni C, Krueger GG, de Vlam K, *et al*. Infliximab improves signs and symptoms of psoriatic arthritis: results of the impact 2 trial. *Ann Rheum Dis* 2005;64:1150–7.
11 Mease PJ, Ory P, Sharp JT, *et al*. Adalimumab for long-term treatment of psoriatic arthritis: 2-year data from the adalimumab effectiveness in psoriatic arthritis trial (ADEPT) 2008.
12 Sterry W, Ortonne J-P, Kirkham B, *et al*. Comparison of two etanercept regimens for treatment of psoriasis and psoriatic arthritis: PRESTA randomised double blind multicentre trial. *BMJ* 2010;340.
13 Kristensen LE, Gülfe A, Saxne T, *et al*. Efficacy and tolerability of anti-tumour necrosis factor therapy in psoriatic arthritis patients: results from the South Swedish arthritis treatment group register. *Ann Rheum Dis* 2008;67:364–9.
14 Cordtz RL, Hawley S, Prieto-Alhambra D, *et al*. Incidence of hip and knee replacement in patients with rheumatoid arthritis following the introduction of biological DMARDs: an interrupted time-series analysis using nationwide Danish healthcare registers. *Ann Rheum Dis* 2018;77:684–9.
15 Egeberg A, Kristensen LE, Thyssen JP, *et al*. Incidence and prevalence of psoriatic arthritis in Denmark: a nationwide register linkage study. *Ann Rheum Dis* 2017;76:1591–7.
16 Glintborg B, Østergaard M, Dreyer L, *et al*. Treatment response, drug survival, and predictors thereof in 764 patients with psoriatic arthritis treated with anti-tumor necrosis factor α therapy: results from the nationwide Danish DANBIO registry. *Arthritis Rheum* 2011;63:382–90.
17 Nystad TW, Husum YS, Furnes ON, *et al*. Incidence and predictive factors for orthopedic surgery in patients with psoriatic arthritis. *J Rheumatol* 2018;45:1532–40.
18 Nicholls S, Langan S, Sørensen HT, *et al*. The record reporting guidelines: meeting the methodological and ethical demands of transparency in research using routinely-collected health data. *Clin Epidemiol* 2016;8:389–92.
19 Schmidt M, Pedersen L, Sørensen HT. The Danish civil registration system as a tool in epidemiology. *Eur J Epidemiol* 2014;29:541–9.
20 NOMESCO. *Classification of surgical procedures. version 115 2011: no.93*. Copenhagen, 2010.
21 Aaalen O, Johansen S. An empirical transition matrix for non-homogeneous Markov chains based on censored observations. *Scand J Stat* 1978.
22 Jørgensen T, Dreyer L, Guðbjörnsson B. Prescription patterns of tumour necrosis factor inhibitor and ustekinumab in psoriatic arthritis: a Nordic population-based cohort study. *Ann Rheum Dis* 2017;76.
23 Coates LC, Navarro-Coy N, Brown SR, *et al*. The TICOPA protocol (tight control of psoriatic arthritis): a randomised controlled trial to compare intensive management versus standard care in early psoriatic arthritis. *BMC Musculoskelet Disord* 2013;14:101.
24 Højgaard P, Christensen R, Dreyer L, *et al*. Pain mechanisms and ultrasonic inflammatory activity as prognostic factors in patients with psoriatic arthritis: protocol for a prospective, exploratory cohort study. *BMJ Open* 2016;6:e010650.
25 Zangger P, Gladman DD, Bogoch ER. Musculoskeletal surgery in psoriatic arthritis. *J Rheumatol* 1998;25:725–9.
26 Haque N, Lories RJ, de Vlam K. Orthopaedic interventions in patients with psoriatic arthritis: a descriptive report from the SPAR cohort. *RMD Open* 2016;2.
27 Lewinson RT, Vallerand IA, LaMothe JM, *et al*. Increasing rates of arthroplasty for psoriatic arthritis in the United Kingdom between 1995 and 2010. *Arthritis Care Res*.
28 Jordan KP, Jöud A, Bergknut C, *et al*. International comparisons of the consultation prevalence of musculoskeletal conditions using population-based healthcare data from England and Sweden. *Ann Rheum Dis* 2014;73:212–8.
29 Cordtz RL, Højgaard P, Zobbe K, *et al*. Predictors of revision, prosthetic joint infection and mortality following total hip or total knee arthroplasty in patients with rheumatoid arthritis: a nationwide cohort study using Danish healthcare registers. *Ann Rheum Dis* 2019;77:281–8.

TRANSLATIONAL SCIENCE

Fexofenadine inhibits TNF signaling through targeting to cytosolic phospholipase A2 and is therapeutic against inflammatory arthritis

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ABSTRACT

Objective Tumour necrosis factor alpha (TNF-α) signalling plays a central role in the pathogenesis of various autoimmune diseases, particularly inflammatory arthritis. This study aimed to repurpose clinically approved drugs as potential inhibitors of TNF-α signalling in treatment of inflammatory arthritis.

Methods In vitro and in vivo screening of an Food and Drug Administration (FDA)-approved drug library; in vitro and in vivo assays for examining the blockade of TNF actions by fexofenadine: assays for defining the anti-inflammatory activity of fexofenadine using TNF-α transgenic (TNF-tg) mice and collagen-induced arthritis in DBA/1 mice. Identification and characterisation of the binding of fexofenadine to cytosolic phospholipase A2 (cPLA2) using drug affinity responsive target stability assay, proteomics, cellular thermal shift assay, information field dynamics and molecular dynamics; various assays for examining fexofenadine inhibition of cPLA2 as well as the dependence of fexofenadine's anti-TNF activity on cPLA2.

Results Serial screenings of a library composed of FDA-approved drugs led to the identification of fexofenadine as an inhibitor of TNF-α signalling. Fexofenadine potently inhibited TNF/nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signalling in vitro and in vivo, and ameliorated disease symptoms in inflammatory arthritis models. cPLA2 was isolated as a novel target of fexofenadine. Fexofenadine blocked TNF-stimulated cPLA2 activity and arachidonic acid production through binding to catalytic domain 2 of cPLA2 and inhibition of its phosphorylation on Ser-505. Further, deletion of cPLA2 abolished fexofenadine's anti-TNF activity.

Conclusion Collectively, these findings not only provide new insights into the understanding of fexofenadine action and underlying mechanisms but also provide new therapeutic interventions for various TNF-α and cPLA2-associated pathologies and conditions, particularly inflammatory rheumatic diseases.

Key messages

What is already known about this subject?

- TNF-α signaling is known to play a central role in the pathogenesis of various autoimmune diseases, particularly inflammatory arthritis, and TNF-α inhibitors (TNFI), including infliximab (Remicade), and adalimumab (Humira), have been accepted as effective anti-inflammatory therapies and are among the most successful biotech pharmaceuticals.

What does this study add?

- This study identifies fexofenadine as an inhibitor of tumour necrosis factor alpha (TNF-α) signalling, and uncovers a new strategy for inhibiting this cardinal pathway of inflammation. Thus, fexofenadine may be used for treating various TNF-associated diseases and conditions, particularly rheumatoid arthritis.
- This study identifies cytosolic phospholipase A2 (cPLA2) as a new target of fexofenadine, thus advancing our understanding of fexofenadine's action and underlying mechanisms, and providing a solid foundation for future discoveries relating to the fexofenadine/cPLA2 interaction in various conditions.

INTRODUCTION

Autoimmune diseases are a series of disorders and conditions caused by immune intolerance to self-antigens which attack specific target organs and display diverse clinical signs.[1 2] Inflammatory arthritis is the most common autoimmune disease, affecting about 1% of the population.[3] Autoimmune diseases are chronic diseases with complicated pathology and diverse clinical signs, underlying which are alterations in cytokine expression and immune cell infiltration. Among the proinflammatory cytokines involved, tumour necrosis factor alpha (TNF-α) has received great attention due to its position at the apex of the proinflammatory cytokine cascade and its dominance in the pathogenesis of various disease processes,[4 5] particularly autoimmune disorders.[6 7] TNF-α inhibitors (TNFI), including etanercept (Enbrel), infliximab (Remicade) and adalimumab (Humira), have been accepted as effective anti-inflammatory therapies and are among the most successful biotech pharmaceuticals.[8–10] Although treatment with TNFI is highly effective in ameliorating disease in some patients, current TNFI fail to provide effective treatment for up to 50% of patients.[11 12] In addition to high cost (upwards of US$20 000 per year per patient using anti-TNF biologics), available TNFI have been found to contribute to infection risk in

Key messages

How might this impact clinical practice or future developments?

- This study identifies fexofenadine as a more effective drug than methotrexate in inflammatory arthritis models. Due to the different mechanisms of action (eg, targeting both cPLA2 and histamine H1 receptor 1), fexofenadine may be effective for patients who fail to respond to current TNF-α blockers. Additionally, fexofenadine is safer (non-prescription medicine), more convenient (taken orally) and cost-effective.
- This study also identifies fexofenadine as a novel antagonist of cPLA2, suggesting that fexofenadine may also be used for treating various cPLA2-associated diseases, including autoimmune diseases, neurodegenerative diseases, cardiovascular diseases and cancers.
- This study revealing that fexofenadine inhibits cPLA2 and is effective in preclinical animal models may overcome current bottlenecks in efforts to develop therapeutic cPLA2 inhibitors.

some patients[13] and association with a slight increased risk of squamous cell cancer has been reported in rheumatoid arthritis patients treated with TNFI.[14] Thus, identification and characterisation of novel, safer and more cost-effective antagonists of TNF-α, in particular antagonists with different inhibitory properties, are of great importance from both a pathophysiological and a therapeutic standpoint. Considering the fact that drug development is time-consuming and extremely expensive, costing ~15 years in time and US$800 million on average,[15] we adopted a strategic approach involving the repurposed use of clinically approved drugs. A drug library composed of FDA-approved drugs was screened both in vitro and in vivo by use of TNF-α/NF-κB reporter constructs and mice, which led to the identification of terfenadine and its active metabolite fexofenadine as inhibitors of TNF-α signalling.

Terfenadine and fexofenadine are two well-known histamine receptor 1 antagonists and used for treating allergic diseases.[16] Terfenadine, a first-generation antihistamine drug, has been clinically suspended due to potential adverse events. In contrast, fexofenadine, the major active metabolite of terfenadine and a non-sedative third-generation antihistamine drug,[17] does not carry the proarrhythmic risk associated with use of terfenadine and is marketed as an over-the-counter (OTC) drug due to its safety. Fexofenadine has been widely used to treat various allergic diseases, like allergic rhinitis, conjunctivitis and chronic idiopathic urticaria.[16–19]

In our efforts to elucidate the molecular mechanisms underlying fexofenadine-mediated inhibition of TNF-α signalling, we identified cytosolic phospholipase A2 (cPLA2) as a novel target of fexofenadine. The major function of cPLA2 is to promote phospholipid hydrolysis-mediated production of arachidonic acid (AA);[20] AA activates NF-κB[21 22] and is involved in the pathogeneses of various conditions, including inflammatory and autoimmune diseases.[23]

Herein, we present comprehensive evidences demonstrating that fexofenadine acts as the inhibitor of TNF/NF-κB signalling and is therapeutic against inflammatory arthritis. Additionally, we also provide evidences revealing that this drug bound to cPLA2 and inhibited its enzymatic activity, which is required for its inhibition of TNF-α signalling.

RESULTS

Fexofenadine is identified as an antagonist of TNF-α and inhibits TNF-α signaling and activity

To isolate the small molecule drugs that inhibit canonical TNF-α/NF-κB signalling pathway, a drug library containing 1046 FDA-approved drugs was initially screened using a NF-κB-*bla* THP-1 cell line in which a NF-κB beta-lactamase reporter gene was stably integrated. Twenty-four drugs that potently inhibited TNF-α/NF-κB activation of beta-lactamase were identified after three independent implementations of this screening scheme (online supplementary figure S1a-b). These 24 isolates were subjected to a second round screen using RAW 264.7 macrophages transiently transfected with an NF-κB luciferase reporter gene. Under such conditions, only the most potent anti-TNF-α/NF-κB signalling drugs are positively screened. Eight drugs among the 24 candidates originally isolated were selected (online supplementary figure S2a-b). In order to identify the drugs that retain anti-TNF-α/NF-κB activity in vivo, we performed a third round screen with NF-κB-Luc reporter mice. We first crossed TNF-α transgenic (TNF-tg) to NF-κB-Luc reporter mice to generate TNF-tg:NF-κB-Luc double mutant mice. Overexpression of TNF-α effectively activated NF-κB luciferase in vivo. In Vivo Imaging System (IVIS) was implemented for whole animal bioluminescence imaging following intraperitoneal injection of eight selected drugs into TNF-tg:NF-κB-Luc double mutant mice. Five drugs, including terfenadine and its active metabolite fexofenadine, were shown to effectively inhibit TNF-tg:NF-κB activated luciferase in vivo (online supplementary figure S3). Among these five drugs, three, including one anticancer drug, are known to have severe side-effects and are not suitable for treating chronic inflammatory diseases, such as rheumatoid arthritis, we accordingly selected fexofenadine and terfenadine (serving as a comparison with fexofenadine) for further analyses (figure 1A).

We first examined the inhibition of fexofenadine on TNF-α-activated NF-κB pathway and downstream genes through RNA-seq with bone marrow-derived macrophages (BMDMs) (figure 1B–C, online supplementary figure S4). Nearly, all TNF-α-induced genes, especially genes encoding inflammatory cytokines, such as IL-1β, IL-6, were clearly downregulated by fexofenadine and terfenadine. The lists of TNF-α inducible genes that were inhibited by fexofenadine were used for transcription factor enrichment analysis with TFactS,[24] which led to the isolation of NF-κB1 p105 and RelA p65 as transcription factors significantly regulated by fexofenadine (figure 1C).

In order to further validate the anti-TNF-α activity of fexofenadine, we next selected a couple of well-known TNF-α downstream inflammatory mediators for further assays. Quantitative real-time PCR revealed that both fexofenadine and terfenadine dose-dependently inhibited TNF-α-induced mRNA expressions of IL-1β, IL-6 and Nos-2 in BMDMs (figure 1D–F). Additionally, ELISA demonstrated that these two drugs abolished TNF-α induced releases of IL-1β and IL-6 in a dose-dependent manner (figure 1G–H). Similar anti-TNF activity of fexofenadine and terfenadine was also observed in RAW264.7 cells (online supplementary figure S5a-b) and BMDMs isolated from TNF-tg mice (online supplementary figure S6a-b). TNF-α is known to enhance RANKL-stimulated osteoclastogenesis.[25] Both fexofenadine and terfenadine markedly inhibited TNF-α-mediated osteoclastogenesis in BMDMs (figure 1I), but not RANKL-induced osteoclastogenesis (online supplementary figure S7). Moreover, in vivo dose-dependent inhibition of the TNF-α/NF-κB pathway by fexofenadine and terfenadine was also revealed by use of

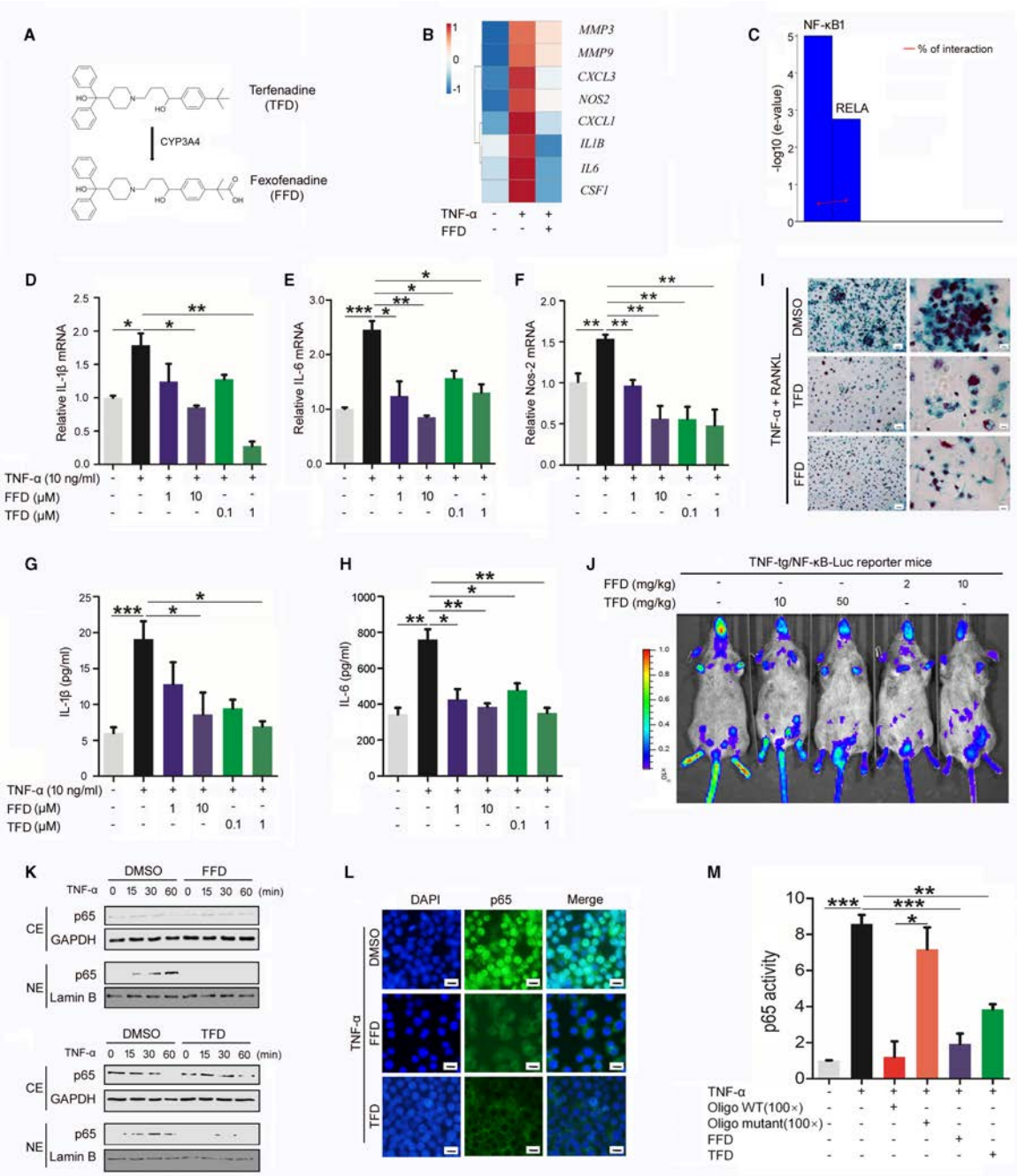

Figure 1 Fexofenadine acts as the antagonists of TNF-α and inhibit TNF-α signalling and activity. (A) The molecular structure of fexofenadine (FFD) and terfenadine (TFD). CYP3A4, the major enzyme responsible for the metabolic process, is indicated. (B) BMDMs were treated without or with (10 ng/mL) in absence or presence of FFD (10 μM) for 24 hours. Total RNA was extracted for RNA-seq. A few typical TNF-α inducible genes that were suppressed by FFD were presented. (C) Transcription factor enrichment analysis from RNA-seq results, indicating the decreased gene expressions resulted from the suppressed activity of transcription factors NF-κB1 and RELA by FFD. (D-F) BMDMs were treated with or without TNF-α (10 ng/mL) in absence or presence of FFD (1 μM, 10 μM)/TFD (0.1 μM, 1 μM) for 24 hours. mRNA expressions of IL-1β, IL-6 and Nos-2 were tested by qRT-PCR. (G–H) BMDMs were treated without or with TNF-α (10 ng/mL) in absence or presence of FFD (1 μM, 10 μM)/TFD (0.1 μM, 1 μM) for 48 hours. The levels of IL-1β and IL-6 in supernatant were detected by ELISA. (I) BMDMs were treated with M-CSF (10 ng/mL) for 3 days, then cultured with receptor activator of nuclear factor kappa-B ligand (RANKL) (100 ng/mL) and TNF-α (10 ng/mL) with or without FFD (10 μM) or TFD (1 μM) for 4 days and tartrate-resistant acid phosphatase (TRAP) staining was performed. Scale bar, 100 μm. (J) TNF-tg/NF-kB-Luc mice were applied to examine the anti-TNF effects of FFD/TFD in vivo. After FFD (2 or 10 mg/kg) and TFD (10 or 50 mg/kg) were orally administrated for 7 days, luciferase signals were detected by IVIS system. (K) BMDMs were treated with TNF-α (10 ng/mL) in the absence or presence of FFD (10 μM)/or TFD (1 μM) for various time points, as indicated. Cytoplasmic extraction (CE) and nuclear extraction (NE) were examined by Western blot with anti-p65 antibody. (L) BMDMs were cultured with TNF-α (10 ng/mL) in the absence or presence of FFD (10 μM) or TFD (1 μM) for 6 hours. Immunofluorescence cell staining was performed to visualise the subcellular localisation of p65. 4′,6-diamidino-2-phenylindole (DAPI) was used to stain the nucleus. Scale bar, 25 μm. (M) p65 DNA binding activity was tested by ELISA. Excess amounts (100×) of wildtype (WT) and mutant oligo were used as positive and negative control, respectively (*p<0.05, **p<0.01, ***p<0.001). BMDMs, bonemarrow-derived macrophages; qRT-PCR, quantitativereal-time PCR; TNF-α, tumournecrosis factor alpha; TNF-tg, TNF-α transgenic.

TNF-tg/NF-κB-Luc reporter double mutant mice (figure 1j). Additionally, the TNF-α-induced nuclear translocation and DNA-binding activity of p65 were also inhibited by fexofenadine and terfenadine (figure 1K–M).

Fexofenadine prevents the spontaneous development of inflammatory arthritis in TNF-tg mice

TNF-tg mice are known to develop an inflammatory arthritis phenotype spontaneously when mice reach 12–16 weeks old.[25] Next, we sought to examine the effects of applying fexofenadine to TNF-Tg mice. First, 8-week-old TNF-tg mice were treated daily with fexofenadine, terfenadine or methotrexate (MTX, serving as a positive control) by oral delivery before the onset of the inflammatory arthritis phenotype. Both fexofenadine and terfenadine treatment resulted in reduction of all visual symptomatic signs (figure 2A) and significant reduction of clinical scores of arthritis; fexofenadine was proven to be more effective than MTX, the current clinically used small molecule drug for treating rheumatoid arthritis (figure 2B–C). In order to observe the response of inflammatory arthritis progression to fexofenadine and terfenadine, we stopped treatment at the 17-week-time point and resumed treatment at the 19-week-time point. Cessation of treatment led to an abrupt increase of the arthritis clinical scores. Once the treatment resumed, there was an immediate reduction in swelling score, indicating that the inflammatory arthritis induced by TNF-α overexpression responds well to both fexofenadine and terfenadine (figure 2B–C). H&E staining of ankle and knee tissues confirmed the inhibition of inflammatory degeneration (figure 2D). TRAP staining of paw and skull showed a preventative effect of treatment on osteoclast differentiation (figure 2E). In addition, the drugs reduced cartilage loss, as revealed by Safranin O staining of ankle and knee (figure 2F). We also measured the serum levels of IL-1β and IL-6 and found that the levels of these inflammatory cytokines were significantly reduced in fexofenadine-treated and terfenadine-treated groups compared with the control group (figure 2G–H).

To determine drug's therapeutic effects, we started treatment when the TNF-tg mice reached an average score of approximately eight points. Both fexofenadine and terfenadine showed effective therapeutic effects in a dose-dependent manner (figure 2I–J). Taken together, data from TNF-tg mice indicate that fexofenadine and terfenadine exert their anti-inflammatory and therapeutic effects through the inhibition of TNF-α activity in vivo.

Fexofenadine prevents the onset and progression of collagen-induced arthritis

To advance understanding of the preventive and therapeutic impact of fexofenadine on inflammatory arthritis in vivo, we utilised another mouse model of rheumatoid arthritis: collagen-induced arthritis (CIA), which has both immunological and pathological features with rheumatoid arthritis. The CIA model was established with 8-week-old male DBA/1J mice. We first started the treatment with fexofenadine, terfenadine, MTX or vehicle by oral delivery at 18 days after immunisation for examining their preventive effects. Severe inflammation and increased thickness in the ankles and paws were observed in the vehicle group compared with intervention groups (figure 3A–B). Analogously, fexofenadine and terfenadine could not only delay the onset of disease but also significantly decrease arthritis clinical scores and incidence (figure 3C–D). Histological analysis revealed less inflammation in treatment groups as compared with control group (figure 3E). Fewer osteoclasts and less bone destruction were detected in the treated groups, as revealed by TRAP staining and microCT images (figure 3F–G). Additionally, fexofenadine and terfenadine also prevented the loss of cartilage (figure 3H), and significantly reduced the serum levels of IL-1β and IL-6 (figure 3I–J).

To determine drug's therapeutic effects, we initiated treatment when the CIA model mice displayed a clinical score of ∼5 points of a maximum 16 points per animal.[26] Both fexofenadine and terfenadine dose-dependently ameliorated disease scores (figure 3K–L). Meanwhile, the serum levels of inflammatory cytokines IL-1β and IL-6 were significantly decreased in the treatment groups versus vehicle (figure 3M–P). Collectively, these data indicate that fexofenadine has both preventive and therapeutic effects in a well-accepted preclinical animal model for testing anti-rheumatoid arthritis (RA) drugs.

cPLA2 is a novel target of fexofenadine

The antihistaminic activity of fexofenadine and terfenadine are known to be mediated by targeting to their selective histamine H1 receptor 1 (H1R1).[27–29] We next sought to determine whether the anti-TNF activity of fexofenadine and terfenadine depends on their known target H1R1. We thus suppressed the expression of H1R1 using its specific siRNAs in RAW264.7 cells, and found, unexpectedly, that suppression of H1R1 did not affect the inhibition of fexofenadine and terfenadine on TNF-induced cytokine release (figure 4A,B). In addition, seven additional accepted H1R1 antagonists did not exhibit anti-TNF activity, with some even associated with increased TNF-induced IL-6 release (figure 4C,D). Collectively, these results indicate that anti-TNF activity of fexofenadine is H1R1 independent. Current clinically employed TNF inhibitors, such as etanercept (Enbrel), infliximab (Remicade) and adalimumab (Humira), exert their anti-TNF activity through disturbing the binding of TNF to its receptor tumour necrosis factor receptor 1 (TNFR1). Therefore, we next examined whether fexofenadine affected the interactions between TNF and TNFR1, leading to its anti-TNF activity. Surprisingly, both solid phase binding and flow cytometry assays showed that fexofenadine and terfenadine did not affect the binding of TNF-α to TNFR1 in vitro and to the cell surface, although anti-TNF antibody completely blocked the binding of TNF to the cell surface (figure 4E–G). These findings led us to propose that fexofenadine may have an additional unidentified target that mediates its anti-TNF activity through a previously unrecognised mechanism. To address this issue, we devoted significant efforts to isolate protein binding partners of fexofenadine. After failure with several approaches, including labelling and biochemical copurification, implementation of drug affinity responsive target stability (DARTS) assay[30] proved successful. We first mixed cell lysate with fexofenadine or terfenadine for 1 hour and protease was added for 15 min. The digested proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), followed by Silver staining (figure 5A), and a band with the molecular weight of ∼80 kDa was found to be protected by fexofenadine and terfenadine. This band was excised from an accompanying Coomassie blue stained gel for protein identification by mass spectrometry (figure 5B), which led to the identification of *PLA2G4A* encoding cPLA2 (figure 5C) and *IKBKB* encoding IKK-β as potential candidates. Both cPLA2 and IKK-β have appropriate molecular weights and are known to be the critical mediators of inflammation.[21 22 31] To determine whether both cPLA2 and IKK-β are the targets of fexofenadine and terfenadine, we performed Western blot of DARTS samples with which a series of protease to cell lysate

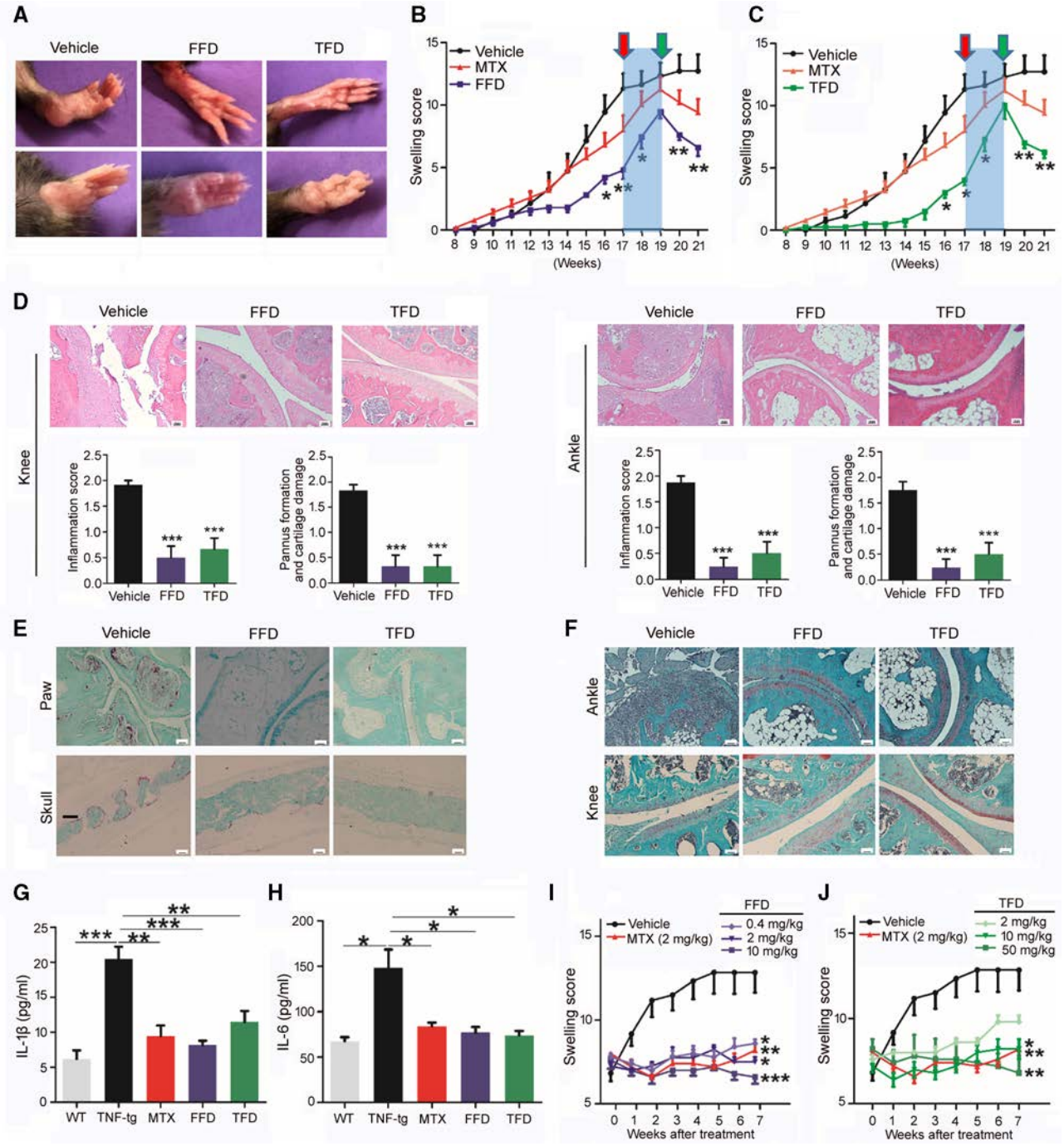

Figure 2 Fexofenadine prevents the spontaneous development of inflammatory arthritis in TNF transgenic mice. (A–H) TNF-tg mice (n=6) were orally administered fexofenadine (FFD, 10 mg/kg), terfenadine (TFD, 50 mg/kg) or methotrexate (MTX, 2 mg/kg, serving as a positive control) daily beginning at 8 weeks of age and continuing for a total of 13 weeks. During this period, treatment was halted at 17-week point indicated by red arrow and resumed at 19-week point indicated by green arrow. (A) Representative images of front paws and hind paws. (B–C) Swelling score. (D) H&E staining and quantification of histological score of knee and ankle samples. (E) TRAP staining of paw and skull samples. (F) Safranin O staining of knee and ankle samples. (G–H) Serum levels of IL-1β and IL-6, assayed by ELISA. (I–J) Therapeutic effects of FFD/TFD were tested by treating the TNF-tg mice with average swelling score reached around eight points (n=6). Swelling scores were recorded weekly (*p<0.05, **p<0.01, ***p<0.001) (scale bar, 100 µm). TNF-tg, TNF-α transgenic.

ratios were implemented (figure 5D), and found that fexofenadine and terfenadine protected cPLA2, but not IKK-β, clearly indicating that cPLA2, but not IKK-β, is a novel target of fexofenadine and terfenadine.

In order to further confirm the associations of cPLA2 with fexofenadine, we employed the cellular thermal shift assay (CETSA),[32 33] which allows for quantification of the change in thermal denaturation temperature of a target protein under different conditions, including those of varying temperature and concentrations of drug of interest. Both fexofenadine and terfenadine, particularly fexofenadine, prevent denaturation of cPLA2 and kept more cPLA2 in the soluble condition under several temperatures, strikingly obvious at 49°C, compared with DMSO (figure 5E, top). The melt curve showed a significant shift and

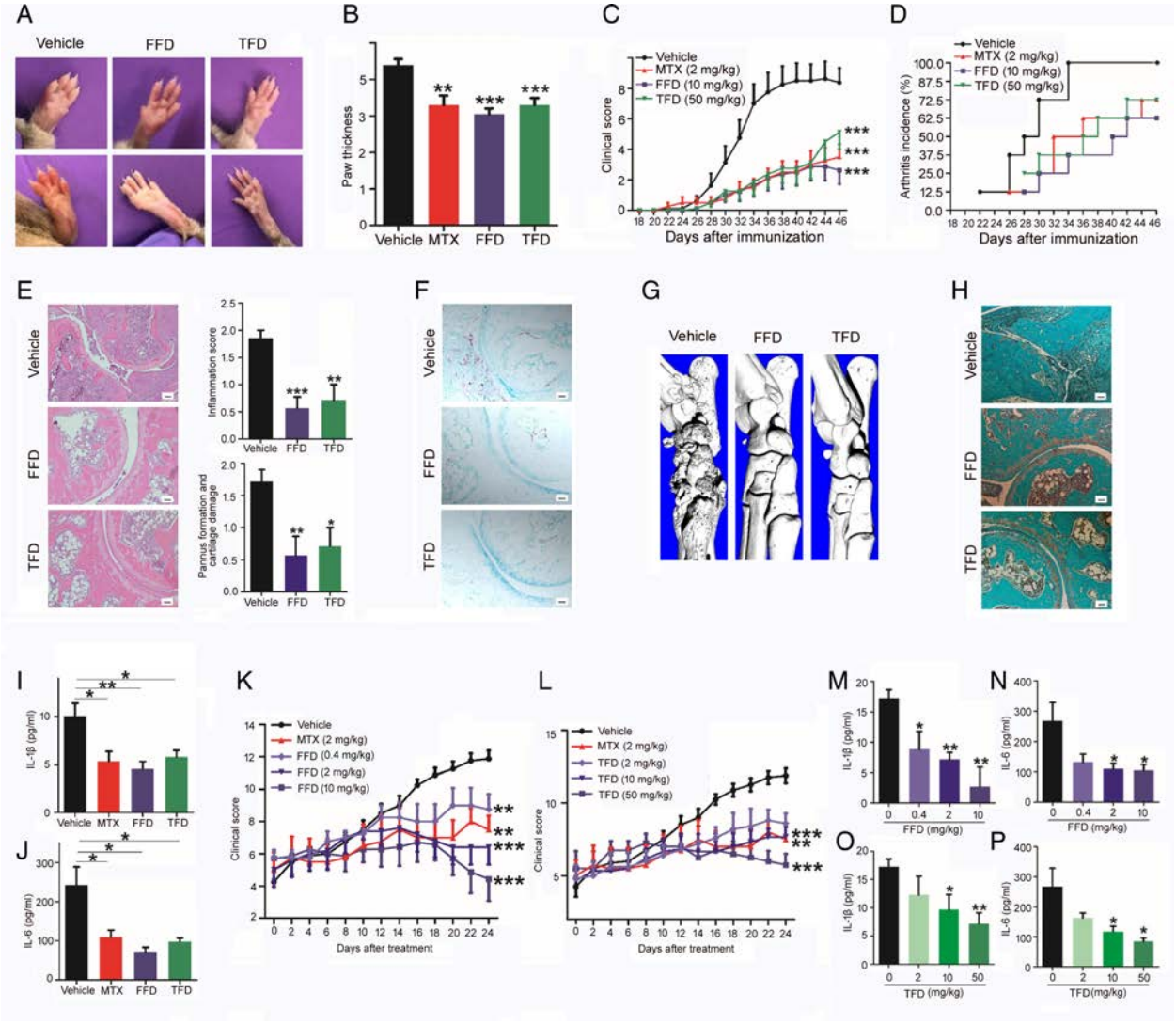

Figure 3 Fexofenadine prevents the onset and progression of collagen-induced arthritis. (A–J) Collagen-induced arthritis (CIA) model of DBA/1J mice was used to test prevention effects of fexofenadine (FFD) and terfenadine (TFD) (n=8). FFD (10 mg/kg), TFD (50 mg/kg) and MTX (2 mg/kg) were orally delivered daily beginning 18 days after immunisation. (A) The representative images of front paws and hind paws. (B) Paw thickness. (C) Clinical score of CIA. (D) The incidence rate of arthritis. (E) H&E staining and quantification of histological score of ankle samples. (F) TRAP staining of ankle samples. (G) microCT of ankles. (H) Safranin O staining of ankle samples. (I–J) The serum levels of IL-1β and IL-6 in CIA models. (K–P) To examine the dosage-dependent therapeutic effects of FFD/TFD, CIA mice were treated with various dose of FFD or TFD, as indicated. FFD, TFD, MTX and vehicle were delivered after the clinical score reached approximately five points. (K) The clinical score of FFD-treated mice. (I) The clinical score of TFD-treated mice. (M–P) The serum levels of IL-1β and IL-6 (n=8) (*p<0.05, **p<0.01, ***p<0.001) (scale bar, 100 µm). MTX,methotrexate.

obvious change of T_m in the presence of fexofenadine and terfenadine (T_m for control DMSO, terfenadine and fexofenadine are 46.09°C, 49.08°C and 51.99°C, respectively) (figure 5E, bottom). Performance of CETSA at 49°C with different dosages of drugs revealed that fexofenadine and terfenadine prevented cPLA2 denaturation in a dose-dependent manner, with the EC50 of 1.025e-007 and 1.449e-009, respectively (figure 5F).

To further characterise the interactions between fexofenadine and cPLA2, we performed both induced-fit docking (IFD) and molecular dynamics (MD) simulations. From IFD simulation, both fexofenadine and terfenadine core structures were predicted to stabilise at the mitogen-activated protein kinases (MAPK) phosphorylation site of cPLA2 at Ser-505 (figure 5G). Docked poses of fexofenadine in cPLA2 reveal that the fexofenadine-binding region was lined by Ser-505 and several residues close to Ser-505 (figure 5H). Fexofenadine was predicted to be involved in two hydrogen bonding interactions with Ser-505. The predicted binding-region and hydrogen bonding interaction for terfenadine were nearly the same as fexofenadine (figure 5I). MD simulations, as a complement to IFD simulation, showed that fexofenadine core structure was majorly stabilised into the binding site predicted by IFD. The protein backbone root mean square deviation (RMSD) deviated up to about 4 Å in the first 3 ns then remained relatively stable until the end of the simulation period, reflecting a relatively stable protein conformation. There was no significant turn-over in the fexofenadine binding pose as the fexofenadine RMSD deviated no more than 2 Å from the initiation of simulation (online supplementary figure S8a). For the cPLA2-terfenadine binding complex, both the binding pocket of cPLA2 and the binding pose of terfenadine showed no significant steric changes with only slight fluctuations on RSMD values after 2 ns (online supplementary figure S8b). The monitored 10 ns MD trajectory of cPLA2-fexofenadine complex are shown in the online supplementary video 1.

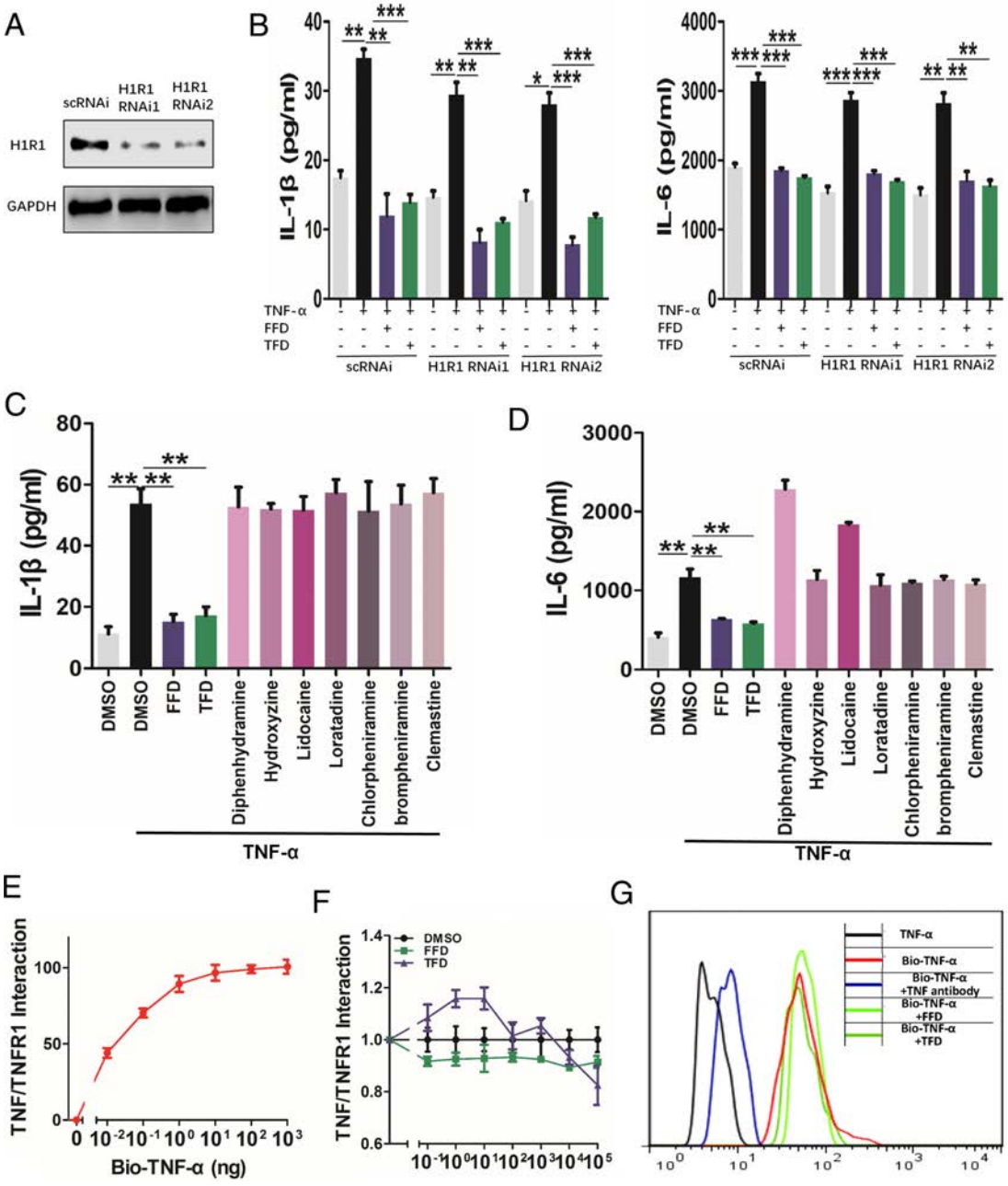

Figure 4 Fexofenadine's anti-TNF activity is H1R1 independant. (A–B) The anti-TNF activity of terfenadine (TFD) and fexofenadine (FFD) does not depend on H1R1. (A) Immunoblotting analysis to examine the knockdown efficacy of siRNA against H1R1. (B) RAW264.7 cells transfected with scrambled control siRNA (scRNAi) or H1R1 RNAis were treated with or without TNF-α (10 ng/mL) in absence or presence of FFD (10 μM)/TFD (1 μM) for 48 hours. The levels of IL-1β and IL-6 in the medium were detected by ELISA. (C–D) Comparison of the anti-TNF activity between terfenadine (TFD)/fexofenadine (FFD) and other known H1R1 inhibitors. BMDM cells were treated without or with TNF-α (10 ng/mL) in absence or presence of various H1R1 inhibitor, as indicated, for 48 hours. The levels of IL-1β and IL-6 in medium were detected by ELISA. (E–G) Terfenadine (TFD) and fexofenadine (FFD) do not affect the binding of TNF-α and TNFR1 and to the cell surface. (E) Solid phase binding was used to reveal the dose-dependent binding of TNF-α to TNFR1. (F) The binding of TNF-α to TNFR1 in the presence of DMSO (negative control), FFD or TFD was also analysed by solid phase binding. (G) RAW264.7 cells were incubated with biotin-labelled TNF-α in the absence or presence of TNF antibody (positive control), FFD (10 μM) or TFD (1 μM) for overnight, then cells were analysed by flow cytometry (*p<0.05, **p<0.01, ***p<0.001). TNF-α,tumour necrosis factor alpha.

Fexofenadine inhibits TNF activity through binding to the catalytic domain 2 of cPLA2 and the inhibition of the phosphorylation of cPLA2 on Ser-505

cPLA2 contains several domains critical for its functions,[34] including Ca^{2+} binding domain (C2D), catalytic domain 1 (CD1) and catalytic domain 2 (CD2) (figure 5J). We sought to identify the domain by which fexofenadine targets to cPLA2. For this purpose, we generated serial N-terminal and C-terminal deletion mutants and tested their interactions with fexofenadine by use of DARTS (figure 5J). Similar to the protective effect seen with intact cPLA2, fexofenadine retained protective effects for mutants with N-terminal C2D deletion (ie, cPLA2 (126–750) and further deletion of CD1 (ie, cPLA2(406–750)), indicating that the CD2 domain is the binding domain of fexofenadine. Indeed, fexofenadine did not show any protective effects on mutants lacking the CD2 domain (ie, cPLA2 (1–479) and cPLA2

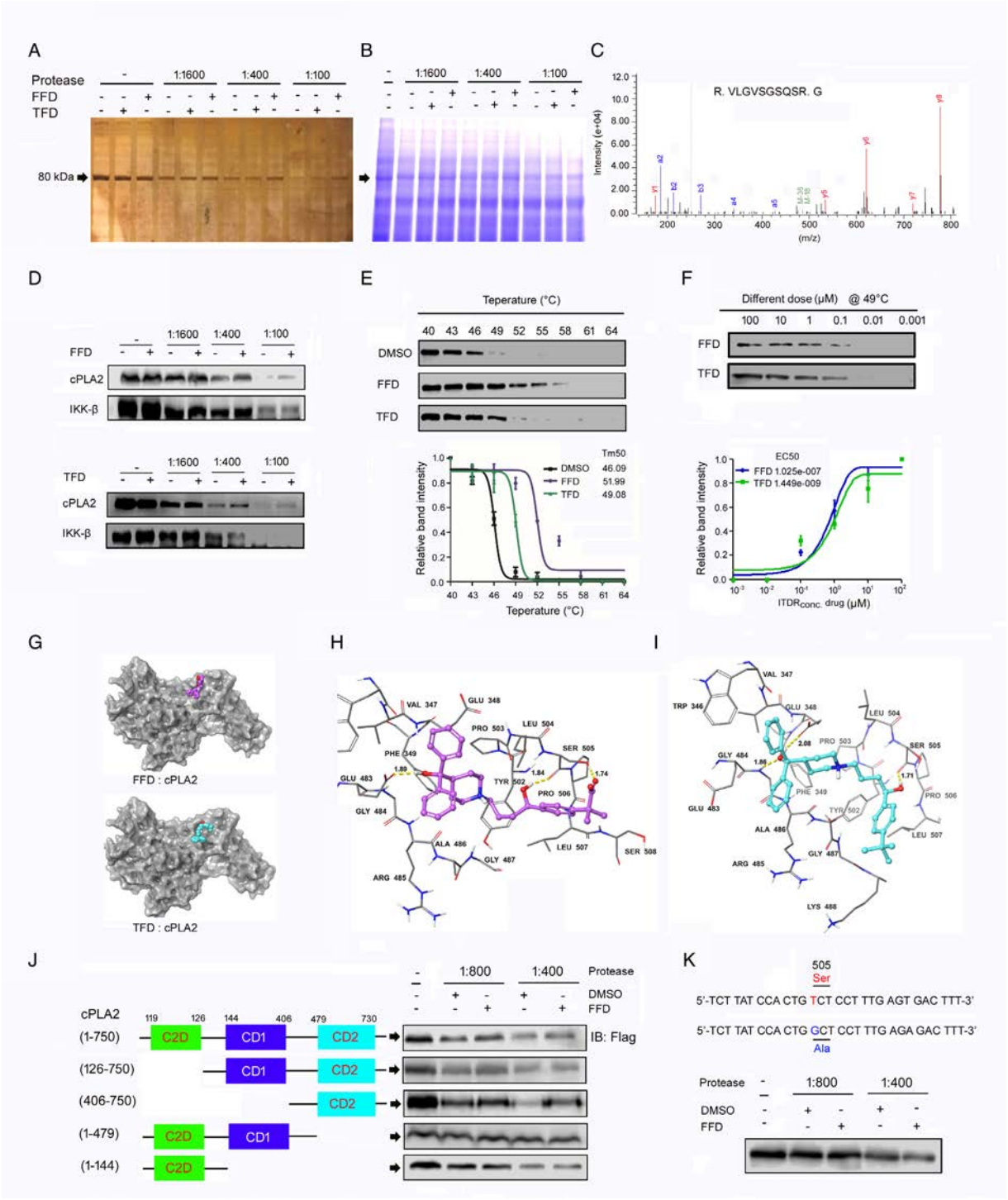

Figure 5 cPLA2 is a novel target of fexofenadine. (A) Silver staining of DARTS assay. (B) Coomassie blue staining of DARTS assay. The band with molecular weight around 80 kDa protected by fexofenadine (FFD)/terfenadine (TFD) was indicated by arrow. (C) *Adapted image of a mass spectra for PLA2G4A*, encoding cPLA2. (D) DARTS and Western blot to confirm FFD/TFD's binding targets. (E) CETSA melt response and associated curve. (F) Isothermal dose response (ITDR) and its curve. (G) IFD simulated binding complexes of cPLA2-FFD and cPLA2-TFD, respectively. cPLA2 is shown by surface representation (grey). FFD and TFD are shown by CPK representation with the atoms coloured as carbon–violet (FFD) or cyan (TFD), oxygen–red, nitrogen–blue, hydrogen–white (only polar hydrogens of ligand are shown). (H–I) Docked poses of FFD and TFD in cPLA2, respectively, predicted by IFD. FFD and TFD are shown as ball and stick model with the same atom colour scheme. Important amino acids are depicted as sticks with the same colour scheme except that carbon atoms are represented in grey. Only polar hydrogens are shown. Dotted yellow lines indicate hydrogen-bonding interactions. Values of the relevant distances are given in Å. (J) DARTS assay with serial deletion constructs encoding Flag-tagged mutants of cPLA2. cPAL2 (aa 1–750), cPLA2 (aa 126–750), cPLA2 (aa 406–750), cPLA2 (aa 1–479), cPLA2 (aa 1–144). 293T cells were transfected with Flag-tagged mutants of cPLA2 plasmids, as indicated. DARTS assay samples were detected by Flag antibody. (K) DARTS assay for Ser-505 point mutant of cPLA2. The Ser-505 of cPLA2 was substituted with Ala505. 293T cells were transfected with the point mutant plasmid and DARTS was performed. Point mutated cPLA2 was detected by Flag antibody. CETSA, cellular thermal shift assay; cPLA2, cytosolic phospholipase A2; DARTS, drug affinity responsive target stability; IFD, induced-fit docking.

(1–144)). Collectively, these sets of assays identify the CD2 domain of cPLA2 as the binding domain of fexofenadine.

Interestingly, both IFD and MD simulations indicated that Ser-505, which is located in the CD2 domain of cPLA2, is the critical amino acid for the interactions between fexofenadine and cPLA2. We next determined whether the substitution of Ser-505 with Ala through the site-directed mutagenesis affected the binding of fexofenadine to cPLA2. DARTS assay clearly demonstrated that fexofenadine lost its protective effect on this point mutant of cPLA2 (figure 5K), further demonstrating that Ser-505 is the critical amino acid required for fexofenadine targeting to cPLA2.

It is well established that the phosphorylation of cPLA2 on Ser-505 by upstream kinases p-p38 and p-REK1/2 is required for its enzymatic activity.[35] Since Ser-505 is an essential amino acid of the binding site for fexofenadine targeting to cPLA2, we next examined whether fexofenadine affected TNF activated phosphorylation of cPLA2 on Ser-505 (figure 6A). As expected, TNF-α activated the phosphorylation of p38 and ERK1/2 as well as cPLA2 in BMDMs. Fexofenadine treatment did not affect the phosphorylation of p38 or ERK1/2, but abolished the phosphorylation of cPLA2 on Ser-505 (figure 6A). These results provide additional evidence that fexofenadine directly targets to cPLA2, without affecting its upstream mediators in the TNF-activated cPLA2 inflammatory pathway.

As mentioned earlier, the phosphorylation of cPLA2 on Ser-505 is required for its enzymatic activity; accordingly, we assessed whether Fexofenadine affected the activity of cPLA2. Similar to arachidonyl trifluoromethyl ketone 27 (ATK), a known cPLA2 inhibitor used here as a positive control, both fexofenadine and terfenadine completely abolished TNF-α induced cPLA2 activity and their inhibitions of cPLA2 activity are dosage dependent (figure 6B). In addition, ATK also inhibited TNF-α-induced cytokine release, but to a lesser degree, when compared with fexofenadine (online supplementary figure S9a).

Inflammatory conditions, including elevated TNF-α, promote the cPLA2 translocation to intracellular phospholipid membrane. The major function of cPLA2 is to promote phospholipid hydrolysis to produce AA,[36] which, in turn, activates NF-κB-mediated inflammation.[21 22] Accordingly, we examined whether fexofenadine and terfenadine inhibited TNF-α induced AA production, the data in figure 6C revealed that this was the case. Moreover, as shown in figure 6D–E, supplementation of medium with AA eliminated the inhibitory influence of fexofenadine on TNF-α induced release of inflammatory cytokines IL-1β and IL6, suggesting that inhibition of AA production by fexofenadine is contributory to its anti-TNF-α activity. In addition, although fexofenadine inhibited stimulation of the TNF-α-activated NF-κB reporter gene, fexofenadine did not constrain AA activation of the NF-κB reporter gene (online supplementary figure S9b), indicating that fexofenadine exerts it role upstream of AA production in NF-κB signalling. To further define the importance of cPLA2 in mediating fexofenadine's anti-TNF-α activity. We deleted *PLA2G4A* gene using the CRISPR-Cas9 technique (figure 6F). This technique produced near complete deletion of cPLA2 (figure 6F). Importantly, fexofenadine-mediated inhibition of TNF-α activity was entirely or almost entirely lost in cPLA2 knockout cells (figure 6G–H). Further, fexofenadine lost its inhibition of TNF-α-activated NF-κB reporter gene in cPLA2 knockout cells. Re-establishing expression of cPLA2 by transfecting cPLA2 knockout cells with a cPLA2 expression plasmid reinstated fexofenadine's anti-TNF/NF-κB activity. However, transfection of cPLA2 knockout cells with an expression plasmid encoding a cPLA2 point mutant Ser-505-Ala (*cPLA2* ^{S505A}), which inactivates cPLA2 enzymatic activity and fails to produce AA, could not rescue fexofenadine's anti-TNF/NF-κB activity (online supplementary figure S9c). Taken together, these findings indicate the dependence of fexofenadine's anti-TNF/NF-κB activity on cPLA2 and cPLA2-mediated AA generation.

DISCUSSION

TNF-α signalling associates with various pathophysiological processes and enormous efforts have been devoted to develop treatments for TNF-α associated diseases and conditions.[6 8] In this study, we performed three rounds of screening using an FDA-approved drug library, and isolated fexofenadine, a selective histamine receptor 1 antagonist, as a novel TNFI (online supplementary figures S1-3). Comprehensive evidences, including RNA-seq, transcription factor enrichment analysis, downstream cytokine expression and release, NF-κB nuclear translocation and activity, osteoclastogenesis as well as in vivo reporter and transgenic mice, validated fexofenadine's anti-TNF-α activities (figure 1, online supplementary figures S4-6). Subsequent in vivo animal models, including TNF-tg, and collagen-induced arthritis, demonstrated that fexofenadine is therapeutic against inflammatory arthritis to a degree better than, or at least as good as, the current small molecule drugs for treating rheumatoid arthritis (figures 2 and 3). Intriguingly, fexofenadine exhibited better anti-TNF-α effects in the therapeutic treatment strategy than that in the preventive TNF-tg model (figure 2), suggesting that fexofenadine may exert a preferential effect during the progression of disease. A detailed analysis of the temporospatial expression and activity of cPLA2 during disease onset and progression in TNF-tg mice may explain this paradoxical difference. Although terfenadine also yielded beneficial effects in inhibiting TNF-α activity in vitro and in the disease models tested, suspension of terfenadine's use due to adverse medical events leads us to discount its utility in inflammatory conditions.[37] Fexofenadine, however, does not produce the significant health risks associated with terfenadine treatment and should be considered a promising drug for treating chronic TNF-α-associated disease.[38] Due to its safety and efficacy, fexofenadine is widely used as a non-prescription medicine, sometimes called an OTC drug, readily available for treating various allergic conditions.

Currently marketed TNF-α blockers, such as etanercept (Enbrel) and adalimumab (Humira), have a demonstrated record of safety and efficacy in the treatment of autoimmune diseases. However, fexofenadine demonstrates features that suggest it may compare favourably to these established biologics. For example, all currently marketed anti-TNF therapies bind to TNF-α and inhibit its binding to TNF receptors; in contrast to these upstream inhibitors, fexofenadine targets the downstream cPLA2 mediator of TNF-α signalling. In addition, it also targets H1R1. Due to this alternate mechanism of action, fexofenadine may be effective for the patients who fail to respond to current TNF-α blockers.[11] As a well-tolerated and generically available oral OTC drug, fexomenadine's safety, convenience and cost-effectiveness suggest that it may be an attractive and viable agent for the clinical treatment of inflammatory autoimmune diseases, particularly rheumatoid arthritis in which fexofenadine has been proven to be effective in the preclinical animal models (figures 1–3).

Fexofenadine is known to be a highly selective antagonist to H1 receptor 1.[27 39] Surprisingly, suppression of H1 receptor 1 does not affect fexofenadine-mediated anti-TNF-α activity. In addition, an additional seven known H1R1 inhibitors do not have anti-TNF activity (figure 4). Although fexofenadine

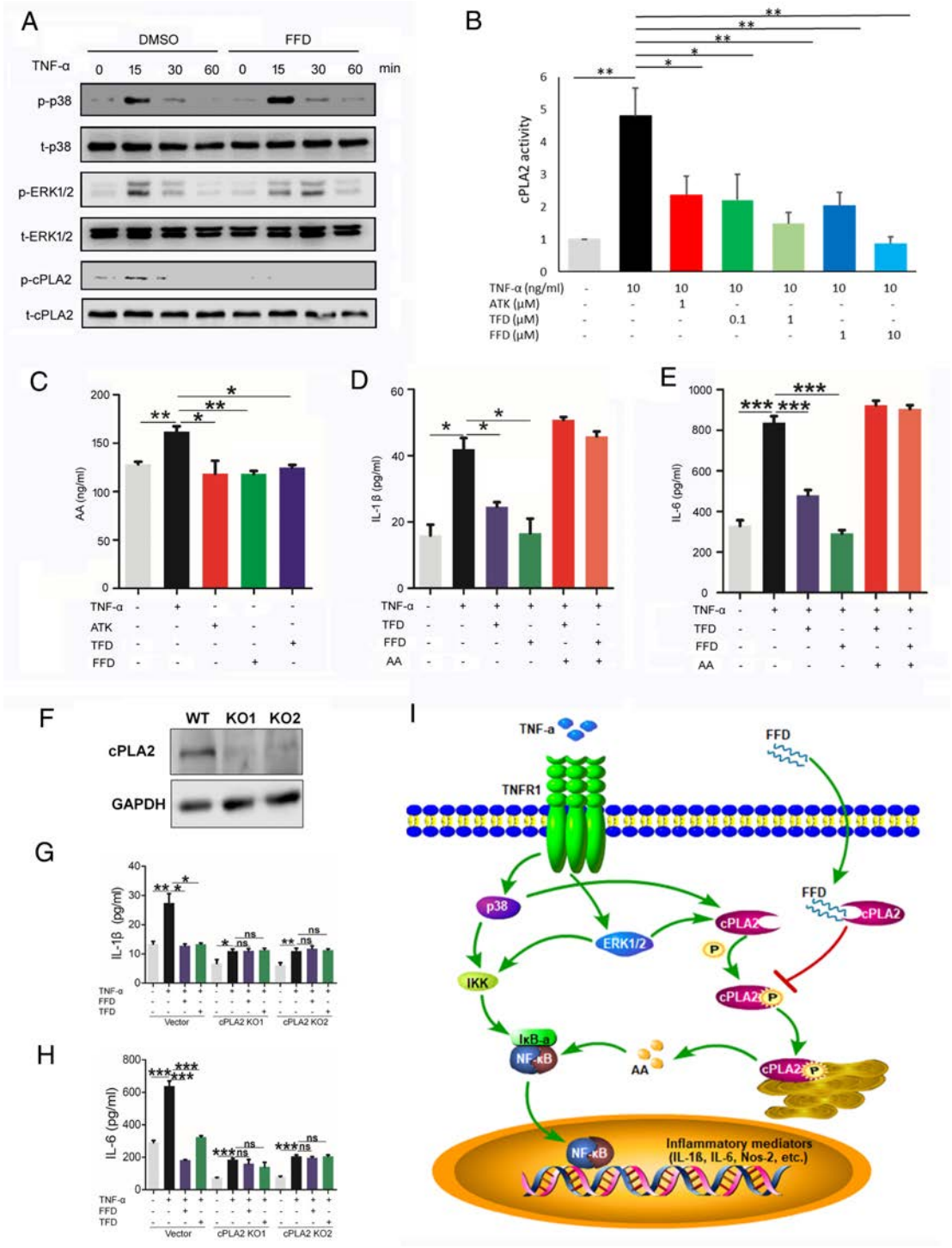

Figure 6 Fexofenadine inhibits TNF activity through binding to the catalytic domain 2 of cPLA2 and inhibition of the phosphorylation of cPLA2 on Ser-505. (A) Fexofenadine (FFD) inhibits the phosphorylation of cPLA2 on Ser-505. BMDM cells were treated with TNF-α (10 ng/mL) in the absence or presence of FFD (10 μM) for various time points, as indicated. p-p38, t-p38, p-ERK1/2, t-ERK1/2, p-cPLA2 (specifically for phosphorylated Ser-505), t-cPLA2 were detected by Western blot with corresponding antibodies. (B) Fexofenadine inhibits TNF-induced cPLA2 activity in living cells. RAW264.7 cells transfected with an expression plasmid encoding cPLA2 were treated with TNF-α and ATK, or terfenadine (TFD), or FFD overnight. Cells lysate was used for cPLA2 activity analysis. (C) FFD inhibits TNF-induced arachidonic acid (AA) production. The AA levels in BMDMs without or with TNF-α (10 ng/mL) in absence or presence of FFD or TFD for 48 hours were examined using a commercial ELISA kit. ATK was used as a positive control. (D-E) Addition of AA abolished FFD inhibition of TNF-induced cytokine release. BMDMs were treated with TNF-α (10 ng/mL), AA (10 μM) and FFD (10 μM)/TFD (1 μM), as indicated. The levels of IL-1β and IL-6 were detected by ELISA. (F) Knockout efficiency of cPLA2 using CRISPR-Cas9 technique in RAW264.7 cells, assayed by Western blot. Two individual knockout clones (KO1 and KO2) were employed. (G–H) Deletion of cPLA2 abolished FFD inhibition of TNF-induced cytokine release. WT and cPLA2 KO RAW264.7 cells were treated without or with TNF-α (10 ng/mL) in absence or presence of FFD (10 μM)/TFD (1 μM) for 48 hours. The levels of IL-1β and IL-6 in medium were detected by ELISA (*p<0.05, **p<0.01, ***p<0.001). (I) A proposed model for explaining the anti-TNF activity of FFD through targeting cPLA2 pathway. ATK, arachidonyl trifluoromethyl ketone 27; cPLA2, cytosolic phospholipase A2; TNF-α,tumour necrosis factor alpha.

potently inhibits TNF-α signalling in vitro and in vivo, it does not affect the binding of TNF-α to its receptors or cell surface, clearly different from clinically used TNFIs (figure 4). Excitingly, through combined use of drug affinity responsive target stability assay, proteomics, CETSA, IFD and MD, we identified cPLA2 as a previously unrecognised target of fexofenadine. Fexofenadine binds to catalytic domain 2 and inhibits the phosphorylation of cPLA2 on Ser-505. Further, deletion of cPLA2 abolished fexofenadine inhibition of TNF-induced AA production and downstream cytokine release (figures 5 and 6). A proposed model for explaining the anti-TNF activity of fexofenadine through directly targeting the cPLA2 pathway is shown in figure 6I. TNF-α binds to TNFR1 and activates p38 and ERK1/2, followed by the phosphorylation of cPLA2 on Ser-505. Phosphorylated cPLA2 then translocates from the cytosol to hydrolyse membrane phospholipids, leading to the production of AA. AA, in turn, actives NF-κB, leading to cytokine release and inflammation. Dissimilar to current TNF inhibitors that disturb TNF/TNFR interactions at the initiation of the signalling cascade, fexofenadine diffuses into the cells and directly binds to cPLA2 and inhibits its phosphorylation on Ser-505, followed by the inhibition of cPLA2/AA/NF-κB inflammatory pathway. It is also noted that fexofenadine inhibited the phosphorylation of NF-κB p65 upstream mediator IκB-α in vivo (data not shown), suggesting effects of fexofenadine on canonical TNF/IκB/NF-κB pathways, possibly through the cross-talk with the TNF/cPLA2/NF-κB pathway.

TNF-α regulation of immune cells, such as macrophage polarisation and differentiation of T cell populations, is a known component in the pathogenesis of autoimmune diseases.[6] We found that both fexofenadine and terfenadine significantly inhibited inflammatory M1 macrophages, while markedly increased anti-inflammatory M2 macrophages (online supplementary figure S10). Furthermore, fexofenadine and terfenadine significantly suppressed the differentiation of IFNϒ-positive Th1 subpopulation in vitro and in vivo, whereas negligible effects were observed with regard to the differentiation of Th2, Th17 and regulatory T cells (online supplementary figure S11). We demonstrate that fexofenadine is therapeutic against inflammatory arthritis spontaneously developed in TNF-tg mice (figure 2) and fexofenadine-mediated anti-TNF-α activity depends on cPLA2 (figure 6F–H), but not histamine H1 receptor (figure 4); however, its anti-histaminic action may also contribute to its therapeutic effects in inflammatory arthritis.

Similar to TNF-α, cPLA2 is also known to play an important role in regulating autoimmune diseases.[40 41] cPLA2 is implicated in synovitis and joint destruction in rheumatoid arthritis by regulating the production of inflammatory mediators.[42] In addition to autoimmune diseases, cPLA2 is also involved in the pathogenesis of many other disorders, particularly neurodegenerative diseases, cardiovascular diseases and cancer.[43–50] Intense efforts have been invested to identify potent cPLA2 inhibitors in the past several decades. Unfortunately, toxicity and poor absorption of anti-cPLA2 compounds from intestine have remained significant challenges for clinical application, although numerous cPLA2 inhibitors have been tested in clinical trials.[51 52] Our findings that the commonly used OTC drug fexofenadine targets and inhibits cPLA2 and is effective in animal models of inflammatory arthritis may provide innovative interventions to overcome the current bottlenecks in the efforts to develop cPLA2 targeting treatments.

In summary, this study identifies fexofenadine as an inhibitor of TNF-α signalling and uncovers a new strategy for inhibiting this cardinal pathway of inflammation. In addition, this study also identifies cPLA2 as a new target of fexofenadine, thus providing new insights into the understanding of fexofenadine's action and underlying mechanisms, and a solid foundation for future discoveries relating to this fexofenadine/cPLA2 interaction. Further, our data also identifies fexofenadine as a novel antagonist of cPLA2, suggesting that fexofenadine can also be used for treating various cPLA2-associated diseases, including autoimmune diseases. With the consideration that both TNF-α and cPLA2 are involved in a plethora of disease processes, the identification of fexofenadine as an inhibitor of both TNF-α and cPLA2, and manipulation of new antagonist of the TNF/cPLA2 pathway may lead to innovative therapeutics for various pathologies and conditions, significantly broadening the application of this OTC drug beyond allergic diseases.

METHODS SUMMARY

In vitro and in vivo screen of FDA-approved drug library

NF-κB-*bla* THP-1 cell line in which NF-κB beta-lactamase reporter gene was stably integrated, RAW 264.7 macrophages in which NF-κB luciferase reporter gene was transiently transfected, and TNF-tg:NF-κB-Luc double mutant reporter mice were employed to screen the drug library.

In vitro and in vivo assays for examining the blockade of TNF actions by fexofenadine

RNA-seq, transcription factor enrichment analysis, downstream cytokine expression and release, NF-κB translocation and activity, osteoclastogenesis with BMDMs and RAW264.7 macrophages, as well as in vivo reporter mice.

In vivo assays for defining the anti-inflammatory activity of fexofenadine using various animal models

Administration of fexofenadine, terfenadine or clinically used positive controls into TNF-Tg mice and CIA of DBA/1 mice.

Identification and characterisation of the binding of fexofenadine to cPLA2

Drug affinity responsive target stability assay, proteomics, CETSA, information field dynamics and MD; solid-phase binding and flow cytometry was used to examine the effects of fexofenadine on TNF/TNFR interactions.

Assays for examining fexofenadine inhibition of cPLA2 as well as the dependence on cPLA2 of fexofenadine's anti-TNF activity

Phosphorylation of p38, Erk1/2 and cPLA2 by TNF-α, activation of cPLA2 activity and AA production by TNF-α, and their inhibition by fexofenadine, dependence of fexofenadine-mediated inhibition of TNF-α on the presence and activity of cPLA2 and AA production were determined.

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Contributors RL and YC designed and performed experiments, collected and analysed data, and cowrote the paper. YC participated in the design of the experiments and analysis of the data, particularly the identification and characterisation of the drug target. WF, SW, XZ, AH, JL, L. Zhang, CW, CZ and YB assisted with experiments, and collected and analysed data. ZL and Z-SC performed the IFD and MD simulations, and also assisted in editing the manuscript. GX assisted in analysing the data and editing the manuscript. CL designed and supervised this study, analysed data, and wrote and edited the manuscript.

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Competing interests None declared.

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REFERENCES

1 McKinney EF, Lee JC, Jayne DRW, *et al*. T-Cell exhaustion, co-stimulation and clinical outcome in autoimmunity and infection. *Nature* 2015;523:612–6.
2 Barturen G, Beretta L, Cervera R, *et al*. Moving towards a molecular taxonomy of autoimmune rheumatic diseases. *Nat Rev Rheumatol* 2018;14:75–93.
3 Surolia I, Pirnie SP, Chellappa V, *et al*. Functionally defective germline variants of sialic acid acetylesterase in autoimmunity. *Nature* 2010;466:243–7.
4 Brenner D, Blaser H, Mak TW. Regulation of tumour necrosis factor signalling: live or let die. *Nat Rev Immunol* 2015;15:362–74.
5 Croft M, Benedict CA, Ware CF. Clinical targeting of the TNF and TNFR superfamilies. *Nat Rev Drug Discov* 2013;12:147–68.
6 Aggarwal BB. Signalling pathways of the TNF superfamily: a double-edged sword. *Nat Rev Immunol* 2003;3:745–56.
7 Kalliolias GD, Ivashkiv LB. Tnf biology, pathogenic mechanisms and emerging therapeutic strategies. *Nat Rev Rheumatol* 2016;12:49–62.
8 Palladino MA, Bahjat FR, Theodorakis EA, *et al*. Anti-Tnf-Alpha therapies: the next generation. *Nat Rev Drug Discov* 2003;2:736–46.
9 Netterwald J. TNF-blocker triple approval. *Nat Biotechnol* 2009;27:495.
10 Taylor PC, Feldmann M. Anti-Tnf biologic agents: still the therapy of choice for rheumatoid arthritis. *Nat Rev Rheumatol* 2009;5:578–82.
11 Nurmohamed MT, Dijkmans BAC. Efficacy, tolerability and cost effectiveness of disease-modifying antirheumatic drugs and biologic agents in rheumatoid arthritis. *Drugs* 2005;65:661–94.
12 Smolen JS, Weinblatt ME. When patients with rheumatoid arthritis fail tumour necrosis factor inhibitors: what is the next step? *Ann Rheum Dis* 2008;67:1497–8.
13 Kim SY, Solomon DH. Tumor necrosis factor blockade and the risk of viral infection. *Nat Rev Rheumatol* 2010;6:165–74.
14 Chen Y, Friedman M, Liu G, *et al*. Do tumor necrosis factor inhibitors increase cancer risk in patients with chronic immune-mediated inflammatory disorders? *Cytokine* 2018;101:78–88.
15 Chong CR, Sullivan DJ. New uses for old drugs. *Nature* 2007;448:645–6.
16 Leurs R, Vischer HF, Wijtmans M, *et al*. En route to new blockbuster anti-histamines: surveying the offspring of the expanding histamine receptor family. *Trends Pharmacol Sci* 2011;32:250–7.
17 Phan H, Moeller ML, Nahata MC. Treatment of allergic rhinitis in infants and children: efficacy and safety of second-generation antihistamines and the leukotriene receptor antagonist montelukast. *Drugs* 2009;69:2541–76.
18 Gelfand EW, Cui Z-H, Takeda K, *et al*. Effects of fexofenadine on T-cell function in a murine model of allergen-induced airway inflammation and hyperresponsiveness. *J Allergy Clin Immunol* 2003;112(4 Suppl):S89–S95.
19 Finn AF, Kaplan AP, Fretwell R, *et al*. A double-blind, placebo-controlled trial of fexofenadine HCl in the treatment of chronic idiopathic urticaria. *J Allergy Clin Immunol* 1999;104:1071–8.
20 Simanshu DK, Kamlekar RK, Wijesinghe DS, *et al*. Non-Vesicular trafficking by a ceramide-1-phosphate transfer protein regulates eicosanoids. *Nature* 2013;500:463–7.
21 Camandola S, Leonarduzzi G, Musso T, *et al*. Nuclear factor kB is activated by arachidonic acid but not by eicosapentaenoic acid. *Biochem Biophys Res Commun* 1996;229:643–7.
22 Ramakers JD, Mensink RP, Schaart G, *et al*. Arachidonic acid but not eicosapentaenoic acid (EPA) and oleic acid activates NF-kappaB and elevates ICAM-1 expression in Caco-2 cells. *Lipids* 2007;42:687–98.
23 Uozumi N, Kume K, Nagase T, *et al*. Role of cytosolic phospholipase A2 in allergic response and parturition. *Nature* 1997;390:618–22.
24 Essaghir A, Toffalini F, Knoops L, *et al*. Transcription factor regulation can be accurately predicted from the presence of target gene signatures in microarray gene expression data. *Nucleic Acids Res* 2010;38:e120.
25 Tang W, Lu Y, Tian Q-Y, *et al*. The growth factor progranulin binds to TNF receptors and is therapeutic against inflammatory arthritis in mice. *Science* 2011;332:478–84.
26 Brand DD, Latham KA, Rosloniec EF. Collagen-Induced arthritis. *Nat Protoc* 2007;2:1269–75.
27 Meeves SG, Appajosyula S. Efficacy and safety profile of fexofenadine HCl: a unique therapeutic option in H1-receptor antagonist treatment. *J Allergy Clin Immunol* 2003;112(4 Suppl):S69–77.
28 Marone G, Granata F, Spadaro G, *et al*. The histamine-cytokine network in allergic inflammation. *J Allergy Clin Immunol* 2003;112(4 Suppl):S83–S88.
29 Shimamura T, Shiroishi M, Weyand S, *et al*. Structure of the human histamine H1 receptor complex with doxepin. *Nature* 2011;475:65–70.
30 Lomenick B, Hao R, Jonai N, *et al*. Target identification using drug affinity responsive target stability (darts). *Proc Natl Acad Sci U S A* 2009;106:21984–9.
31 Ruocco MG, Karin M. IKK{beta} as a target for treatment of inflammation induced bone loss. *Ann Rheum Dis* 2005;64 Suppl 4:iv81–5.
32 Martinez Molina D, Jafari R, Ignatushchenko M, *et al*. Monitoring drug target engagement in cells and tissues using the cellular thermal shift assay. *Science* 2013;341:84–7.
33 Jafari R, Almqvist H, Axelsson H, *et al*. The cellular thermal shift assay for evaluating drug target interactions in cells. *Nat Protoc* 2014;9:2100–22.
34 Clark JD, Lin LL, Kriz RW, *et al*. A novel arachidonic acid-selective cytosolic PLA2 contains a Ca(2+)-dependent translocation domain with homology to PKC and GAP. *Cell* 1991;65:1043–51.
35 Lin LL, Wartmann M, Lin AY, *et al*. cPLA2 is phosphorylated and activated by MAP kinase. *Cell* 1993;72:269–78.
36 Korotkova M, Lundberg IE. The skeletal muscle arachidonic acid cascade in health and inflammatory disease. *Nat Rev Rheumatol* 2014;10:295–303.
37 Burkhart GA, Freiman J. The risk of life-threatening cardiovascular events with terfenadine. *Am J Cardiol* 1995;75:213–4.
38 Ando K, Nakamura Y, Hagiwara-Nagasawa M, *et al*. Comparison of electropharmacological effects between terfenadine and its active derivative fexofenadine using a cross-over study in halothane-anesthetized dogs to analyze variability of pharmacodynamic and pharmacokinetic profiles of terfenadine and torsadogenic risk of fexofenadine. *J Toxicol Sci* 2018;43:183–92.
39 Thurmond RL, Gelfand EW, Dunford PJ. The role of histamine H1 and H4 receptors in allergic inflammation: the search for new antihistamines. *Nat Rev Drug Discov* 2008;7:41–53.
40 Marusic S, Leach MW, Pelker JW, *et al*. Cytosolic phospholipase A2 alpha-deficient mice are resistant to experimental autoimmune encephalomyelitis. *J Exp Med* 2005;202:841–51.
41 Dennis EA, Norris PC. Eicosanoid storm in infection and inflammation. *Nat Rev Immunol* 2015;15:511–23.
42 Sommerfelt RM, Feuerherm AJ, Jones K, *et al*. Cytosolic phospholipase A2 regulates TNF-induced production of joint destructive effectors in synoviocytes. *PLoS One* 2013;8:e83555.
43 Kalyvas A, David S. Cytosolic phospholipase A2 plays a key role in the pathogenesis of multiple sclerosis-like disease. *Neuron* 2004;41:323–35.
44 Park JB, Lee CS, Jang J-H, *et al*. Phospholipase signalling networks in cancer. *Nat Rev Cancer* 2012;12:782–92.
45 Bonventre JV, Huang Z, Taheri MR, *et al*. Reduced fertility and postischaemic brain injury in mice deficient in cytosolic phospholipase A2. *Nature* 1997;390:622–5.
46 Sanchez-Mejia RO, Newman JW, Toh S, *et al*. Phospholipase A2 reduction ameliorates cognitive deficits in a mouse model of Alzheimer's disease. *Nat Neurosci* 2008;11:1311–8.
47 Farooqui AA, Ong W-Y, Horrocks LA. Inhibitors of brain phospholipase A2 activity: their neuropharmacological effects and therapeutic importance for the treatment of neurologic disorders. *Pharmacol Rev* 2006;58:591–620.
48 Tosato G, Segarra M, Salvucci O. Cytosolic phospholipase A2{alpha} and cancer: a role in tumor angiogenesis. *J Natl Cancer Inst* 2010;102:1377–9.
49 Patel MI, Singh J, Niknami M, *et al*. Cytosolic phospholipase A2-alpha: a potential therapeutic target for prostate cancer. *Clin Cancer Res* 2008;14:8070–9.
50 Falik Zaccai TC, Savitzki D, Zivony-Elboum Y, *et al*. Phospholipase A2-activating protein is associated with a novel form of leukoencephalopathy. *Brain* 2017;140:370–86.
51 Kokotou MG, Limnios D, Nikolaou A, *et al*. Inhibitors of phospholipase A_2 and their therapeutic potential: an update on patents (201_2-2016). *Expert Opin Ther Pat* 2017;27:217–25.
52 Soubhye J, van Antwerpen P, Dufrasne F. Targeting cytosolic phospholipase A2α for novel anti-inflammatory agents. *Curr Med Chem* 2018;25:2418–47.

EPIDEMIOLOGICAL SCIENCE

Treatment response and drug retention rates in 24 195 biologic-naïve patients with axial spondyloarthritis initiating TNFi treatment: routine care data from 12 registries in the EuroSpA collaboration

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ABSTRACT

Objective To study drug retention and response rates in patients with axial spondyloarthritis (axSpA) initiating a first tumour necrosis factor inhibitor (TNFi).

Methods Data from 12 European registries, prospectively collected in routine care, were pooled. TNFi retention rates (Kaplan-Meier statistics), Ankylosing Spondylitis Disease Activity Score (ASDAS) Inactive disease (<1.3), Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) <40 mm and Assessment of SpondyloArthritis International Society responses (ASAS 20/40) were assessed at 6, 12 and 24 months.

Results A first TNFi was initiated in 24 195 axSpA patients. Heterogeneity of baseline characteristics between registries was observed. Twelve-month retention was 80% (95% CI 79% to 80%), ranging from 71% to 94% across registries. At 6 months, ASDAS Inactive disease/BASDAI<40 rates were 33%/72% (LUNDEX-adjusted: 27%/59%), ASAS 20/40 response rates 64%/49% (LUNDEX-adjusted 52%/40%). In patients initiating first TNFi after 2009, 6097 patients was registered to fulfil ASAS criteria for axSpA, 2935 was registered to fulfil modified New York Criteria for Ankylosing Spondylitis and 1178 patients was registered as having non-radiographic axSpA. In nr-axSpA patients, we observed lower 12-month retention rates (73% (70%–76%)) and lower 6-month LUNDEX adjusted response rates (ASDAS Inactive disease/BASDAI40 20%/50%, ASAS 20/40 45%/33%). For patients initiating first TNFi after 2014, 12-month retention rate, but not 6-month response rate, was numerically higher compared with patients initiating TNFi in 2009–2014.

Conclusion A large European database of patients with axSpA initiating a first TNFi treatment in routine care, demonstrated that 27% of patients achieved ASDAS inactive disease after 6 months, while 59% achieved BASDAI <40. Four of five patients continued treatment after 1 year.

Key messages

What is already known about this subject?

- Single countries have reported effectiveness of tumour necrosis factor inhibitor (TNFi) treatment in axial spondyloarthritis (axSpA), but the generalisability of the findings is unknown.

What does this study add?

- This study of 24 195 European axSpA patients in a pooled dataset offered large-scale real-world evidence on the effectiveness of TNFi across 12 European countries.
- Overall, ~1/4 of patients achieved ASDAS inactive disease after 6 months and 80% were still receiving the same TNFi after 1 year.
- Patients with non-radiographic AxSpA showed numerically lower ASDAS inactive disease rates (1/5 at 6 months) and 12-month retention rates (73%).

How might this impact on clinical practice or future developments?

- The EuroSpA collaboration offers unprecedented opportunities for providing real-world evidence on European patients with axSpA, including drug effectiveness, predictors thereof and differences between countries.

INTRODUCTION

Tumour necrosis factor inhibitors (TNFi) improve symptoms of axial spondyloarthritis (axSpA), such as inflammatory back pain, stiffness and range of motion.[1–3] Their effects have been demonstrated in randomised controlled trials (RCTs) and TNFi are now an essential part of axSpA treatment.[4–7]

However, in contrast to the RCTs with strict inclusion and exclusion criteria, a more heterogeneous group of patients with a broad spectrum of various comorbidities, concomitant medications and atypical disease manifestations is treated in routine care. Thus, up to 80% of patients receiving TNFi in routine care would not have been eligible to be enrolled in the RCTs that led to approval of the agents.[8] This observation emphasises the need for real-world observational studies as a valuable supplement to RCTs.

To date, single countries have reported real-world effectiveness of TNFi treatments in axSpA.[9–11] Investigation of characteristics of patients exposed to TNFi, treatment adherence and response rates of TNFi across countries would improve our knowledge of the effectiveness of TNFi treatment in axSpA patients treated in routine care.

The EuroSpA collaboration is a research network of 15 European registries that has been created to strengthen research on patients with spondyloarthritis in the real-world setting. In this first study of axSpA patients in which 12 of the registries participated, we aimed to investigate retention and response rates among TNFi-naive axSpA patients initiating a first TNFi treatment. Analyses were performed in a pooled dataset of axSpA patients across all registers, in data from individual registries as well as in subgroups of patients registered as fulfilling Assessment of Spondyloarthritis International Society (ASAS) criteria for axSpA, fulfilling modified New York Criteria for Ankylosing Spondylitis (AS) and as having non-radiographic axSpA.[1–3] We also investigated potential heterogeneity of patient characteristics between registries at treatment initiation.

METHODS

The EuroSpA research collaboration

The present study included secondary use of data on patients registered with an axSpA diagnosis from 12 European registries up to 2018 and data were uploaded from the following registries: ARTIS (Sweden), DANBIO (Denmark), SCQM (Switzerland), ATTRA (Czech Republic), TURKBIO (Turkey), NOR-DMARD (Norway), ROB-FIN (Finland), Reuma.pt (Portugal), RRBR (Romania), BIOBADASER (Spain), biorx.si (Slovenia) and ICEBIO (Iceland).

Data sources

In all registries, data are collected prospectively as part of routine clinical practice. Based on a predefined study protocol, a list of study variables including definitions for each variable was sent to data managers in each registry. The data managers created anonymised datasets and uploaded these through secure Virtual Private Network pipelines to the EuroSpA server. Statistical analyses were predefined in the study protocol and statistical analysis plan.

Patients

Patients were included if they had a diagnosis of axSpA as judged by the treating rheumatologist, were aged ≥18 years at diagnosis, had received treatment with at least one TNFi after diagnosis and were registered with start and (if relevant) stop dates of TNFi. We conducted analyses separately for patients initiating a first TNFi since registry start, and for three subcohorts of patients initiating a first TNFi after 1 January 2009 with available registration of classification criteria: patients registered to fulfil ASAS criteria for axSpA (ASAS cohort), patients registered to fulfil the modified New York criteria for AS (NY cohort) and patients registered to fulfil ASAS criteria and to NOT fulfil the modified New York criteria for AS (nr-axSpA cohort).

Clinical variables

Baseline variables included age, gender, human leucocyte antigen B27 (HLA-B27) status, body mass index, time since diagnosis, smoking status, current treatment with conventional synthetic disease-modifying antirheumatic drugs (csDMARDs) as well as TNFi agent. At baseline and after 6, 12 and 24 months' follow-up, the following disease scores were included: Bath Ankylosing Spondylitis Disease Activity Index (BASDAI)[12] and Bath Ankylosing Spondylitis Functional Index (BASFI), both on a 0–100 mm scale.[13] Furthermore, fatigue and global scores on visual analogue scales (VAS) were obtained as well as the components required for calculation of the Ankylosing Spondylitis Disease Activity Score (ASDAS).[14] The 6-month visit was defined as a registered visit from 90 to 270 days after baseline, the 12-month visit as a registered visit from 271 to 545 days after baseline and a 24-month visit as a registered visit from 546 to 910 days after baseline.

Retention rates

The treatment period (time on drug) was defined as the number of days between the registered date of treatment start and the registered stop date for the individual patients. A treatment without a registered stop date was assumed to have been discontinued if a new biologic DMARD (bDMARD) was recorded in the registry and the stop date was defined as the date of next bDMARD start. If no new bDMARD was registered the treatment was assumed to have ended 12 months after the last registered visit. If the same drug was restarted within 3 months of the recorded treatment stop date, with no other bDMARD recorded in-between, the treatment periods were considered as one period.

Retention rates were calculated as the percentage of patients still on TNFi 6, 12 and 24 months after treatment start. Observations were censored by: (1) the date of data extraction; (2) date of death; or (3) end of registry follow-up, whichever came first; (4) withdrawal from treatment for other reasons than lack of efficacy (LOE) and adverse events (AE), that is, remission or other reasons such as planning for pregnancy.

Treatment response

At 6, 12 and 24 months' follow-up, clinical response was evaluated as achievement of ASDAS inactive disease (<1.3), BASDAI <40 or achievement of ASAS 20/40 response.

Primary and secondary outcomes

The primary study outcome was the overall 12-month TNFi drug retention rate. Secondary outcomes were overall 6 and 24 months' retention rates and proportions of axSpA patients achieving ASDAS inactive disease, BASDAI <40 and ASAS 20/40 response at 6, 12 and 24 months. Explorative outcomes were retention and response rates in the individual registries at 6, 12 and 24 months, and differences in retention and crude and LUNDEX-adjusted response rates by calendar year.

Ethics

When required, the registries obtained the necessary approvals from national data protection agencies and/or local research ethics boards prior to data transfer. Data from the participating

registries were sent to the coordinating centre in accordance with legal, compliance and regulatory requirements. This study was designed and is reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology guidelines and with the ethical principles according to the Declaration of Helsinki.

Statistical analysis

R V.3.4.3 software was used for statistical analyses. All calculations were based on observed data. No imputation of missing data was performed. The number of patients with available data at baseline and follow-up are shown in table 1 and online supplementary tables S1-S4 and S6-S11.

Descriptive statistics (median, IQR and/or percentage) were applied for demographics and patient characteristics. Comparisons between individual registries and baseline characteristics were tested with analysis of variance (ANOVA). For baseline variables that showed significant heterogeneity, pairwise comparison was performed with χ^2 tests (for categorical variables), Mann-Whitney tests (for non-normally distributed continuous data) and two-tailed Student's t-test (for normally distributed continuous data).

TNFi retention rates (in the pooled cohort and stratified per registry) were investigated by Kaplan-Meier estimation. Age-standardised and gender-standardised drug retention rates were calculated by using the WHO European standard population.[15]

Response rates (crude and adjusted according to LUNDEX[16]) were calculated for ASDAS inactive disease, BASDAI <40 and ASAS 20/40 response. Differences between registries were tested by χ^2 test for crude and LUNDEX-adjusted response rates and logrank for retention rates.

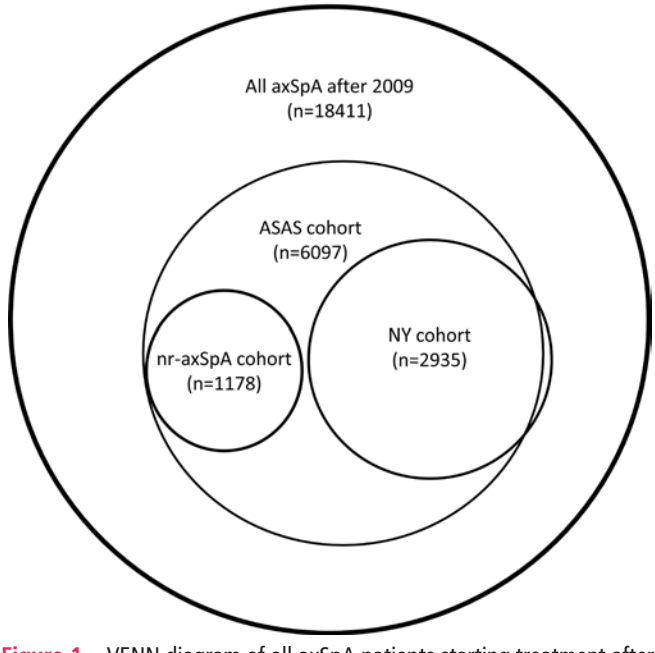

Figure 1 VENN diagram of all axSpA patients starting treatment after 2009. axSpA, axial spondyloarthritis; ASAS cohort, patients registered to fulfill the Assessment of Spondyloarthritis International Sciety (ASAS) criteria for axSpA; Ny cohort, patients registered to fulfill the Modified New York criteria for Ankylosing Spondylitis (AS); nr-axSpA cohort, patients registered to fulfill the ASAS criteria for axSpA and NOT fulfill the Modified New York criteria for AS.

RESULTS

Patient characteristics

A total of 24 195 patients with axSpA initiating their first TNFi were identified from the 12 participating registries and data were pooled. Registration of classification criteria was available in 35% of patients, and the following subcohorts were identified: ASAS cohort (n=6097), NY cohort (n=2935) and nr-axSpA cohort (n=1178) (figure 1).

Most patients were prescribed infliximab (28%), adalimumab (29%) or etanercept (25%), while 14% were treated with golimumab and 5% with certolizumab (table 1). During the study period, some drugs, for example, infliximab, were used off label in the treatment of nr-axSpA. Thirty-one per cent of patients received csDMARDs. At baseline, median (IQR) time since diagnosis was 2 (1-9) years, BASDAI 59 mm (44-72) and BASFI 46 mm (26-66).

Table 2 shows baseline variables for the 12 registries. Statistically (and clinically) significant differences between the registries were observed for all baseline variables (ANOVA; p<0.001). Subsequent pairwise comparison of registries showed statistically significant differences for most baseline variables (data not shown). Patients with AS (fulfilling modified New York criteria) compared with nr-axSpA patients were more often men (67% vs 52%), had longer time since diagnosis (3 (1–10) vs 1 (0–3) years) and higher CRP (13 (5-27) vs 7 (2-19) mg/L). Median BASDAI scores at first TNFi initiation were very similar (66 (52-77) vs 65 (50-78), respectively).

TNFi retention rates at 6, 12 and 24 months

For the entire cohort, the 12-month TNFi retention rate was 80% (95% CI 79% to 80%). Corresponding 12-month retention rates for patients in the ASAS cohort, NY cohort and nr-axSpA cohort were 81% (80% to 82%), 83% (82% to 85%) and 73% (70% to 76%), respectively. At 6/24 months, retention rates for all patients were 88% (87% to 88%) / 73% (72% to 73%), for ASAS cohort 89% (88% to 90%) / 74% (73% to 76%), for NY cohort 90% (89% to 91%) / 76% (74% to 78%) and for nr-axSpA cohort 84% (82% to 86%) / 64% (62% to 67%) (table 3).

The 12-month retention rate in the individual registries differed significantly (p<0.001, logrank) and ranged from 71% to 94% (table 4 and figure 2). Retention rates in the individual registries after 6 and 24 months ranged from 81% to 98% and 62% to 92% (p<0.001, logrank), respectively.

Standardised retention rates (age and gender) for the individual registries at 6, 12 and 24 months were similar to the non-standardised retention rates (table 4).

Over calendar years, retention rates tended to decrease from before 2009 to 2009 to 2014 and to increase again after 2014 (online supplementary table S6).

Achievement of ASDAS inactive disease, BASDAI <40 and ASAS 20/40 response at 6, 12 and 24 months

Overall, ASDAS inactive disease was achieved in 33%, 35% and 38% of the patients at 6, 12 and 24 months, respectively. For BASDAI <40 the proportions were 72%, 75% and 77%, whereas ASAS 20/40 response rates were achieved in 64%/49% at 6 months, 67%/53% at 12 months and 68%/54% at 24 months. Corresponding LUNDEX adjusted rates at 6, 12 and 24 months were 27%, 24% and 19% for ASDAS inactive disease, 59%, 51% and 38% for BASDAI <40. LUNDEX adjusted ASAS 20/40 response rates were 52%/40% at 6

Table 1 Baseline characteristics of all patients and the axSpA subcohorts

	All patients		ASAS cohort*		NY cohort†		nr-axSpA cohort‡	
	No of patients with available data, n	Median (IQR) or percentage	No of patients with available data, n	Median (IQR) or percentage	No of patients with available data, n	Median (IQR) or percentage	No of patients with available data, n	Median (IQR) or percentage
Age, years	24195	41 (33–50)	6097	41 (33–50)	2935	43 (34–52)	1178	39 (31–48)
Male	24195	61%	6097	63%	2935	67%	1178	52%
HLA-B27	12620	74%	5645	76%	2595	70%	1104	69%
BMI, kg/m^2	10418	26 (23–29)	4699	26 (23–29)	2266	26 (23–29)	753	25 (23–29)
Concomitant csDMARD	23984	31%	6002	29%	2865	26%	1169	25%
Time since diagnosis, years	19091	2 (1–9)	5971	2 (1–8)	2884	3 (1–10)	1155	1 (0–3)
Current smoking	21049	23%	5755	27%	2822	27%	1121	26%
Infliximab	6874	28%	1143	19%	580	20%	194	16%
Etanercept	6034	25%	1165	19%	647	22%	230	20%
Adalimumab	6936	29%	2176	36%	1050	36%	410	35%
Certolizumab	1107	5%	240	4%	77	3%	60	5%
Golimumab	3244	14%	1373	23%	581	20%	284	24%
Start before 2009	5784	24	0	0	0	0	0	0
Start 2009–2011	5061	21	1586	26	941	32	345	29
Start 2012–2014	6092	25	2123	35	932	32	500	42
Start 2015–2017	7258	30	2388	39	1062	36	333	28
CRP, mg/L	18552	10 (4–23)	5159	13 (5–26)	2446	13 (5–27)	1003	7 (2–19)
CRP >10 mg/L	18552	48%	5159	56%	2446	56%	1003	40%
BASDAI, mm	14442	59 (44–72)	5014	64 (51–76)	2442	66 (52–77)	994	65 (50–78)
BASMI, mm	4551	24 (10–40)	1937	20 (10–40)	1037	30 (10–50)	605	20 (10–30)
BASFI, mm	11548	46 (26–66)	4187	51 (33–69)	1840	53 (34–70)	762	48 (29–68)
ASDAS, units	7678	3.6 (2.9–4.2)	3139	4.0 (3.3–4.6)	1394	4.1 (3.3–4.7)	561	3.9 (3.1–4.6)
VAS pain, mm	15332	65 (45–80)	4249	70 (52–82)	1927	75 (58–90)	911	70 (52–86)
VAS fatigue, mm	10718	69 (49–80)	3650	70 (51–85)	1434	77 (60–90)	853	72 (51–86)

Data are as observed, median (IQR) or percentage.
*Patients registered as fulfilling the ASAS criteria for axSpA, initiating treatment after 2009.
†Patients registered as fulfilling the modified New York Criteria for ankylosing spondyloarthritis (AS), initiating treatment after 2009.
‡Patients registered to fulfil the ASAS criteria for axSpA and to NOT fulfil the modified New York criteria for AS (nr-axSpA), initiating treatment after 2009.
BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASMI, Bath Ankylosing Spondylitis Metrology Index; BMI, body mass index; INF, infliximab; TNFi, tumour necrosis factor inhibitor; VAS, visual analogue scale; csDMARD, conventional synthetic disease-modifying antirheumatic drug.

months, 46%/36% at 12 months and 34%/27% at 24 months (table 4).

For the subcohorts of patients in the ASAS, NY and nr-axSpA cohort, ASDAS inactive disease response rates at 6, 12 and 24 months were 30%, 25% and 26%, respectively, and corresponding LUNDEX-adjusted rates were 25%, 21% and 20%. BASDAI <40 for the three subgroups of patients at 6, 12 and 24 months were 73%, 71% and 64%, and corresponding LUNDEX adjusted rates were 60%, 60% and 50%.

The 6-month response rates (ASDAS inactive disease and BASDAI <40) in the individual registries ranged from 29% to 53% (LUNDEX adjusted from 17% to 46%) and 61% to 86% (LUNDEX adjusted from 50% to 72%), respectively (p<0.001) (table 4).

The crude and LUNDEX adjusted response rates for BASDAI <40, ASDAS inactive disease and ASAS 20/40 did not show any consistent differences when comparing patients initiating first TNFi before 2009, from 2009 to 2014 or after 2014 (online supplementary S6).

The relation between four selected baseline parameters in different countries and LUNDEX-adjusted response rate (BASDAI ≤40) at 6 months were explored (online supplementary figure S1).

Details about number of patients are found in online supplementary S1, S2, S3 and S4.

Reasons for withdrawal of TNFi treatment

During 24 months of follow-up, a total of 5673 patients (23%) withdrew from treatment. Of these, 3654 patients stopped due to LOE and 2019 due to AE. For patients who withdrew during the 24 months' follow-up period, the median (IQR) time to withdrawal was 7 months (4-13).

For patients in the ASAS, NY and nr-axSpA cohort, the reasons for withdrawal and median time to withdrawal were comparable to the pooled cohort (online supplementary S5).

DISCUSSION

This is the first study of patients with axSpA initiating a first TNFi in routine care, using data from the EuroSpA research collaboration and includes data from 12 countries and over 24000 patients.

Overall, 80% of patients remained on the TNFi 12 months after treatment start. This is comparable to retention rates reported for earlier observational studies of patients with axSpA or AS initiating treatment with a TNFi. In a Swedish study from 2014 of 112 patients with nr-axSpA (patients fulfilling modified New York criteria for AS were excluded), the 12-month retention rate was 76% and 2-year retention rate 65%.[17] In a study from 2010 of 842 Danish TNFi naive patients with AS, 12-month and 24-month retention rates were 74% and 63%, respectively.[18] The 12-month retention rates in individual registries varied from 71% to 94%. Different prescription patterns across countries may explain part of this variability. Despite international recommendations regarding treatment strategies,[19] they may be overruled by national guidelines. These guidelines may have changed over time. Currently, an overview of the guidelines in individual European countries and the changes therein over time does not exist. Examples of differences between countries are different initial doses and/or step up

Table 2 Baseline characteristics of all axSpA patients, stratified by registry

	ARTIS	BIOBADASER	Biorx.si	DANBIO	ICEBIO	NOR-DMARD	Reuma.pt	RRBR	ROB-FIN	SCQM	TURKBIO	ATTRA
Country	Sweden	Spain	Slovenia	Denmark	Iceland	Norway	Portugal	Romania	Finland	Switzerland	Turkey	Czech Republic
All patients (n)	6945	662	615	3897	316	1562	1156	672	1367	2578	2095	2330
ASAS cohort* (n)	NA	NA	503	1229	30	113	700	672	56	1212	351	1231
NY cohort† (n)	NA	NA	467	512	30	38	563	524	NA	559	242	NA
nr-axSpA cohort‡ (n)	NA	NA	37	544	4	68	82	148	NA	221	74	NA
Age, years	41 (32–51)	45 (38–55)	45 (36–55)	41 (32–50)	43 (35–53)	40 (33–50)	41 (34–51)	43 (34–52)	41 (33–51)	42 (33–51)	38 (31–46)	39 (33–48)
Year of first patient initiating TNFi	1999	2000	2002	2000	2001	2001	2001	2015	2000	2002	2001	2002
Male	58%	69%	64%	61%	66%	60%	56%	74%	59%	53%	60%	72%
HLA-B27	NA	81%	80%	71%	92%	82%	77%	51%	87%	64%	62%	91%
BMI, kg/m^2	NA	26 (24–30)	26 (23–29)	26 (23–29)	26 (24–29)	25 (23–29)	25 (23–29)	26 (24–29)	26 (23–29)	25 (23–29)	27 (24–30)	26 (23–29)
Concomitant csDMARD, pct	33%	30%	15%	27%	22%	18%	42%	42%	72%	19%	20%	41%
Time since diagnosis, years	1 (0–7)§	5 (1–13)	5 (1–12)	1 (0–6)	3 (0–11)	4 (1–14)	4 (1–10)	3 (1–10)	4 (1–11)	1 (0–7)	3 (1–7)	5 (2–10)
Current smoking	12%	31%	22%	33%	24%	29%	29%	12%	23%	25%	41%	24%
Infliximab	34%	25%	15%	35%	83%	18%	22%	17%	30%	21%	27%	23%
Etanercept	29%	20%	23%	14%	8%	32%	27%	27%	33%	25%	29%	22%
Adalimumab	27%	30%	43%	29%	2%	18%	31%	36%	27%	33%	28%	37%
Certolizumab	3%	7%	1%	7%	0%	19%	1%	0%	1%	2%	5%	2%
Golimumab	10%	19%	17%	15%	7%	12%	18%	20%	8%	19%	11%	15%
Start before 2009	25%	23%	18%	25%	36%	26%	18%	0%	43%	27%	11%	23%
Start 2009–2011	21%	9%	29%	22%	16%	24%	24%	0%	25%	25%	15%	22%
Start 2012–2014	25%	15%	35%	26%	21%	30%	26%	0%	20%	26%	31%	26%
Start 2015–2017	29%	53%	18%	27%	28%	20%	32%	100%	12%	22%	43%	28%
CRP, mg/L	8 (3–21)	3 (1–9)	11 (3–25)	8 (3–19)	7 (3–19)	6 (3–15)	11 (4–26)	24 (16–41)	8 (3–20)	7 (3–15)	12 (4–26)	20 (12–32)
CRP >10 mg/L	43%	22%	51%	42%	38%	33%	52%	84%	42%	33%	55%	80%
BASDAI, mm	56 (40–69)	55 (43–70)	69 (57–80)	61 (47–73)	60 (46–76)	50 (33–65)	62 (49–76)	74 (66–82)	39 (14–58)	57 (42–71)	49 (35–62)	65(52–76)
BASFI, mm	38 (20–59)	NA	58 (40–73)	49 (31–67)	43 (32–58)	NA	61 (40–75)	NA	27 (7–49)	38 (19–60)	31 (17–51)	54 (38–70)
BASMI, mm	NA	NA	NA	30 (10-40)	20 (10-30)	NA	40 (28-54)	NA	NA	20 (10-30)	30 (10-50)	NA
ASDAS, units	3.1 (2.5–3.8)	3.4 (2.6–4.0)	NA	3.5 (2.9–4.2)	3.7 (3.2–4.2)	NA	3.7 (3.1–4.3)	4.6 (4.2–5.0)	2.8 (2.0–3.5)	NA	3.5 (2.9–4.0)	4.0 (3.5–4.5)
VAS pain, mm	62 (43-75)	NA	70 (50–80)	66 (49–78)	65 (46–78)	50 (31–68)	50 (10–60)	90 (80–100)	58 (31–72)	70 (50–80)	71 (51–80)	70 (50–80)
VAS fatigue, mm	63 (36–78)	NA	NA	71 (52–84)	65 (45–80)	58 (30–75)	NA	90 (80–90)	NA	70 (50–80)	70 (50–75)	65 (50–80)

Data are as observed, median (IQR) or percentage.

*Patients registered as fulfilling the ASAS criteria for axSpA, initiating treatment after 2009.

†Patients registered as fulfilling the modified New York Criteria for ankylosing spondyloarthritis (AS), initiating treatment after 2009.

‡Patients registered to fulfil the ASAS criteria for axSpA and to NOT fulfil the modified New York criteria for AS (nr-axSpA), initiating treatment after 2009.

§Time since inclusion in ARTIS.

BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASMI, Bath Ankylosing Spondylitis Metrology Index; BMI, body mass index; CRP, C reactive protein; TNFi, tumour necrosis factor inhibitor; VAS, visual analogue scale; csDMARD, conventional synthetic disease-modifying antirheumatic drug.

Table 3 Retention and response rates in patients with axial spondyloarthritis

	All patients	ASAS cohort*	NY cohort†	nr-axSpA cohort‡
	Retention rates	Retention rates	Retention rates	Retention rates
6 months (95% CI)	88% (87% to 88%)	89% (88% to 90%)	90% (89% to 91%)	84% (82% to 86%)
12 months (95% CI)	80% (79% to 80%)	81% (80% to 82%)	83% (82% to 85%)	73% (70% to 76%)
24 months (95% CI)	73% (72% to 73%)	74% (73% to 76%)	76% (74% to 78%)	64% (62%–67%)

	Response rates		Response rates		Response rates		Response rates	
	Crude§	LUNDEX adjusted ¶	Crude§	LUNDEX adjusted¶	Crude§	LUNDEX adjusted¶	Crude§	LUNDEX adjusted¶
ASDAS inactive disease at 6 months	33%	27%	30%	25%	25%	21%	26%	20%
ASDAS inactive disease at 12 months	35%	24%	33%	23%	29%	21%	31%	19%
ASDAS inactive disease at 24 months	38%	19%	38%	18%	32%	16%	37%	15%
BASDAI <40 at 6 months	72%	59%	73%	60%	71%	60%	64%	50%
BASDAI <40 at 12 months	75%	51%	76%	52%	73%	52%	70%	43%
BASDAI <40 at 24 months	77%	38%	79%	37%	76%	37%	71%	29%
ASAS 20/40 at 6 months	64%/49%	52%/40%	68%/54%	56%/45%	64%/48%	54%/41%	57%/42%	45%/33%
ASAS 20/40 at 12 months	67%/53%	46%/36%	70%/57%	48%/39%	66%/52%	47%/37%	63%/49%	39%/30%
ASAS 20/40 at 24 months	68%/54%	34%/27%	73%/59%	34%/28%	67%/51%	33%/25%	69%/53%	28%/22%

Data are as observed, median (IQR) or percentage.
*Patients registered as fulfilling ASAS criteria, initiating treatment after 2009.
†Patients registered as fulfilling the modified New York Criteria for AS, initiating treatment after 2009.
‡Patients registered to fulfil the ASAS criteria for axSpA and to NOT fulfil the modified New York criteria for AS (nr-axSpA), initiating treatment after 2009.
§Crude value: the fraction responding of those still on drug at 6, 12 and 24 months, respectively.
¶LUNDEX adjusted: crude value adjusted for drug retention.
ASAS, Assessment of Spondylo Arthritis International Society; ASDAS, Ankylosing Spondylitis Disease Activity Score; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index.

strategies,[20–22] comedication with csDMARDs[23–25] and mandatory elevation of CRP or evidence of inflammation on MRI for patients with nr-axSpA.[26] Those differences in access to therapy and treatment guidelines between countries probably account for some of the variation in both baseline variables and treatment outcomes observed in the present study.

Further, patients selected to receive their first TNFi varied across the countries, which was not unexpected. For instance, the disease activity (BASDAI) at baseline in individual countries varied from 39 to 74 mm. Moreover, HLA-B27-positive patients at baseline varied from 51% to 92%, and the percentage of patients treated with concomitant csDMARD varied from 15% to 72%. Although no consistent pattern was found, it appeared that countries with a high proportion of HLA-B27-positive patients and a high proportion of patients receiving concomitant csDMARDs achieved higher LUNDEX-adjusted BASDAI remission rates. Further studies of the predictive value of baseline characteristics in individual countries for crude and LUNDEX-adjusted response and remission rates are needed. Interestingly, adjustment for age and gender did not change retention and response rates substantially.

Different response measures have been applied in previous studies, which makes comparisons difficult. A Danish study of 842 Danish TNFi naive patients with AS evaluated drug effectiveness by reduction in BASDAI of at least 50% or >20 mm compared with baseline according to the ASAS guidelines (BASDAI 50%/20 mm response) and reported a response rate of 63%.[18] Landewe *et al* investigated ASAS20 response in 325 axSpA patients treated with certolizumab. At week 12, ASAS20 response was 58%–64%, and no differences were observed between patients with AS and nr-axSpA.[27] This is in line with our findings where ASAS20 response at 6-month follow-up was 64%. The study of Sieper *et al* evaluated the efficacy of adalimumab in 185 patients who fulfilled ASAS criteria.[28] They found 36% of patients achieved ASAS40 at week 12, which is comparable to the percentage of patients achieving ASAS40 in our pooled population.

The large number of patients allowed us to identify and analyse data on three subcohorts of axSpA patients, who had started treatment after 2009. These subcohorts were patients who were explicitly registered as (1) fulfilling the ASAS criteria for axSpA (ASAS cohort) or (2) the modified New York criteria for AS (NY cohort). The nr-axSpA cohort was constructed of patients registered to fulfil ASAS criteria and to NOT fulfil the Modified New York criteria. This information was only available in a minority of patients, which explains the lower patient numbers in the subcohorts. The subcohorts were investigated to clarify if they displayed marked differences in retention and response rates. Interestingly, nr-axSpA subcohort had lower retention rates at 12 months. Furthermore, we found numerically lower LUNDEX-adjusted response rates (BASDAI <40 and ASAS 20/40 response at 6 months) for nr-axSpA patients compared with the other subcohorts. This observation may reflect that the subcohort of nr-axSpA patients include some patients with a less certain diagnosis, which may consequently respond poorly and discontinue their TNFi more frequently. However, our data showed no differences in crude response rates.

Table 4 Retention rates at 6, 12 and 24 months and response rates at 6 months stratified by registry

	All patients		ASAS cohort*		NY cohort†		nr-axSpA cohort‡		All patients			
	Retention rates (6/12/24 months), pct		Retention rates (6/12/24 months), pct		Retention rates (6/12/24 months), pct		Retention rates (6/12/24 months), pct		Rates of ASDAS inactive disease (6 months)		Rates of BASDAI <40 (6 months)	
Registry		95% CI		95% CI		95% CI		95% CI	Crude§	LUNDEX adjusted¶	Crude¶	LUNDEX adjusted**
ARTIS	87/79/71	(86 to 88)/ (78 to 80)/ (69 to 72)	NA	NA	NA	NA	NA	NA	41%	33%	71%	56%
BIOBADASAR	92/86/79	(90 to 94)/ (83 to 89)/ (76 to 83)	NA	NA	NA	NA	NA	NA	20%	17%	61%	52%
Biorx.si	93/86/80	(90 to 95)/ (83 to 89)/ (76 to 83)	91/83/75	(88 to 93)/ (80 to 86)/ (71 to 79)	91/83/76	(88 to 94)/ (80 to 87)/ (72 to 80)	89/77/68	(79 to 100)/ (65 to 93)/ (53 to 86)	NA	NA	67%	61%
DANBIO	81/71/62	(80 to 82)/ (70 to 73)/ (61 to 64)	80/70/59	(78 to 83)/ (68 to 73)/ (57 to 62)	83/75/65	(80 to 87)/ (71 to 79)/ (61 to 70)	79/66/55	(75 to 82)/ (62 to 70)/ (51 to 60)	29%	22%	64%	48%
ICEBIO	89/79/70	(86 to 93)/ (74 to 84)/ (65 to 75)	80/70/63	(67 to 96)/ (55 to 88)/ (48 to 83)	80/70/63	(67 to 96)/ (55 to 88)/ (48 to 83)	75/50/50	(43 to 100)/ (19 to 100)/ (19 to 100)	42%	36%	74%	63%
NOR-DMARD	82/72/63	(80 to 84)/ (70 to 75)/ (61 to 66)	92/86/80	(87 to 97)/ (80 to 93)/ (72 to 87)	95/92/84	(88 to 100)/ (84 to 100)/ (73 to 97)	90/81/75	(83 to 97)/ (72 to 91)/ (65 to 86)	NA	NA	76%	61%
Reuma.pt	93/87/82	(91 to 94)/ (85 to 89)/ (79 to 84)	94/87/82	(92 to 96)/ (84 to 90)/ (79 to 85)	94/88/82	(92 to 96)/ (86 to 91)/ (79 to 86)	92/78/69	(87 to 98)/ (69 to 88)/ (59 to 81)	23%	20%	64%	57%
ROB-FIN	95/92/89	(94 to 96)/ (91 to 94)/ (87 to 91)	91/87/75	(83 to 99)/ (78 to 96) (63 to 90)	NA	NA	NA	NA	53%	46%	79%	68%
RRBR	98/94/92	(97 to 99)/ (92 to 96)/ (89 to 95)	98/94/92	(97 to 99)/ (92 to 96)/ (89 to 95)	98/93/91	(97 to 99)/ (91 to 96)/ (88 to 94)	99/97/95	(98 to 100)/ (94 to 100)/ (91 to 99)	22%	18%	86%	69%
SCQM	83/74/66	(82 to 85)/ (73 to 76)/ (65 to 68)	83/75/68	(81 to 86)/ (72 to 77)/ (65 to 71)	86/77/69	(83 to 88)/ (74 to 81)/ (65 to 73)	80/71/66	(75 to 86)/ (65 to 77)/ (60 to 73)	NA	NA	62%	50%
TURKBIO	92/85/77	(91 to 93)/ (83 to 86)/ (75 to 79)	89/82/75	(85 to 92)/ (78 to 86)/ (70 to 80)	89/84/78	(85 to 93)/ (80 to 90)/ (73 to 84)	87/76/67	(80 to 95)/ (66 to 87)/ (56 to 81)	27%	22%	79%	65%
ATTRA	93/88/83	(92 to 94)/ (87 to 90)/ (82 to 85)	94/88/84	(92 to 95)/ (87 to 90)/ (82 to 86)	NA	NA	NA	NA	38%	34%	81%	72%

Data are as observed, median (95% CI); ARTIS (Sweden); BIOBADASAR (Spain); Biorx.si (Slovenia); DANBIO (Denmark); ICEBIO (Iceland); NOR-DMARD (Norway); Reuma.pt (Portugal); ROB-FIN (Finland); RRBR (Romania); SCQM (Switzerland); TURKBIO (Turkey); ATTRA (Czech Republic).
*Patients registered as fulfilling ASAS criteria, initiating treatment after 2009.
†Patients registered as fulfilling the modified New York Criteria for AS, initiating treatment after 2009.
‡Patients registered to fulfil the ASAS criteria for axSpA and to NOT fulfil the modified New York criteria for AS (nr-axSpA), initiating treatment after 2009.
§Standardised (age and gender) drug retention rates.
¶Crude value: the fraction responding of those still on drug at 6, 12 and 24 months, respectively.
**LUNDEX adjusted: crude value adjusted for drug retention.

Also, differences in baseline characteristics were found between the subgroups of patients registered with AS according to New York criteria and patients with nr-axSpA. This is in line with earlier studies. A Danish study of 1250 axSpA patients found that patients with nr-axSpA were more frequently women, HLA-B27 negative, had shorter time since diagnosis, higher VAS scores and BASDAI, but lower CRP and Bath Ankylosing Spondylitis Metrology Index than patients with AS.[11] A French observational study of 361 axSpA patients and a study from the German GESPIC cohort found the same differences in AS versus nr-axSpA regarding gender, disease duration and CRP.[29 30]

The strengths of this study are the generalizability of the results, which we consider to be high due to the inclusion of data from 12 registries across Europe, and a total of over 24 000 patients. A limitation is that selection bias based on data availability cannot be ruled out. Compliant subjects may be more likely to visit their doctor regularly and may therefore have more complete registry data, which could potentially lead to overestimation of drug retention rates. However, data have been collected prospectively and independently of the current research study.

Collaboration across registries may provide knowledge regarding prescription patterns, which has an interest in its own, but may also allow for investigation of heterogeneity across countries. The differences in prescription patterns and access to treatment imply that the results of pooling of data across countries should be interpreted with caution.[20]

In conclusion, data from 24195 European patients with axSpA who received their first TNFi were pooled, and the

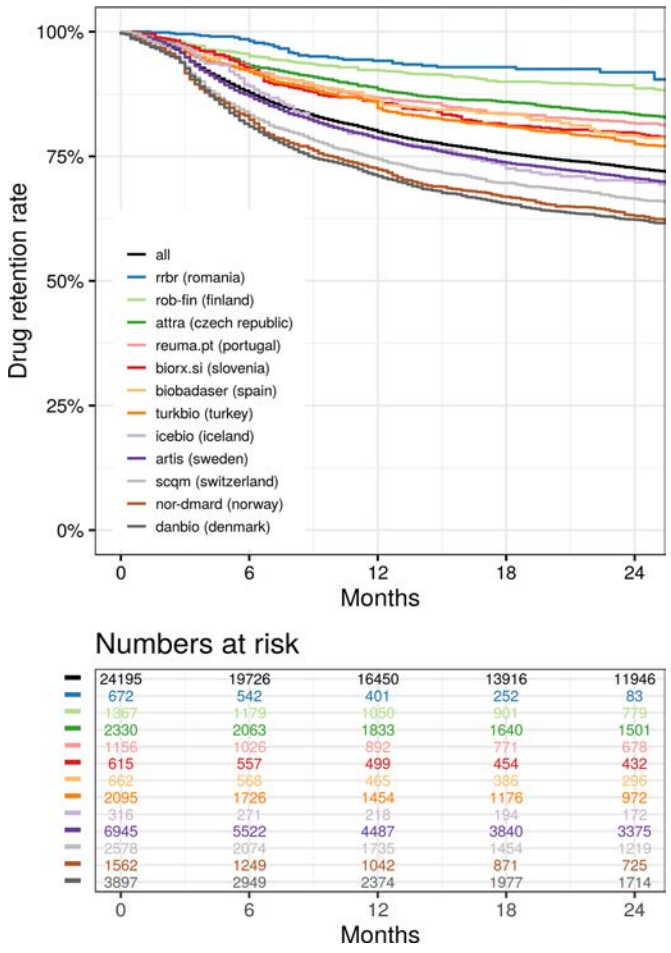

Figure 2 Kaplan-Meier curves (top) showing drug retention rates up to 24 months for pooled data and per register. The table (bottom) shows the number of patients who were still being treated at the corresponding time points.

retention and response rates were reported. Approximately a third of patients were in ASDAS inactive disease state after 6 months, and 80% were still receiving the same TNFi after 1 year. The EuroSpA collaboration offers unprecedented opportunities for providing real-world evidence on the effectiveness of biological drugs in European patients with axSpA.

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REFERENCES

1. van der Linden S, Valkenburg HA, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the new York criteria. *Arthritis Rheum* 1984;27:361–8.
2. Rudwaleit M, van der Heijde D, Landewe R, *et al*. The development of assessment of spondyloarthritis International Society classification criteria for axial spondyloarthritis (Part II): validation and final selection. *Ann Rheum Dis* 2009;68:777–83.

3. Sieper J, Rudwaleit M, Baraliakos X, *et al*. The assessment of spondyloarthritis International Society (ASAS) Handbook: a guide to assess spondyloarthritis. *Ann Rheum Dis* 2009;68(Suppl 2):ii1–44.
4. Braun J, Davis J, Dougados M. First update of the International ASAS consensus statement for the use of anti-TNF agents in patients with ankylosing spondylitis. *Ann Rheum Dis* 2006;65:316–20.
5. Guideline for treatment of axial spondyloarthritis in Denmark. Dansk Reumatologisk Selskab, Dansk Radiologisk Selskab (Danish language). Available: http://www.danskreumatologisksel skab.dk/fileadmin/DRS/kliniskeretningslinjer/SpA_retningslinie_DRS.pdf
6. Braun J*et al*. International ASAS consensus statement for the use of anti-tumour necrosis factor agents in patients with ankylosing spondylitis. *Ann Rheum Dis* 2003;62:817–24.
7. Braun J, van den Berg R, Baraliakos X, *et al*. 2010 update of the ASAS/EULAR recommendations for the management of ankylosing spondylitis. *Ann Rheum Dis* 2011;70:896–904.
8. Zink A, Askling J, Dixon WG, *et al*. European biologicals registers: methodology, selected results and perspectives. *Ann Rheum Dis* 2009;68:1240–6.
9. Glintborg B, Sørensen IJ, Østergaard M, *et al*. Ankylosing spondylitis versus Nonradiographic axial spondyloarthritis: comparison of tumor necrosis factor inhibitor effectiveness and effect of HLA-B27 status. An observational cohort study from the nationwide DANBIO registry. *J Rheumatol* 2017;44:59–69.
10. Glintborg B, Østergaard M, Krogh NS, *et al*. Clinical response, drug survival and predictors thereof in 432 ankylosing spondylitis patients after switching tumour necrosis factor α inhibitor therapy: results from the Danish nationwide DANBIO registry. *Ann Rheum Dis* 2013;72:1149–55.
11. Lindström U, Olofsson T, Wedrén S, *et al*. Impact of extra-articular spondyloarthritis manifestations and comorbidities on drug retention of a first TNF-inhibitor in ankylosing spondylitis: a population-based nationwide study. *RMD Open* 2018;4.
12. Garrett S, Jenkinson T, Kennedy LG, *et al*. A new approach to defining disease status in ankylosing spondylitis: the Bath ankylosing spondylitis disease activity index. *J Rheumatol* 1994;21:2286–91.
13. van der Heijde D, Landewe R, Feldtkeller E. Proposal of a linear definition of the Bath ankylosing spondylitis Metrology index (BASMI) and comparison with the 2-step and 10-step definitions. *Ann Rheum Dis* 2008;67:489–93.
14. Machado PM, Landewé R, Heijde DV. Ankylosing spondylitis disease activity score (ASDAS): 2018 update of the nomenclature for disease activity states. *Ann Rheum Dis* 2018;77:1539–40.
15. Eurostat. Revision of the European standard population: report of Eurostat's task force, 2013. Available: https://ec.europa.eu/eurostat/documents/3859598/5926869/KS-RA-13-028-EN.PDF/e713fa79-1add-44e8-b23d-5e8fa09b3f8f
16. Kristensen LE, Saxne T, Geborek P. The LUNDEX, a new index of drug efficacy in clinical practice: results of a five-year observational study of treatment with infliximab and etanercept among rheumatoid arthritis patients in southern Sweden. *Arthritis Rheum* 2006;54:600–6.
17. Gulfe A, Kapetanovic MC, Kristensen LE. Efficacy and drug survival of anti-tumour necrosis factor-alpha therapies in patients with non-radiographic axial spondyloarthritis: an observational cohort study from southern Sweden. *Scand J Rheumatol* 2014;43:493–7.
18. Glintborg B, Ostergaard M, Krogh NS, *et al*. Predictors of treatment response and drug continuation in 842 patients with ankylosing spondylitis treated with anti-tumour necrosis factor: results from 8 years' surveillance in the Danish nationwide DANBIO registry. *Ann Rheum Dis* 2010;69:2002–8.
19. van der Heijde D, Ramiro S, Landewé R, *et al*. 2016 update of the ASAS-EULAR management recommendations for axial spondyloarthritis. *Ann Rheum Dis* 2017;76:978–91.
20. Martinez-Cutillas J, Alerany-Pardo C, Borrás-Blasco J, *et al*. The use of adalimumab, etanercept, golimumab and infliximab in rheumatic pathologies: variation between label dosage and real-world use. *Expert Rev Pharmacoecon Outcomes Res* 2015;15:851–8.
21. Tenga G, Goëb V, Lequerré T, *et al*. A 3mg/kg starting dose of infliximab in active spondyloarthritis resistant to conventional treatments is efficient, safe and lowers costs. *Joint Bone Spine* 2011;78:50–5.
22. Glintborg B, Gudbjörnsson B, Steen Krogh N, *et al*. Impact of different infliximab dose regimens on treatment response and drug survival in 462 patients with psoriatic arthritis: results from the nationwide registries DANBIO and ICEBIO. *Rheumatology* 2014;53:2100–9.
23. Nissen MJ, Ciurea A, Bernhard J, *et al*. The effect of comedication with a conventional synthetic disease-modifying antirheumatic drug on drug retention and clinical effectiveness of anti-tumor necrosis factor therapy in patients with axial spondyloarthritis. *Arthritis Rheumatol* 2016;68:2141–50.
24. Sepriano A, Ramiro S, van der Heijde D, *et al*. Effect of comedication with conventional synthetic disease-modifying antirheumatic drugs on retention of tumor necrosis factor inhibitors in patients with spondyloarthritis: a prospective cohort study. *Arthritis Rheumatol* 2016;68:2671–9.
25. Lie E, Kristensen LE, Forsblad-d'Elia H, *et al*. The effect of comedication with conventional synthetic disease modifying antirheumatic drugs on TNF inhibitor drug survival in patients with ankylosing spondylitis and undifferentiated spondyloarthritis: results from a nationwide prospective study. *Ann Rheum Dis* 2015;74:970–8.
26. Poddubnyy D, Sieper J. Similarities and differences between nonradiographic and radiographic axial spondyloarthritis: a clinical, epidemiological and therapeutic assessment. *Curr Opin Rheumatol* 2014;26:377–83.
27. Landewé R, Braun J, Deodhar A, *et al*. Efficacy of certolizumab pegol on signs and symptoms of axial spondyloarthritis including ankylosing spondylitis: 24-week results of a double-blind randomised placebo-controlled phase 3 study. *Ann Rheum Dis* 2014;73:39–47.
28. Sieper J, van der Heijde D, Dougados M, *et al*. Efficacy and safety of adalimumab in patients with non-radiographic axial spondyloarthritis: results of a randomised placebo-controlled trial (ABILITY-1). *Ann Rheum Dis* 2013;72:815–22.
29. Corli J, Flipo R-M, Philippe P, *et al*. Tumor necrosis factor-α inhibition in ankylosing spondylitis and Nonradiographic axial spondyloarthritis: treatment response, drug survival, and patient outcome. *J Rheumatol* 2015;42:2376–82.
30. Rudwaleit M, Haibel H, Baraliakos X, *et al*. The early disease stage in axial spondylarthritis: results from the German spondyloarthritis inception cohort. *Arthritis Rheum* 2009;60:717–27.

CLINICAL SCIENCE

Do patients with axial spondyloarthritis with radiographic sacroiliitis fulfil both the modified New York criteria and the ASAS axial spondyloarthritis criteria? Results from eight cohorts

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ABSTRACT

Background Patients with spondyloarthritis with radiographic sacroiliitis are traditionally classified according to the modified New York (mNY) criteria as ankylosing spondylitis (AS) and more recently according to the Assessment of SpondyloArthritis international Society (ASAS) criteria as radiographic axial spondyloarthritis (r-axSpA).

Objective To investigate the agreement between the mNY criteria for AS and the ASAS criteria for r-axSpA and reasons for disagreement.

Methods Patients with back pain ≥3 months diagnosed as axSpA with radiographic sacroiliitis (mNY radiographic criterion) were selected from eight cohorts (ASAS, Esperanza, GESPIC, OASIS, Reuma.pt, SCQM, SPACE, UCSF). Subsequently, we calculated the percentage of patients who fulfilled the ASAS r-axSpA criteria within the group of patients who fulfilled the mNY criteria and vice versa in six cohorts with complete information.

Results Of the 3882 patients fulfilling the mNY criteria, 93% also fulfilled the ASAS r-axSpA criteria. Inversely, of the 3434 patients fulfilling the ASAS r-axSpA criteria, 96% also fulfilled the mNY criteria. The main cause for discrepancy between the two criteria sets was the reported age at onset of back pain.

Conclusion Almost all patients with axSpA with radiographic sacroiliitis fulfil both ASAS and mNY criteria, which supports the interchangeable use of the terms AS and r-axSpA.

Key messages

What is already known about this subject?

► Patients with spondyloarthritis with radiographic sacroiliitis can be classified using the modified New York (mNY) criteria for ankylosing spondylitis (AS) or the Assessment of SpondyloArthritis international Society (ASAS) criteria for radiographic axial spondyloarthritis (r-axSpA). However, discussion remains whether these criteria define the same patients.

What does this study add?

► This study demonstrates that patients with axSpA classified as AS according to the mNY criteria and those classified as r-axSpA according to the ASAS criteria are mostly the same. These findings support the interchangeable use of the terms r-axSpA and AS.

How might this impact on clinical practice or future developments?

► Acknowledging that r-axSpA and AS are interchangeable increases comparability between studies, since both terms describe the same patients (ie, patients with axSpA with radiographic sacroiliitis). This also ensures that results from older research on AS cohorts can be directly compared with more recently published articles on r-axSpA cohorts.

BACKGROUND

Traditionally, patients with axial spondyloarthritis (axSpA) with definite structural changes on conventional radiographs are classified according to the modified New York (mNY) criteria as ankylosing spondylitis (AS). However, they may also be classified according to the more recent Assessment of SpondyloArthritis international Society (ASAS) axSpA criteria as radiographic axSpA (r-axSpA).

Both the mNY and the ASAS axSpA classification criteria use the radiographic criterion as defined by the mNY criteria (ie, sacroiliitis of at least grade 2 bilaterally or at least grade 3 unilaterally). However, the additionally required (clinical) features of the classification criteria differ (table 1). Importantly, patients with age at onset of back pain ≥45 years cannot fulfil the ASAS criteria, but there is no age limit for the mNY criteria.[1 2] Patients without the inflammatory character of back pain fulfil the ASAS criteria if another SpA feature is present, but only fulfil the mNY criteria if there is limitation in spinal mobility. These differences in the clinical part of both criteria sets raise the question whether the two sets classify the same patients with axSpA with radiographic sacroiliitis.

The aim of this study was to investigate if patients who fulfil the mNY criteria also fulfil the ASAS

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Table 1 Classification of axSpA with radiographic sacroiliitis using the mNY criteria for the classification of AS[17] and the ASAS criteria for the classification of r-axSpA[1]

mNY criteria for the classification of AS	ASAS criteria for the classification of radiographic axSpA
1. Low back pain and stiffness for at least 3 months, which improves with exercise and is not relieved by rest 2. Limitation of lumbar spine motion in the sagittal and frontal planes 3. Decreased chest expansion compared with age-matched and sex-matched controls 4a. Unilateral sacroiliitis grade 3 or 4 4b. Bilateral sacroiliitis grade 2 to 4	1. Back pain ≥3 months 2. Age at onset <45 years 3. Definite radiographic sacroiliitis according to mNY criteria 4. ≥1 SpA feature ► Inflammatory back pain ► Arthritis ► Enthesitis ► Uveitis ► Dactylitis ► Psoriasis ► Crohn's/colitis ► Good response to NSAIDs ► Family history for SpA ► HLA-B27 positive ► Elevated CRP (or ESR)
Definite AS if sacroiliitis as described in 4a or 4b and any of the clinical symptoms (1–3)	Definite r-axSpA if fulfilment of 1 and 2, sacroiliitis as described in 3 and at least one of the clinical SpA features as described in 4

AS, ankylosing spondylitis; ASAS, Assessment of SpondyloArthritis international Society; axSpA, axial Spondyloarthritis; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; HLA-B27, human leucocyte antigen B27; mNY, modified New York; NSAID, non-steroidal anti-inflammatory drugs;r-axSpA, radiographic axial spondyloarthritis.

criteria for r-axSpA and vice versa. The second objective was to investigate reasons for disagreement.

METHODS

Patients diagnosed with axSpA who had back pain for at least 3 months and definite radiographic sacroiliitis based on local reading, according the mNY radiographic criterion (#4a or 4b in table 1) were selected from eight cohorts (ASAS, Esperanza, GErman SPondyloarthritis Inception Cohort (GESPIC), Outcome in Ankylosing Spondylitis International Study (OASIS), Reuma.pt, Swiss Clinical Quality Management (SCQM), SPondyloArthritis Caught Early cohort (SPACE) and University of California San Francisco (UCSF) axSpA cohort[1 3–9]). The ASAS cohort included patients with undiagnosed axSpA irrespective of symptom duration in 25 ASAS centres across 16 countries in Western Europe, Turkey, Asia, Colombia and Canada between 2005 and 2009.[1] Esperanza is a Spanish national health programme for early SpA, which started inclusion in 2007.[6] GESPIC started in 2000 and consists of patients with axSpA and symptom duration of up to 10 years.[7] OASIS consists of Dutch, Belgian and French patients with established AS, which started in 1996.[8] Since 2008, Reuma.pt started with the inclusion of Portuguese rheumatic patients of various diseases and disease stages in a national register, including patients with early and established axSpA.[3] The SCQM axSpA cohort started in Switzerland in 2005 including patients with early and established disease.[4] SPACE is an early chronic back pain cohort including European patients since 2009.[9] Patients in the UCSF axSpA cohort started enrolling in 2007; patients with early and established disease from the UCSF clinic are included.[5] Approval from the medical ethical committees was obtained per cohort, and for all patients written informed consent was obtained prior to inclusion.

For these cohorts, we calculated how many patients with SpA with radiographic sacroiliitis fulfil the mNY criteria (mNY+) and the ASAS r-axSpA criteria (ASAS+). Subsequently, we calculated the percentage of patients who fulfil the ASAS r-axSpA criteria within the group of patients who fulfil the mNY criteria. In six cohorts, we were also able to calculate the percentage of patients fulfilling the mNY criteria within the group fulfilling the ASAS r-axSpA criteria. For the Esperanza and OASIS cohorts, specific information on the individual items of the mNY clinical criteria was unavailable. Consequently, it was not possible to calculate the percentage of patients fulfilling the mNY criteria within the subgroup fulfilling the ASAS criteria. Flowcharts were used to visualise fulfilment of the criteria sets (online supplementary figure S1).

For the patients with axSpA with radiographic sacroiliitis, the first step was to determine whether a patient had inflammatory back pain (IBP). For the purpose of this study, the first clinical criterion of the mNY was equated to IBP according to the ASAS definition.[10] The second step was to determine the number of SpA features (<1 vs ≥1) as well as whether the patient had mobility restrictions. Mobility restrictions were defined using the age-adjusted fifth percentile scores of healthy individuals from Ramiro *et al*[11]; if the Schober's test and lateral spinal flexion were below the age-adjusted fifth percentile value or chest expansion was below the age-adjusted and height-adjusted fifth percentile value, mobility was considered restricted. The final step was to look at age at onset of back pain (<45 vs ≥45 years old).

RESULTS

A total of 7636 patients with a SpA diagnosis and back pain >3 months were included in these eight cohorts. Of these, 4041 patients had a diagnosis of axSpA with radiographic sacroiliitis and were available for analysis. In total, 3882 patients fulfilled the mNY criteria, of which 3607 (93%; range 88%–100%) also fulfilled the ASAS r-axSpA criteria (figure 1A). From the six cohorts (N=3721) in which the fulfilment of the mNY criteria in the subgroup of patients fulfilling the ASAS r-axSpA criteria (N=3434) could be analysed, 3300 (96%; range 84%–98%) also fulfilled the mNY criteria (figure 1B).

For all 4041 patients with r-axSpA fulfilment of the criteria sets was determined (online supplementary tables S1-S3). In total, 3607 (89%) of patients fulfilled both criteria sets; 275 (7%) only the mNY criteria; 134 (3%) only the ASAS criteria and 25 (1%) neither set (table 2).

The main difference between the two criteria sets was caused by the reported age at onset of back pain; 99.7% of the patients fulfilling the mNY criteria could potentially fulfil the ASAS criteria except for registered age at onset (online supplementary figure S4).

Out of the 275 mNY+patients not fulfilling the ASAS criteria (7% of all included patients), 265 (96%) cases were due to the age criterion and 10 (4%) due to the absence of SpA features including IBP (online supplementary table 1). These 10 patients had spinal mobility limitation as the only clinical feature. The 134 mNY-/ASAS+ did not have mobility restriction or IBP but another SpA feature instead. For the cohorts that had data available (N=1833), the human leucocyte antigen B27 (HLA-B27) status was determined in each of the subgroups. In the mNY+/ASAS+ group, HLA-B27 positivity was 68%. In the mNY-/ASAS+ group, a similar percentage was found (72%), whereas in the mNY+/ASAS- group this percentage was only 46%, thus

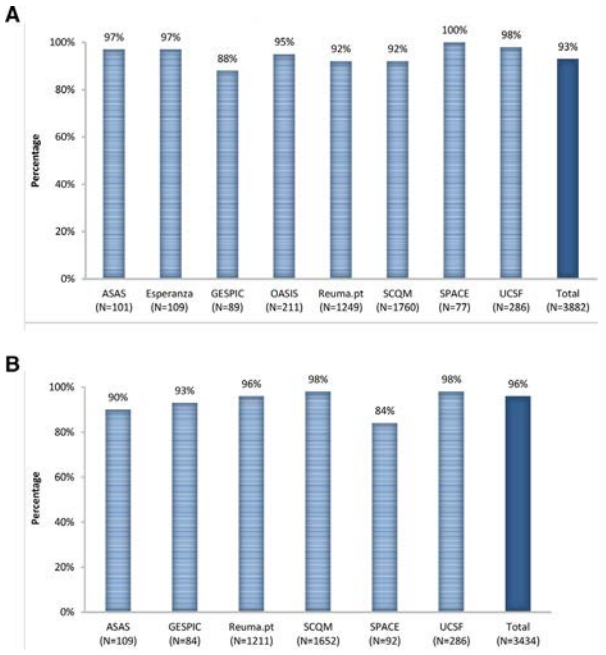

Figure 1 Percentage of patients fulfilling ASAS r-axSpA within subgroup fulfilling mNY criteria (3607/3882) (A) and percentage of patients fulfilling mNY criteria within subgroup fulfilling ASAS r-axSpA (3300/3434) (B), per cohort and overall. ASAS, Assessment of SpondyloArthritis international Society cohort; Esperanza, Spanish national health programme for early SpA; GESPIC, GErman SPondyloarthritis Inception Cohort; mNY, modified New York; OASIS, Outcome in Ankylosing Spondylitis International Study; r-axSpA, radiographic axial spondyloarthritis; Reuma.pt, Portuguese Register for Rheumatic Diseases; SCQM, Swiss Clinical Quality Management cohort; SPACE, SPondyloArthritis Caught Early cohort; UCSF, University of California San Francisco axSpA cohort.

only slightly higher than the mNY-/ASAS- group (42%) (online supplementary table S2).

DISCUSSION

'Classification criteria are standardised definitions that are primarily intended to create well-defined, relatively homogeneous cohorts of patients for clinical research; they are not intended to capture every single patient but rather to capture the majority of patients who share key features of the condition'.[12] Patients with axSpA with radiographic sacroiliitis are traditionally classified according to the mNY criteria and more recently according to the ASAS criteria. The data presented in this study show that patients with axSpA classified as AS according to mNY criteria and those classified as r-axSpA according to ASAS criteria are mostly the same. Nonetheless, there is minor disagreement, mainly due to age at onset of back pain. The latter is reported by patients at the time of diagnosis in almost all cohorts and therefore susceptible to recall bias, a valid concern especially for the cohorts containing patients with a long disease duration and long gap between symptom onset and diagnosis. The age criterion was introduced with the implementation of the ASAS criteria in 2009; this was mainly based on data from Feldtkeller *et al*,[13] which showed that 95% of AS patients reported an age of onset <45 years. Based on this fact, one would expect around 5% of the patients fulfilling the mNY criteria not to fulfil the ASAS criteria. In this study, this percentage is 7%.

Due to the nature of the data and the slight differences between the two criteria sets some assumptions had to be made, which is a limitation to this study. The first assumption concerns IBP; in general, the ASAS definition of IBP[10] was used. However, if this was unavailable (and could not be defined from individual components of IBP), the rheumatologist's assessment as provided in the dataset was used instead. The second assumption regards mobility limitations; according to the mNY criteria, mobility limitations are to be identified based on age-adjusted and gender-adjusted comparisons; however, in the original publication no reference values were provided. Therefore, reference values resulting from the MOBILITY study[11] were used. If information on mobility was unavailable, the rheumatologist's judgement of 'restricted mobility' as provided in the dataset was used. Both assumptions may have influenced the proportion of patients fulfilling either of the criteria sets.

As shown in the HLA-B27 analysis, the mNY+/ASAS- group showed a lower percentage of HLA-B27 positives. HLA-B27 positivity is associated with earlier disease onset,[13–15] which may explain the low percentage of HLA-B27+ in the mNY+/ASAS- group (48%) and is in line with the highest HLA-B27 positivity (72%) in the mNY-/ASAS+ group. An alternative explanation may be that patients in the mNY+/ASAS- group are misclassified

Table 2 Percentage of patients with axSpA with radiographic sacroiliitis fulfilling both sets of criteria, either criteria set or neither

	mNY+ASAS+	mNY+ASAS-	mNY- ASAS+	mNY- ASAS-	Total mNY+*	Total ASAS+†
ASAS (N=114)	86% (98)	2% (3)	10% (11)	2% (2)	89% (101)	96% (109)
GESPIC (N=96)	81% (78)	12% (11)	6% (6)	1% (1)	93% (89)	88% (84)
Esperanza (N=109)	97% (106)	3% (3)	NA‡	NA‡	100% (109)	–
OASIS (N=211)	95% (201)	5% (10)	NA‡	NA‡	100% (211)	–
Reuma.pt (N=1320)	88% (1156)	7% (93)	4% (55)	1% (16)	95% (1249)	92% (1211)
SCQM (N=1806)	89% (1612)	8% (148)	2% (40)	0.3% (6)	97% (1760)	91% (1652)
SPACE (N=92)	84% (77)	0% (0)	16% (15)	0% (0)	84% (77)	100% (92)
UCSF (N=293)	95% (279)	2.5% (7)	2.5% (7)	0% (0)	98% (286)	98% (286)
Total (N=4041)	89% (3607)	7% (275)	3% (134)	1% (25)	96% (3882)	–

*The total percentage of patients who fulfil the mNY criteria per cohort and in total.
†The total percentage of patients who fulfil the ASAS r-axSpA criteria per cohort and in total.
‡Specific information on the individual items of the mNY clinical criteria was unavailable, it was therefore not possible to accurately calculate the number of patients fulfilling the mNY in the subgroup fulfilling the ASAS r-axSpA criteria.
ASAS, Assessment of SpondyloArthritis international Society cohort; Esperanza, Spanish national health programme for early SpA; GESPIC, GErman SPondyloarthritis Inception Cohort; NA, not available; OASIS, Outcome in Ankylosing Spondylitis International Study; Reuma.pt, Portuguese Register for Rheumatic Diseases; SCQM, Swiss Clinical Quality Managementcohort; SPACE, SPondyloArthritis Caught Early cohort; UCSF, University of California San Francisco axSpA cohort; axSpA, axial spondyloarthritis; mNY, modified New York; r-axSpA, radiographic axial spondyloarthritis.

as having r-axSpA as a higher HLA-B27 percentage is expected in mNY+ patients. The overall percentage of HLA-B27 found in this study is relatively low, which may be due to the local readings of the radiographs that may have resulted in false classifications for both classification sets.[16]

In conclusion, this study found that agreement between the mNY and ASAS r-axSpA criteria is very high, which supports the interchangeable use of the terms AS and r-axSpA. This has important implications for the axSpA research field, since older literature used mNY and AS, whereas more recent literature often uses ASAS criteria and r-axSpA. Acknowledging that both criteria sets identify the same patients implies that older literature on AS and newer literature on r-axSpA can be directly compared.

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REFERENCES

1 Rudwaleit M, van der Heijde D, Landewé R, *et al*. The development of assessment of spondyloarthritis International Society classification criteria for axial spondyloarthritis (Part II): validation and final selection. *Ann Rheum Dis* 2009;68:777–83.
2 Malaviya AN, Rawat R, Agrawal N, *et al*. The Nonradiographic axial spondyloarthritis, the radiographic axial spondyloarthritis, and ankylosing spondylitis: the Tangled Skein of rheumatology. *Int J Rheumatol* 2017;2017:1–9.
3 Canhão H, Faustino A, Martins F, *et al*. Reuma.pt - the rheumatic diseases portuguese register. *Acta Reumatol Port* 2011;36:45–56.
4 Ciurea A, Scherer A, Exer P, *et al*. Tumor necrosis factor α inhibition in radiographic and nonradiographic axial spondyloarthritis: results from a large observational cohort. *Arthritis Rheum* 2013;65:3096–106.
5 Lee W, Reveille JD, Davis JC, *et al*. Are there gender differences in severity of ankylosing spondylitis? results from the psoas cohort. *Ann Rheum Dis* 2007;66:633–8.
6 Muñoz-Fernández S, Carmona L, Collantes E, *et al*. A model for the development and implementation of a national plan for the optimal management of early spondyloarthritis: the Esperanza program. *Ann Rheum Dis* 2011;70:827–30.
7 Rudwaleit M, Haibel H, Baraliakos X, *et al*. The early disease stage in axial spondylarthritis: results from the German spondyloarthritis inception cohort. *Arthritis Rheum* 2009;60:717–27.
8 Spoorenberg A, van der Heijde D, de Klerk E, *et al*. Relative value of erythrocyte sedimentation rate and C-reactive protein in assessment of disease activity in ankylosing spondylitis. *J Rheumatol* 1999;26:980–4.
9 van den Berg R, de Hooge M, van Gaalen F, *et al*. Percentage of patients with spondyloarthritis in patients referred because of chronic back pain and performance of classification criteria: experience from the spondyloarthritis caught early (space) cohort. *Rheumatology* 2013;52:1492–9.
10 Sieper J, van der Heijde D, Landewé R, *et al*. New criteria for inflammatory back pain in patients with chronic back pain: a real patient exercise by experts from the assessment of spondyloarthritis International Society (ASAS). *Ann Rheum Dis* 2009;68:784–8.
11 Ramiro S, van Tubergen A, Stolwijk C, *et al*. Reference intervals of spinal mobility measures in normal individuals: the mobility study. *Ann Rheum Dis* 2015;74:1218–24.

12 Aggarwal R, Ringold S, Khanna D, *et al*. Distinctions between diagnostic and classification criteria? *Arthritis Care Res* 2015;67:891–7.
13 Feldtkeller E, Khan MA, van der Heijde D, *et al*. Age at disease onset and diagnosis delay in HLA-B27 negative vs. positive patients with ankylosing spondylitis. *Rheumatol Int* 2003;23:61–6.
14 Bennett AN, McGonagle D, O'Connor P, *et al*. Severity of baseline magnetic resonance imaging-evident sacroiliitis and HLA-B27 status in early inflammatory back pain predict radiographically evident ankylosing spondylitis at eight years. *Arthritis Rheum* 2008;58:3413–8.
15 Endo Y, Fujikawa K, Koga T, *et al*. Characteristics of late-onset spondyloarthritis in Japan: a retrospective cohort study. *Medicine* 2019;98:e14431.
16 van den Berg R, Lenczner G, Feydy A, *et al*. Agreement between clinical practice and trained central reading in reading of sacroiliac joints on plain pelvic radiographs. results from the DESIR cohort. *Arthritis Rheumatol* 2014;66:2403–11.
17 van der Linden S, Valkenburg HA, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the new York criteria. *Arthritis Rheum* 1984;27:361–8.

CLINICAL SCIENCE

MRI lesions in the sacroiliac joints of patients with spondyloarthritis: an update of definitions and validation by the ASAS MRI working group

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ABSTRACT

Objectives The Assessment of SpondyloArthritis international Society (ASAS) MRI working group (WG) was convened to generate a consensus update on standardised definitions for MRI lesions in the sacroiliac joint (SIJ) of patients with spondyloarthritis (SpA), and to conduct preliminary validation.

Methods The literature pertaining to these MRI lesion definitions was discussed at three meetings of the group. 25 investigators (20 rheumatologists, 5 radiologists) determined which definitions should be retained or required revision, and which required a new definition. Lesion definitions were assessed in a multi-reader validation exercise using 278 MRI scans from the ASAS classification cohort by global assessment (lesion present/absent) and detailed scoring (inflammation and structural). Reliability of detection of lesions was analysed using kappa statistics and the intraclass correlation coefficient (ICC).

Results No revisions were made to the current ASAS definition of a positive SIJ MRI or definitions for subchondral inflammation and sclerosis. The following definitions were revised: capsulitis, enthesitis, fat lesion and erosion. New definitions were developed for joint space enhancement, joint space fluid, fat metaplasia in an erosion cavity, ankylosis and bone bud. The most frequently detected structural lesion, erosion, was detected almost as reliably as subchondral inflammation (κappa/ICC:0.61/0.54 and 0.60/0.83) . Fat metaplasia in an erosion cavity and ankylosis were also reliably detected despite their low frequency (κappa/ICC:0.50/0.37 and 0.58/0.97).

Conclusion The ASAS-MRI WG concluded that several definitions required revision and some new definitions were necessary. Multi-reader validation demonstrated substantial reliability for the most frequently detected lesions and comparable reliability between active and structural lesions.

Key messages

What is already known?

- Growing evidence demonstrates an increased spectrum of MRI lesions in the sacroiliac joint related to spondyloarthritis (SpA) and more clearly defined associations between inflammatory and structural lesions over time but standardisation of sacroiliac joint (SIJ) lesion definitions has not been updated for a decade.

What does this study add?

- The Assessments in SpondyloArthritis international Society (ASAS) MRI Working Group reports a consensus-based update of standardised MRI SIJ lesion definitions relevant to SpA, which includes several new definitions for both inflammatory and structural lesions.
- These definitions were validated in a multi-reader exercise using 278 scans from the ASAS classification cohort which demonstrated acceptable reliability for most inflammatory and structural lesions, even for those occurring at a low frequency.

How might this impact on clinical practice or future developments?

- The updated definitions are aimed at enhancing educational and research initiatives towards improving early diagnosis, classification and prognostic assessment.

INTRODUCTION

MRI is now an established tool in the assessment of the sacroiliac joint (SIJ) in patients with spondyloarthritis (SpA), especially in early disease.[1] Both active and structural lesions may be observed prior to the appearance of radiographic findings,[2] and SIJ MRI is being used for studies of disease pathogenesis and as a quantitative measure for assessment of therapeutics. It is therefore essential that there is widespread understanding of each

Box 1 Assessments in SpondyloArthritis international Society MRI Working Group consensus definitions for MRI lesions in the sacroiliac joint of patients with spondyloarthritis

A. Overarching principles

1. When interpreting medical studies of the SIJ in SpA for diagnostic or classification purposes, all available images for that modality should be reviewed at the same time as different slice orientations or sequences may provide additional information that is important for the correct interpretation of the findings. MR images that illustrate different features of sacroiliitis compatible with (or highly suggestive of) SpA, such as active disease and structural damage, should be simultaneously reviewed and interpreted in the context of all findings.
2. Many artefacts occur on MRI of the SIJ, and when a feature of uncertain significance is seen in one orientation then, if possible, the feature should be verified on a second orientation. This may be more important for imaging studies used for diagnostic or classification purposes.
3. The SIJ lesion(s) must be clearly present, located in a typical anatomical location and its appearance must be highly suggestive of SpA. The presence of any small solitary lesion should be interpreted with caution. It is rare for a lesion to be 'clearly present' if small and solitary, and it is expected that relevant lesions will be either multiple or seen on multiple images (slices, sequences, orientation). If a lesion appears to be present but it is hard to determine whether the lesion is 'highly suggestive of SpA', then the decision may be influenced by the presence of other concomitant lesions.[4]
4. The visual interpretation of an MRI scan of the SIJ should be performed objectively. In the research setting, the interpretation will usually be done in the absence of patient data. But the clinician should interpret the MRI report in the total context of the demographic, clinical and laboratory information from the patient, and although the MRI of the SIJ may be reported as suggestive of SpA, the final decision can still be that the patient has no SpA. Other conditions of the SIJ such as fracture, osteoarthritis, sepsis, trauma, neoplasia and artefacts may resemble lesions observed on MRI in patients with SpA.

B. MRI SIJ lesion definitions indicating signs of activity

These observations are made on MRI sequences that are sensitive for the detection of disease activity, such as T2W sequences with FS that are sensitive for free water (eg, STIR), or T1W sequences with fat suppression that are sensitive for contrast enhancement such as T1WFS post-Gd.

1. ASAS definition of positive MRI for the classification of SpA (figures 1A–C and 2)[3 4]: MRI evidence of bone marrow inflammation must be present, and the features required for the definition of active sacroiliitis on MRI are as follows:
   a. BME on a T2W sequence sensitive for free water (eg, STIR and T2FS) or bone marrow contrast enhancement on a T1W sequence (eg, T1FS post-Gd). BME is depicted as a hyperintense signal on STIR images and usually as a hypointense signal on T1 images. A hyperintense signal on contrast-enhanced, T1-weighted, fat-saturated images (T1 post-Gd) reflects increased vascularisation and is referred to as osteitis. The sacral interforaminal bone marrow signal forms the reference for assignment of normal signal in the bone marrow.[7]
   b. Inflammation must be clearly present and located in a typical anatomical area (subchondral bone).
   c. MRI appearance must be highly suggestive of SpA.
2. Capsulitis (figure 1A): Increased signal on STIR and/or T1FS post-Gd, which is observed at the perimeter of the joint (anterior or posterior on axial images, cranial or caudal on semicoronal images).
3. Joint space enhancement (online supplementary figure): Increased signal on contrast-enhanced images in the joint space of the cartilaginous portion of the SIJ.
4. Inflammation at the site of erosion (figure 1B): Increased signal on STIR and/or T1FS post-Gd at the site of erosion.
5. Enthesitis (figure 1C): Increased signal in bone marrow and/or soft tissue on STIR and/or T1FS post-Gd at sites where ligaments and tendons attach to bone, but not including the inter-osseous ligaments of the sacroiliac joint.
6. Joint space fluid (figure 1D): Bright signal in the joint space on STIR images equivalent to cerebrospinal fluid.

C. MRI SIJ lesion definitions indicating signs of structural change

These observations are made on MRI sequences that are sensitive for the detection of structural change. Most of the observations can only be seen clearly on sequences sensitive for fat signal, specifically T1W spin echo without fat suppression.

1. Erosion (figures 1A,B and 2): A defect in subchondral bone associated with full-thickness loss of the dark appearance of the subchondral cortex at its expected location, with loss of signal on a T1W non-fat-suppressed sequence compared with the normal bright appearance of adjacent bone marrow.
2. Fat lesion (also known as Fat metaplasia) (figure 3A): Bright signal seen on a T1W non-fat-suppressed sequence that is brighter than normal bone marrow, which meets the following requirements:
   a. Homogeneously bright.
   b. Located in a typical anatomical area (subchondral bone).
   c. Sharply defined along its non-articular border with normal bone marrow.
3. Fat metaplasia in an erosion cavity (also known as 'backfill') (figure 3B): Bright signal on a T1-weighted sequence in a typical location for an erosion or confluent erosions, with signal intensity greater than normal bone marrow, which meets the following requirements:
   a. Associated with complete loss of the dark appearance of the subchondral cortex at its expected location.

Continued

Box 1 Continued

b. Clearly demarcated from adjacent bone marrow by an irregular band of dark signal reflecting sclerosis at the border of the original erosion.

4. Sclerosis (figure 2): Very low signal on all sequences located in a typical anatomical area (subchondral bone).
5. Ankylosis (figure 3C): Abnormal bright signal on a T1W non-fat-suppressed sequence with similar signal intensity to bone marrow, which is in the expected location of the sacroiliac joint space and bridges the joint so that there is continuity of bone marrow signal between the ilium and sacrum. It is associated with full-thickness loss of the dark appearance of the subchondral cortex on both sides of the joint.
6. Bone bud (figure 3C): Abnormal bright signal on a T1W non-fat-suppressed sequence with similar signal intensity to bone marrow, which is in the expected location of the sacroiliac joint space but does not bridge the joint so that it is continuous with the subchondral bone of either the ilium or the sacrum but not both. It is associated with full-thickness loss of the dark appearance of the subchondral cortex on the corresponding side of the joint, at its expected location.

ASAS, Assessment of SpondyloArthritis international Society; BME, bone marrow oedema; FS, fat suppression; Gd, gadolinium; SIJ, sacroiliac joint; SpA, spondyloarthritis; STIR, short tau inversion recovery; T2W, T2-weighted.

lesion based on a standardised descriptive terminology generated by international consensus. Moreover, comprehension of these definitions should be validated in reading exercises.

It has been a decade since the first international consensus culminated in published definitions for MRI lesions in the SIJ.[3] This described active and structural lesion definitions but did not include any reading exercises. In addition, this group also formulated a provisional definition of an MRI that could be considered positive for SpA for the purposes of disease classification, which was based on the clear presence of bone marrow oedema (BME)/osteitis in subchondral bone marrow. A recent consensus from the Assessment of SpondyloArthritis international Society (ASAS) further elaborated the descriptive terminology of this definition emphasising the importance of contextual interpretation of both active and structural lesions to enhance confidence in interpretation.[4] However, this exercise also did not include any readings of MRI scans.

Over the past decade, our understanding of MRI lesions in the SIJ has increased substantially, and there have been further descriptions of structural lesions, which have been assessed longitudinally to understand their origin and association with active lesions. This progress led to the decision by ASAS to convene the ASAS-MRI working group (WG) to discuss progress in the field, review existing definitions, determine the necessity for updated or new lesion definitions and conduct a multi-reader exercise by expert readers from the ASAS-MRI WG to examine the performance of all lesion definitions in the SIJ.

METHODS

Consensus exercises

Rheumatologists (n=20) and radiologists (n=5) of the ASAS-MRI WG were invited to participate in the consensus exercises and an initiative to validate lesion definitions using the available MRI scans from patients recruited to the ASAS classification cohort.[5] Published definitions for active and structural MRI lesions in the SIJ were reviewed at two face-to-face meetings in 2016, in Gent, Belgium and Leiden, the Netherlands. A summary of these deliberations was presented at the *Annual Meeting of ASAS* on 20 January 2017. Feedback from ASAS members was taken into consideration during a webex on 21 March 2017 where the ASAS-MRI WG finalised consensus wording of these definitions and agreed on a set of reference images that depict each lesion.[6] The group also agreed on a study design, the ASAS MRImagine study, for multi-reader assessment of lesion definitions and a study-specific interactive electronic case report form (eCRF).

ASAS eCRF for evaluation of MRI lesions in the SIJ

The online-available[6] CRF comprised two sections: (1) Data were first entered onto a global scoring page where readers recorded the presence/absence of each lesion in iliac and sacral portions of each SIJ and whether the scan met the ASAS definition for a positive MRI.[3 4] (2) After global assessment, data were entered onto a granular scoring web-based interface where inflammatory and structural lesions were recorded in each SIJ quadrant on consecutive semicoronal slices.[6] All slices with a minimum 1 cm vertical height of visible SIJ were scored, and SIJ quadrants were defined according to established rules.[7] Structural lesions were recorded in either SIJ quadrants (erosion, fat, sclerosis) or upper and lower SIJ halves (fat metaplasia in an erosion cavity, ankylosis), as previously defined.[8]

ASAS classification cohort MRI resource

MRI scans of the SIJ were available from 278 cases recruited to the ASAS classification cohort. Of these, 175 cases were in standard digital imaging and communications in medicine (DICOM) format, 71 were in JPEG format and in 32 cases images were derived from hard copy film. Global assessment for active lesions on a fat-suppressed scan was possible for all available cases (263 semi-coronal and 15 axial scans). Global assessment for structural lesions on a T1W scan was possible for 238 cases (189 semi-coronal and 49 axial scans). Granular assessment for active and structural lesions was conducted only in cases where a DICOM series was available in semi-coronal orientation (160 for fat-suppressed scans and 148 for T1W scans, respectively).

Reading exercises

Seven readers from the ASAS-MRI WG with over 10 years experience evaluating SIJ lesions for SpA assessed the MRI scans. Validated calibration modules aimed at standardisation of slice selection and defining SIJ quadrants were provided online for review prior to the readings.[9 10]

Statistics

Frequencies of each SIJ lesion were assessed descriptively according to individual as well as majority reader (≥4/7 readers) data. Reliability for presence/absence of each lesion was assessed using the kappa statistic. Reliability for the number of SIJ

quadrants or halves with SIJ lesions was assessed by intraclass correlation coefficient (ICC 2.1 (two-way random effects, absolute agreement, single rater/measurement MedCalc V.12.6)).

RESULTS

Overarching considerations

The ASAS-MRI WG consensus emphasised several overarching recommendations pertaining to optimal interpretation of MRI lesions in the SIJ (box 1). Definitions were categorised according to lesions indicating signs of activity and structural lesions.

Lesion definitions indicating signs of activity

Bone marrow oedema

The ASAS definition for subchondral BME in the SIJ indicative of SpA was not revised from the definition reported previously.[3 4]

Capsulitis

The wording of this definition was revised from the original description[3] to clarify its location (new reference image figure 1A).

Joint space enhancement

This is a new definition to replace the original lesion definition named 'synovitis'[3] and applies only to contrast-enhanced sequences (supplementary Figure). A new definition was considered necessary because of the observation that synovium is only present at the perimeter of the lower third of the cartilaginous portion of the joint.[11] Concomitant enhancement of tissue at the perimeter of the joint is captured in the definition of capsulitis.

Inflammation at the site of erosion

This is a new definition describing inflammation within an erosion (new reference image figure 1B).

Enthesitis

This definition has been revised from the original definition[3] to exclude the inter-osseous soft tissues in the ligamentary portion of the SIJ because this could be difficult to distinguish from vascular signal (new reference image figure 1C).

Joint space fluid

This is a new definition to describe bright signal in the joint space on a T2-weighted fat suppressed sequence (new reference image figure 1D).

Lesion definitions indicating signs of structural change

Erosion

This definition was revised to include wording that not only describes a breach in cortical bone but also loss of adjacent marrow matrix (new reference images figures 1A, B and 2). It was noted that erosions have variable signal intensity on water-sensitive sequences, and the defect may be small (discrete erosion) or large (multiple confluent erosions along the iliac and/or sacral bone) causing pseudo-widening of the joint.

Fat lesion (also known as fat metaplasia)

This definition was revised to include morphological characteristics that indicate fat metaplasia after resolution of an inflammatory lesion, such as distinct border and location adjacent to subchondral bone, rather than the fat infiltration that may be seen in healthy individuals (new reference image figure 3A).

Fat metaplasia in an erosion cavity (also known as 'backfill')

This is a new definition and was considered necessary to define a distinctive structural lesion that may develop following resolution of inflammation in an erosion cavity and comprises two components: (1) a bright signal within the erosion cavity, signifying a reparative process previously termed backfill resembling the transformation of subchondral BME into fat metaplasia that occurs in bone marrow; (2) an irregular band of dark signal reflecting sclerosis at the border of the original erosion. This composite lesion may be observed along the vertical height of the joint cavity on a semi-coronal scan after erosions have become confluent (new reference image figure 3B).

Sclerosis

This definition has not been revised from the previous ASAS definition (new reference image figure 2).[3]

Ankylosis

A new definition was considered necessary to clearly indicate that ankylosis is considered present when there is continuity of bright bone marrow signal across the joint space (new reference image figure 3C).

Non-bridging bone bud

This is a new definition to describe new bone formation in the SIJ that has not bridged the joint cavity (new reference image figure 3C).

Frequency of SIJ MRI lesions in patients from the ASAS classification cohort

Subchondral BME was the most frequent lesion detected according to majority reader agreement (≥4 of 7) and was observed in 40.3% of the 278 cases (table 1). However, only 31.3% of cases were deemed to have subchondral BME that would meet the ASAS definition of a positive MRI. Other inflammatory lesions were observed in <10% of cases. Descriptive data based on individual reader assessments were comparable to the majority reader data (online supplementary table 1). Subchondral BME was also the most frequently detected MRI lesion on granular assessment (online supplementary table 2).

Erosion was the most frequent structural lesion in the SIJ being observed in 28.3% of cases (table 1). Fat lesion and sclerosis were observed in 19.8% and 16.9% of cases, respectively, while fat metaplasia in an erosion cavity was observed in 7.6%. Ankylosis and bone bud were observed in <5% of cases. Fat lesion was the most frequently scored structural lesion according to granular assessment, followed by erosion and sclerosis (online supplementary table 2).

Reliability of detection of SIJ MRI lesions in patients from the ASAS classification cohort

Reliability for detection of active and structural MRI lesions in the SIJ was broadly comparable when all available MRI scans were assessed (table 2). In particular, reliability (mean kappa (95% CI)) for detection of erosion (0.55 (0.44–0.66)) and fat lesion (0.59 (0.47–0.71)) was almost at the same level as for subchondral BME (0.65 (0.56–0.74)) but less than the reliability for detection of an ASAS-positive MRI (0.75 (0.66–0.83)). Reliability for detection of all structural lesions, with the exception of bone bud, was improved when images from the 175 cases with DICOM scans were assessed, which were judged to be better quality images. Reliability for detection of inflammatory lesions was also greater when these DICOM scans were assessed

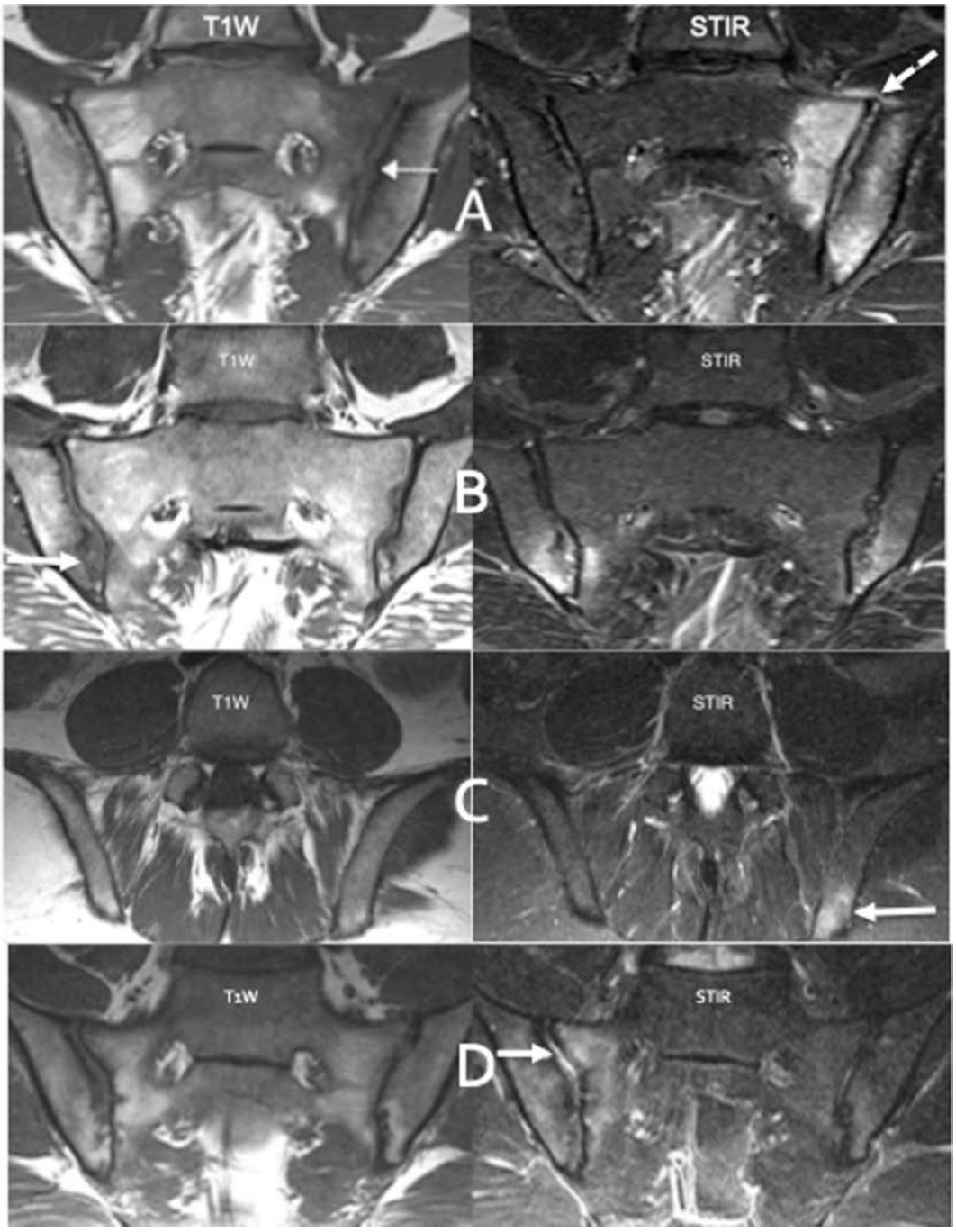

Figure 1 ASAS consensus reference images for active MRI lesions in the sacroiliac joints of patients with spondyloarthritis. All images have been acquired in the semicoronal orientation. (A) MRI scans of a 42-year-old man with a 1-year history of inflammatory back pain, a single episode of acute anterior uveitis, HLA-B27 positivity and a CRP level of 67.5 mg/L. Extensive bone marrow oedema in the left iliac and sacral subchondral bone marrow is depicted as bright signal on the STIR MRI scan meeting the ASAS definition of a positive MRI. There is loss of the bone marrow fat signal in the corresponding location on the T1W scan and erosion of the entire vertical height of the left iliac cortical bone with loss of adjacent marrow matrix leading to an appearance of widening of the joint space (arrow). This meets the ASAS definition for erosion. The dashed arrow points to bright signal in the anterosuperior joint capsule on the STIR scan meeting the ASAS definition of capsulitis. (B) MRI scans of a 35-year-old man with a 5-year history of inflammatory back pain, lack of response to NSAID therapy, HLA-B27 positivity and a CRP level of 6.8 mg/L. The arrow on the T1W scan points to erosion of the right iliac bone. There is bright signal in the cavity of the erosion on the STIR scan, indicating that there is inflammation within the erosion cavity meeting the ASAS definition. There is also bright signal in the right iliac, left iliac and right sacral subchondral bone marrow, indicating bone marrow oedema meeting the ASAS definition for a positive MRI. (C) MRI scan of the same patient as in (B). The arrow on the STIR scan points to bright signal in the bone marrow of the left iliac bone several slices posterior to the sacroiliac joint. This meets the ASAS definition of enthesitis. (D) MRI scan of a 33-year-old man with a 2-year history of inflammatory back pain, HLA-B27 positive and a CRP level of 18.6 mg/L. The arrow on the STIR scan points to bright signal, with intensity comparable to vascular signal, in the right sacroiliac joint space. This meets the ASAS definition of joint fluid. There is also subchondral bone marrow oedema in the right sacral and iliac bones meeting the ASAS definition of a positive MRI. ASAS, Assessment of SpondyloArthritis international Society; CRP, C-reactive protein; NSAID, nonsteroidal anti-inflammatory drug; STIR, short tau inversion recovery; T1W, T1-weighted.

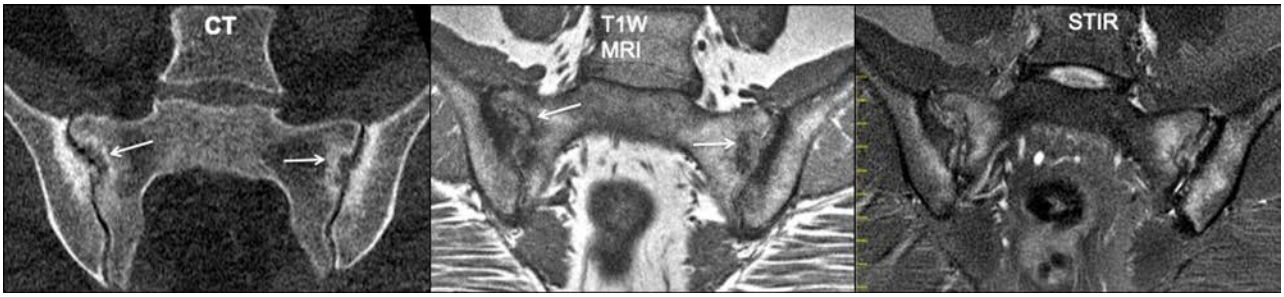

Figure 2 ASAS consensus reference images for active and structural lesions in the sacroiliac joints of a patient with spondyloarthritis. MRI scans and CT scan reconstructed in the semicoronal orientation from a 39-year-old man with a 4-year history of inflammatory back pain unresponsive to NSAID therapy, B27 positivity and a CRP level of 18.8 mg/L. The arrows on the T1W scan point to large sacral erosions with loss of cortical bone and adjacent marrow matrix meeting the ASAS definition for erosion. This is clearly evident on the CT scan slice corresponding to the MRI slice. On the STIR scan, bone marrow oedema is visible in all four bones meeting the ASAS definition of a positive MRI. There is dark signal in both iliac bones on both MRI sequences meeting the ASAS definition for bone sclerosis. This is also apparent in the CT scan. ASAS, Assessment of SpondyloArthritis international Society; CRP, C-reactive protein; NSAID, nonsteroidal anti-inflammatory drug; STIR, short tau inversion recovery; T1W, T1-weighted.

except for detection of subchondral BME and ASAS-positive MRI. Acceptable reliability (κ≥0.50) was attained for ASAS-positive MRI, subchondral BME, capsulitis, erosion, fat lesion, fat metaplasia in an erosion cavity and ankylosis. Reliability (mean ICC (95% CI)) for granular assessment of lesions according to affected number of SIJ quadrants or halves per case was excellent for ankylosis (0.97 (0.96–0.98)), very good for subchondral BME (0.83 (0.78–0.88)) and acceptable (≥0.50) for erosion, fat lesion and sclerosis (online supplementary table 3).

DISCUSSION

The primary objective of this ASAS initiative was to provide an updated international consensus for MRI lesion definitions in the SIJ of patients with SpA and to conduct a first validation exercise assessing the reliability of detection of these lesions in patients from the ASAS classification cohort. Structural lesions occurred almost as frequently as inflammatory lesions and were detected to a comparable degree of reliability as subchondral BME. Certain newly defined lesions were detected less reliably although all occurred at low frequency (<10%) in this cohort.

Reliable detection of subchondral BME meeting the ASAS definition of a positive MRI was comparable to a previous evaluation of MRI scans from cases with early SpA.[12] Subchondral BME was recorded in about 10% more cases than an ASAS-positive MRI indicating that readers distinguished between BME indicative of SpA and BME unrelated to SpA. The definition for capsulitis was revised to include further details regarding its location, and a new reference image was provided where this lesion is more clearly evident than in the prior publication.[3] Although reliability for its detection was poor when all available scans were evaluated, this was substantially greater when only DICOM images were assessed.

The definition for joint space enhancement generated considerable debate prior to attainment of consensus. Some considered that the only lesion reflecting increased signal in the joint space on contrast-enhanced sequences is synovitis and argued for no revision to the original definition, which had been named 'synovitis', or a minor revision to 'synovial enhancement'. Others considered that the increased signal may reflect inflammation in other tissues, for example, cartilage, osteochondral interface, or may even occur after trauma. Detailed post-mortem analysis of the healthy sacroiliac joint has shown that synovium is present only in the lower third of the joint and only at the periphery of the joint.[11] Since there is no synovium in the joint space in the interior of the joint, increased signal in the joint space on contrast-enhanced images without any concomitant peripheral enhancement cannot be considered as reflecting synovitis. Moreover, synovitis on contrast-enhanced sequences will usually only be visible as enhancement at the periphery of the joint in its lower one-third and not as enhancement of effusion because this often requires delayed imaging to fully capture gradual leakage of contrast material into joint fluid. These MRI appearances most likely reflect inflammation at the osteochondral interface as this would be consistent with our understanding of early sacroiliitis and histopathological data indicating that the primary lesion in early disease is subchondral inflammation.[13 14] High signal in the joint space on the STIR sequence is not synonymous with joint space enhancement on contrast MRI, and a recent report described high STIR signal in the joint space of up to a third of healthy sports enthusiasts.[15]

Inflammation at the site of erosion is a newly defined lesion and a well-known feature of SpA on MRI. Its inclusion as a distinct entity reflects recent work demonstrating that the appearance of erosive lesions on both STIR and T1W sequences changes as the inflammation resolves (vide infra). Its contribution to sensitivity and specificity for SpA is unknown, and it is possible that even a small erosion with a focus of inflammation in the erosion cavity may be specific for SpA. It is unclear why this lesion was not reliably detected, and it may reflect the complexity of detecting both an erosion on the T1W scan and unequivocally bright signal within the erosion cavity on the STIR scan, which could resemble blood vessel if small.

It is now well established that resolution of subchondral BME in the SIJ may be associated with the appearance of bright tissue on a T1W scan indicating the expression of fatty acids although the histopathology of this tissue is unknown.[16–18] While the appearance of fat in the bone marrow may also be physiological,[19 20] previous reports have shown that the appearance of post-inflammatory fat metaplasia has characteristic features defined by a distinct border, homogeneous increase in T1W signal and proximity to subchondral bone.[21] This lesion has previously been shown to be reliably detected and to be highly specific for SpA,[21 22] although questionable to what degree it enhances diagnosis because of the concomitant presence of lesions such as BME and erosion. The presence of this lesion in the SIJ has been associated with a worse prognostic phenotype characterised by an increased propensity to new bone formation in both the SIJ and spine.[23–25]

It has been shown that resolution of inflammatory lesions in erosions is also associated with the appearance of bright tissue on a T1W scan at the site of erosion.[8 17 18] Since erosions in the

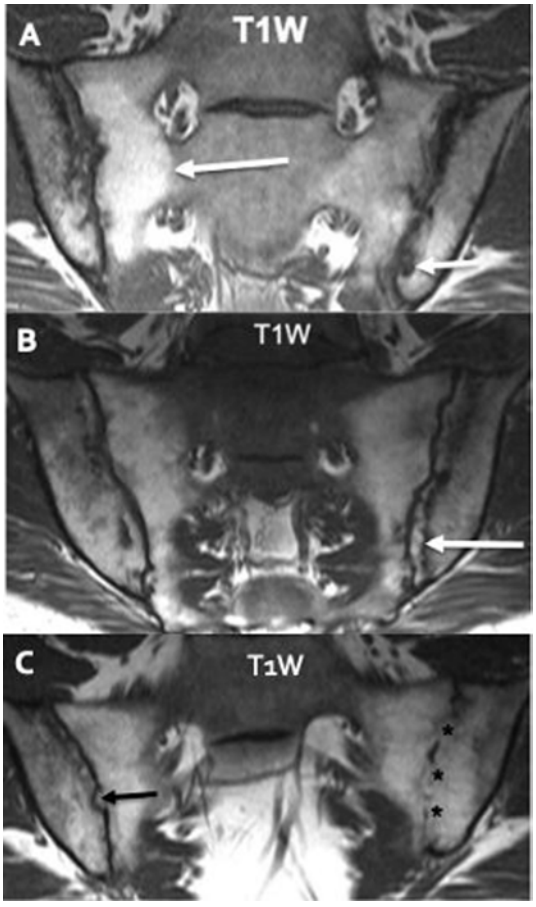

Figure 3 ASAS consensus reference images for structural MRI lesions in the sacroiliac joints of patients with spondyloarthritis. All images have been acquired in the semicoronal orientation. (A) MRI scan of a 32-year-old man with a 3-year history of inflammatory back pain, Crohn's colitis controlled with TNF inhibitor therapy, HLA-B27 negativity and a CRP level of 3.5 mg/L. The arrow points to a region of homogeneously increased signal in the right sacral bone marrow on the T1W scan that has a distinct border and is adjacent to subchondral bone and erosion of the right sacral cortex. This appearance meets the ASAS definition for fat metaplasia related to SpA. There are smaller areas of fat metaplasia in both lower iliac bones adjacent to areas of erosion, especially the left iliac cortex (arrow). (B) ASAS consensus reference image for fat metaplasia in an erosion cavity of the sacroiliac joint of a patient with spondyloarthritis. Semicoronal T1W MRI scan of a 48-year-old man with a 14-year history of inflammatory back pain, symptoms controlled by NSAID therapy, HLA-B27 positivity and a CRP level of 5.7 mg/L. The arrow on the T1W scan points to bright signal on the joint surface of the left iliac bone bordered laterally by a vertical irregular dark band. This meets the ASAS definition for fat metaplasia in an erosion cavity (backfill). (C) ASAS consensus reference image for ankylosis and bone bud in a patient with spondyloarthritis. MRI scans of a 54-year-old woman with a 23-year history of inflammatory back pain responsive to NSAID therapy, HLA-B27 positivity and a CRP level of 4.2 mg/L. The arrow on the T1W scan points to abnormal bright signal which protrudes into the sacroiliac joint space but does not bridge the joint. This meets the ASAS definition for bone bud. The asterisks on the T1W scan point to several regions of the left sacroiliac joint where there is continuity of bright marrow signal from ilium to sacrum across the joint space meeting the ASAS definition for ankylosis. ASAS, Assessment of SpondyloArthritis international Society; CRP, C-reactive protein; NSAID, nonsteroidal anti-inflammatory drug; STIR, short tau inversion recovery; T1W, T1-weighted.

Table 1 Descriptive data for global evaluation of active and structural MRI lesions in the SIJ generated by the majority (four or more) of seven readers from the ASAS MRI group who assessed 278 scans from the ASAS Classification cohort*

Variable	Number (%) of cases
Subchondral BME and meets ASAS definition for positive MRI	87 (31.3%)
Subchondral BME	112 (40.3%)
Inflammation at site of erosion	20 (7.2%)
Capsulitis	8 (2.9%)
Joint fluid	18 (6.5%)
Enthesitis	14 (5.0%)
Subchondral sclerosis	40 (16.9%)
Erosion	67 (28.3%)
Fat lesion	47 (19.8%)
Bone bud	1 (0.4%)
Fat metaplasia in an erosion cavity	18 (7.6%)
Ankylosis	6 (2.5%)

*Data for structural lesions available from 238 scans.
ASAS, Assessment of SpondyloArthritis international Society; BME, bone marrow oedema; SIJ, sacroiliac joint.

SIJ often extend along the vertical height of the iliac and/or sacral joint surface, this leads to the appearance of bright signal in the joint space. A band of tissue that is dark on all MRI sequences may be seen at the border of an erosion and is thought to reflect reactive new bone formation. The variable appearance of erosions on T1W scans was first recognised almost a decade ago following assessment of scans from a cross-sectional cohort,[26] and a clearer understanding of the evolution of these MRI appearances was then determined in a prospective cohort analysis of patients with SpA.[18] The characteristic appearance of bright signal in the erosion cavity on T1W MRI with a dark irregular band lateral to it was termed backfill to denote the 'filling-in' of the erosion cavity with reparative tissue resembling fat metaplasia.[26] In the ASAS-MRI WG, it was decided to name this 'fat metaplasia in an erosion cavity', rather than backfill, to emphasise the descriptive character of the definition, as its histopathological correlate is not fully known. This lesion has also been associated with the development of ankylosis in the SIJ and may therefore have prognostic significance.[18] Reliable detection is challenging because this requires agreement that there is both unequivocally bright signal in the joint space and an adjacent irregular dark band on the T1W scan. However, acceptable reliability was attainable when DICOM images were available despite a low frequency of only 8% in this cohort. Reliability can likely be enhanced with more intensive calibration using web-based DICOM images that provide examples of lesions at the threshold of detection.[9 10 27]

Reliability for detection of erosion has varied widely, and this may reflect both the complexity of the lesion and the application of different lesion definitions.[8 26–29] Reliability is enhanced when readers are calibrated with a validated online DICOM-based calibration module similar to the one available for readers of the ASAS MRI-WG.[9 10 27] The degree to which readers used this calibration module prior to the scoring exercise was not documented, although reliability was greater when DICOM images were available.

The new definition for ankylosis stresses the importance of continuity of bright marrow signal across the joint space on a T1W scan as a defining feature. Reliability was comparatively high when taken in the context of the low frequency of this lesion in this cohort and comparable to a previous report.[8] Reliability

Table 2 Reliability (κappa values) for detection of active and structural MRI lesions in the SIJ by seven readers from the ASAS MRI group who assessed MRI scans from the ASAS Classification cohort

	Mean κ of all reader pairs (95% CI)	Median κ of all reader pairs	Range of κ for all reader pairs
All available MRI scans (n=278) (Data for structural lesions available from 238 scans)			
ASAS-positive MRI	0.75 (0.66 to 0.83)	0.75	0.64–0.86
Subchondral bone marrow oedema	0.65 (0.56 to 0.74)	0.65	0.53–0.75
Inflammation at the site of erosion	0.30 (0.13 to 0.47)	0.30	0.15–0.53
Capsulitis	0.40 (0.14 to 0.66)	0.42	0.19–0.72
Joint fluid	0.36 (0.21 to 0.50)	0.36	0.18–0.53
Enthesitis	0.21 (0.05 to 0.37)	0.17	0.03–0.56
Sclerosis	0.43 (0.30 to 0.55)	0.42	0.20–0.62
Erosion	0.55 (0.44 to 0.66)	0.53	0.40–0.69
Fat lesion	0.59 (0.47 to 0.71)	0.58	0.41–0.72
Fat metaplasia in an erosion cavity	0.46 (0.27 to 0.66)	0.47	0.22–0.65
Bone bud	0.13 (–0.05 to 0.30)	0.12	–0.04–0.56
Ankylosis	0.53 (0.21 to 0.83)	0.49	0.28–0.91
MRI scans with consecutive DICOM images (Data for active and structural lesions available from 160 and 148 scans, respectively)			
ASAS-positive MRI	0.73 (0.61 to 0.84)	0.73	0.60–0.86
Subchondral bone marrow oedema	0.60 (0.49 to 0.72)	0.60	0.47–0.69
Inflammation at site of erosion	0.37 (0.15 to 0.58)	0.37	0.18–0.73
Capsulitis	0.55 (0.18 to 0.90)	0.56	0.29–0.80
Joint fluid	0.41 (0.23 to 0.59)	0.41	0.27–0.57
Enthesitis	0.23 (0.03 to 0.45)	0.20	–0.04–0.66
Sclerosis	0.48 (0.33 to 0.63)	0.52	0.25–0.68
Erosion	0.61 (0.47 to 0.75)	0.64	0.41–0.74
Fat lesion	0.61 (0.46 to 0.76)	0.61	0.32–0.78
Fat metaplasia in an erosion cavity	0.50 (0.26 to 0.74)	0.47	0.25–0.71
Bone bud	0.11 (–0.06 to 0.29)	0.06	–0.06–0.60
Ankylosis	0.58 (0.25 to 0.89)	0.59	0.32–0.91

ASAS, Assessment of SpondyloArthritis international society; DICOM, digital imaging and communications in medicine ; SIJ, sacroiliac joint.

for detection of bone bud and enthesitis was low, though this may reflect their very low frequency (≤5%) in this cohort.

In conclusion, the ASAS MRI-WG provides a consensus-based update of MRI lesion definitions in the SIJ of patients with SpA. Testing of these definitions in scans from the ASAS classification cohort for agreement among seven expert readers demonstrated acceptable reliability for most inflammatory and structural lesions, even among some lesions that occurred at a frequency of <10%. Validation of these lesions for diagnostic, classification and prognostic utility is warranted.

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REFERENCES

1 Oostveen J, Prevo R, den Boer J, *et al*. Early detection of sacroiliitis on magnetic resonance imaging and subsequent development of sacroiliitis on plain radiography. A prospective, longitudinal study. *J Rheumatol* 1999;26:1953–8.
2 Maksymowych WP, Wichuk S, Dougados M, *et al*. Mri evidence of structural changes in the sacroiliac joints of patients with non-radiographic axial spondyloarthritis even in the absence of MRI inflammation. *Arthritis Res Ther* 2017;19.
3 Rudwaleit M, Jurik AG, Hermann K-GA, *et al*. Defining active sacroiliitis on magnetic resonance imaging (MRI) for classification of axial spondyloarthritis: a consensual approach by the ASAS/OMERACT MRI group. *Ann Rheum Dis* 2009;68:1520–7.

4 Lambert RGW, Bakker PAC, van der Heijde D, *et al*. Defining active sacroiliitis on MRI for classification of axial spondyloarthritis: update by the ASAS MRI Working group. *Ann Rheum Dis* 2016;75:1958–63.
5 Rudwaleit M, van der Heijde D, Landewé R, *et al*. The development of assessment of spondyloarthritis International Society classification criteria for axial spondyloarthritis (Part II): validation and final selection. *Ann Rheum Dis* 2009;68:777–83.
6 ASAS MRImagine Module. Available: https://www.carearthritis.com/mriportal/mrimagine/index/
7 Maksymowych WP, Inman RD, Salonen D, *et al*. Spondyloarthritis research Consortium of Canada magnetic resonance imaging index for assessment of sacroiliac joint inflammation in ankylosing spondylitis. *Arthritis Rheum* 2005;53:703–9.
8 Maksymowych WP, Wichuk S, Chiowchanwisawakit P, *et al*. Development and preliminary validation of the spondyloarthritis research Consortium of Canada magnetic resonance imaging Sacroiliac joint structural score. *J Rheumatol* 2015;42:79–86.
9 Maksymowych M, Boutrup H, Cheah J, *et al*. Validation of online calibration modules for the spondyloarthritis research Consortium of Canada MRI scores based on real-time experiential learning. *Arthritis Rheumatol* 2017;69(suppl 10).
10 Canadian Research and Education. Mri scoring modules. Available: www.carearthritis.com/MRI_scoring_modules.php
11 Puhakka KB, Melsen F, Jurik AG, *et al*. Mr imaging of the normal sacroiliac joint with correlation to histology. *Skeletal Radiol* 2004;33:15–28.
12 van den Berg R, Lenczner G, Thévenin F, *et al*. Classification of axial spa based on positive imaging (radiographs and/or MRI of the sacroiliac joints) by local rheumatologists or radiologists versus central trained readers in the DESIR cohort. *Ann Rheum Dis* 2015;74:2016–21.
13 Bollow M, Fischer T, Reisshauer H, *et al*. Quantitative analyses of sacroiliac biopsies in spondyloarthropathies: T cells and macrophages predominate in early and active sacroiliitis- cellularity correlates with the degree of enhancement detected by magnetic resonance imaging. *Ann Rheum Dis* 2000;59:135–40.
14 Schichikawa K, Tsyimoto M, Nishioka J, *et al*. Histopathology of early sacroilitis and enthesitis in ankylosing spondylitis. In: Ziff M, Cohen SB, eds. *Advances in inflammation research, volume 9: the spondyloarthropathies*. New York: Raven Press, 1985: 15–24.
15 Weber U, Jurik AG, Zejden A, *et al*. Frequency and Anatomic Distribution of Magnetic Resonance Imaging Features in the Sacroiliac Joints of Young Athletes: Exploring "Background Noise" Toward a Data-Driven Definition of Sacroiliitis in Early Spondyloarthritis. *Arthritis Rheumatol* 2018;70:736–45.
16 Song I-H, Hermann KG, Haibel H, *et al*. Relationship between active inflammatory lesions in the spine and sacroiliac joints and new development of chronic lesions on whole-body MRI in early axial spondyloarthritis: results of the ESTHER trial at week 48. *Ann Rheum Dis* 2011;70:1257–63.
17 Pedersen SJ, Wichuk S, Chiowchanwisawakit P, *et al*. Tumor necrosis factor inhibitor therapy but not standard therapy is associated with resolution of erosion in the sacroiliac joints of patients with axial spondyloarthritis. *Arthritis Res Ther* 2014;16.
18 Maksymowych WP, Wichuk S, Chiowchanwisawakit P, *et al*. Fat metaplasia and backfill are key intermediaries in the development of sacroiliac joint ankylosis in patients with ankylosing spondylitis. *Arthritis Rheumatol* 2014;66:2958–67.
19 Ziegeler K, Eshkal H, Schorr C, *et al*. Age- and sex-dependent frequency of fat metaplasia and other structural changes of the sacroiliac joints in patients without axial spondyloarthritis: a retrospective, cross-sectional MRI study. *J Rheumatol* 2018;45:915–21.
20 Weber U, Lambert RGW, Østergaard M, *et al*. The diagnostic utility of magnetic resonance imaging in spondylarthritis: an international multicenter evaluation of one hundred eighty-seven subjects. *Arthritis Rheum* 2010;62:3048–58.
21 Weber U, Pedersen SJ, Zubler V, *et al*. Fat infiltration on magnetic resonance imaging of the sacroiliac joints has limited diagnostic utility in nonradiographic axial spondyloarthritis. *J Rheumatol* 2014;41:75–83.
22 de Hooge M, van den Berg R, Navarro-Compán V, *et al*. Patients with chronic back pain of short duration from the space cohort: which MRI structural lesions in the sacroiliac joints and inflammatory and structural lesions in the spine are most specific for axial spondyloarthritis? *Ann Rheum Dis* 2016;75:1308–14.
23 Maksymowych WP, Wichuk S, Chiowchanwisawakit P, *et al*. Fat metaplasia on MRI of the sacroiliac joints increases the propensity for disease progression in the spine of patients with spondyloarthritis. *RMD Open* 2017;3:e000399.
24 Chiowchanwisawakit P, Lambert RGW, Conner-Spady B, *et al*. Focal fat lesions at vertebral corners on magnetic resonance imaging predict the development of new syndesmophytes in ankylosing spondylitis. *Arthritis Rheum* 2011;63:2215–25.
25 Machado PM, Baraliakos X, van der Heijde D, *et al*. Mri vertebral corner inflammation followed by fat deposition is the strongest contributor to the development of new bone at the same vertebral corner: a multilevel longitudinal analysis in patients with ankylosing spondylitis. *Ann Rheum Dis* 2016;75:1486–93.
26 Weber U, Pedersen SJ, Østergaard M, *et al*. Can erosions on MRI of the sacroiliac joints be reliably detected in patients with ankylosing spondylitis? - A cross-sectional study. *Arthritis Res Ther* 2012;14:R124–R24.
27 Maksymowych WP, Pedersen SJ, Navarro-Compán V, *et al*. Can structural progression on MRI of sacroiliac joints in patients with spondyloarthritis be reliably detected and what type of calibration is necessary to achieve this? *Arthritis Rheumatol* 2015;67(suppl 10).
28 Wick MC, Weiss RJ, Jaschke W, *et al*. Erosions are the most relevant magnetic resonance imaging features in quantification of sacroiliac joints in ankylosing spondylitis. *J Rheumatol* 2010;37:622–7.
29 Poddubnyy D, Gaydukova I, Hermann K-G, *et al*. Magnetic resonance imaging compared to conventional radiographs for detection of chronic structural changes in sacroiliac joints in axial spondyloarthritis. *J Rheumatol* 2013;40:1557–65.

TRANSLATIONAL SCIENCE

Integrin and transcriptomic profiles identify a distinctive synovial CD8+ T cell subpopulation in spondyloarthritis

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ABSTRACT

Objectives Current evidence suggests that immune events in the gut may impact joint inflammation in ankylosing spondylitis (AS) but the expression of gut-related trafficking molecules in the inflammed joint is poorly characterised. We aimed to (1) assess differential expression patterns of trafficking molecules between patients and controls, (2) generate joint-specific cellular signatures and (3) obtain transcriptomic profiles of noteworthy cell subpopulations.

Methods Male subjects under 40 years of age fulfilling the mNY criteria were recruited. The following cells were surface stained using a 36-marker mass cytometry antibody panel: (1) peripheral blood mononuclear cells from AS patients, and healthy controls; (2) synovial fluid mononuclear cells from AS and rheumatoid arthritis (RA) patients. Additionally, RNA-seq was performed on CD8+ T cell subpopulations from the synovial fluid (SF).

Results Mature CD8+ T cells were enriched in AS SF, with a distinct pattern of integrin expression (β7, CD103, CD29 and CD49a). RNA-seq analysis of SF-derived CD103+CD49a+CD8+ T cells revealed elevated *TNFAIP3*, *GZMB*, *PRF1* and *IL-10*.

Conclusions We have identified a novel integrin-expressing mature CD8+ T cell population (CD49a+CD103+β7+CD29+) that appears to be more prevalent in AS SF than RA SF. These cells seem to possess dual cytotoxic and regulatory profiles which may play a role in AS pathogenesis.

Key messages

What is already known about this subject?

- Axial spondyloarthritis (AS) has clinical, genetic and immunological overlaps with inflammatory bowel disease, thereby making the gut–joint axis of inflammation an emerging area of research and therapy.
- Phenotypic knowledge of immune cells, implicated in inflammation of the gut and joint, is unclear.

What does this study add?

- Proteomic and transcriptomic analyses helped identify a mature CD8+ T cell subpopulation, enriched in gut-associated trafficking molecules (CD49a+CD103+β7+CD29+), which was elevated in AS synovial fluid.
- This supopulation appeared to have a dual cytotoxic and regulatory profile, which may play a role in AS pathogenesis.

How might this impact on clinical practice or future developments?

- Therapeutic intervention using integrin-based blocking agents have had inconsistent results in proof-of-concept studies in AS patients, mandating a more thorough understanding of trafficking molecules in disease pathogenesis.

INTRODUCTION

Ankylosing spondylitis (AS), is characterised by chronic inflammation of the axial skeleton and often coexists with inflammatory bowel disease (IBD).[1] AS prevalence ranges from 0.1% to 1.4%[1] and male sex influences clinical disease expression.[2] HLA-B27, an major histocompatibility complex (MHC) class I molecule, remains the strongest genetic risk factor for AS, with 80%–90% AS patients testing positive for HLA-B27.[1] Since there is a strong clinical and genetic association between AS and IBD, the gut–joint axis of inflammation has become an important area of research.[3] One hypothesis states that inflammation iniating in the gut is transferred to the joint due to the abberant trafficking of immune cells. Nonetheless, the trafficking molecules shared between cells in the gut and joint are poorly defined.

Leukocytes employ various trafficking molecules, such as integrins and chemokine receptors, to orchestrate their exit from the circulation and retention in tissue.[4] Adhesion molecules and chemokine receptors expressed by immune cells programme targeting to specific tissues. To date, studies have identified shared trafficking molecules between gut and joint tissue. For example, $\alpha_4\beta_7$, the prototypic gut homing integrin, is also expressed by immune cells in synovial tissues.[5] The $\alpha_4\beta_7$ ligand, MAdCAM-1, is expressed on endothelial cells at sites such as joints, eyes, skin and liver.[6] Moreover, mucosal lymphocytes isolated from IBD patients bind to inflamed synovial vessels in vitro via distinct adhesion receptors.[4 7]

Integrin-blocking agents, approved for IBD, have been used in AS patients with coexisting IBD with conflicting case reports with regards to affects on arthritis.[8–12] A major limitation to our understanding as to whether integrin blockade could be effective or exacerbative of arthritis is our incomplete understanding of the trafficking molecules associated with AS. For this reason, we screened gut-related trafficking molecule expression at the cellular level in AS patients using multidimensional

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mass cytometry (CyTOF). We sought to identify trafficking marker combinations, using multiple computational methods, to identify novel cell populations in AS. We demonstrate disease specificity of trafficking marker expression to the joint and we have identified a population of mature CD8+ T cells that expresses CD103 (αE integrin), β7, CD29 (β1 integrin) and CD49a (α1 integrin) and which are enriched in AS. These integrin-expressing (InEx) cells possess a dual cytotoxic and regulatory transcriptomic profile, as evidenced by elevated *TNFAIP3*, *GZMB*, *PRF1* and *IL-10*.

METHODS

Patient cohorts. CyTOF, RNA sequencing, NanoString, protein–protein interactions and cytokine multiplex assay

Detailed experimental procedures are provided in the online supplementary files 1; 2.

RESULTS

Broad cell population changes in SFMC compared with PBMC

We began analysis of our CyTOF samples using spanning-tree progression analysis of density-normalized events (SPADE) on all live cells (selections strategy displayed in online supplementary figure S1). SPADE is an unsupervised hierarchical clustering algorithm which provides an overview of cell populations. Major cell populations appeared similar in the peripheral blood mononuclear cell (PBMC) of patients and controls; however, differences were seen in the number of cells in each node, and in surface marker expression when comparing patient synovial fluid mononuclear cell (SFMC) to PBMC (figure 1A). These qualitative SPADE observations were used to direct FlowJo-based quantitative analysis of PBMCs and SFMCs from healthy controls (HC), AS and rheumatoid arthritis (RA) samples (figure 1B). For example, CD14+CD56- cells are expanded in synovial fluid (SF), which likely represent macrophage-like cells seen frequently on histological examination (online supplementary figure S2). CD19+ expression was similar between HC and AS blood, although the frequency of CD19+ B cells was decreased in AS-SF. Further, there was a trend towards expansion of natural killer (NK) cells in SF versus blood.

Using heat maps, distinctive trafficking molecule expression profiles were seen on major non-T cell PBMC populations independent of disease status (figure 1C). For example, monocytes expressed CD61, CCR1 and CCR2, whereas B and NK cells did not. On the other hand, B cells expressed CCR6, while monocytes and NK cells were limited in their CCR6 expression. Notably, there were few differences in trafficking marker expression between AS and control PBMC, with respect to CD14+ monocytes, CD19+ B cells and CD56+ NK cells.

Heatmap analysis further revealed marked differences between cells in AS SF compared with blood, many of which were significant (figure 1C and online supplementary figure S3A and S3B). For example, CD14+ monocytes showed higher CD4, CD16, HLA-DR and lowered CD61 expression in SF (figure 1C and online supplementary figure S3A). CD56+ NK cells in SFMC displayed characteristically high β7, CCR1, CD49a and CD103 expression compared with PBMC, but lower CD16 and CXCR4 (figure 1C and online supplementary S3B), indicating a tissue-resident phenotype in SF.[13] Some of these SF profiles were disease specific. For example, CD14+ cells in AS SF expressed more CD4+, CD16+ and HLA-DR+ than those in RA SF. Likewise, there was a trend for RA SF NK cells expressing more trafficking molecules, such as β7, CCR1 and CCR2, when compared with their AS SF counterparts.

$CD56^{hi}$ NK cells, a population of cytokine-expressing NK,[14] were more common in the AS SF than blood and were at a higher frequency than RA SF (online supplementary figure S4A). Stratification by CD16 indicates NK cell cytotoxicity.[14] $CD56^{hi}$CD16+ NK cells were markedly reduced in AS SF, while $CD56^{hi}$CD16- NK cells were enriched in AS SF, suggesting NK cells in SF have a less cytotoxic phenotype. The more cytotoxic[14] NK cell subtype, $CD56^{dim}$ NK cells, were infrequent in AS versus HC blood, with a trend towards reduced frequency in AS SF. These cells appeared to be less cytotoxic, owing to the less common occurrence of $CD56^{dim}$CD16+ cells and the increased $CD56^{dim}$CD16+ cells (online supplementary figure S4B).

In summary, we observed few changes to non-T cell frequency and phenotype in the blood of AS patients and controls. Large changes were seen in the frequency and phenotype of these cells in the SF, highlighting the importance of studying the target site of inflammation.

Mature CD8+ T cells are enriched in AS SF

Owing to the high diversity of T cells, and their role in AS, we performed SPADE analysis by pregating on whole T cells (figure 2A). Here, it was evident that the SF was enriched with mature CD45RO+ T cells, with certain cell populations being more common in SF, as indicated by arrows in figure 2A.

Statistical analysis of these major cell subsets revealed no differences in whole CD3+ T cell and γδ T cell frequencies in the blood. On the contrary, CD4+ T cells were elevated in RA patient blood, which was accompanied by a reduction in CD8+ T cells (figure 2B). RA blood CD4+ T cells had a more mature phenotype than AS and HC, and RA blood CD8+ T cells were more mature than AS (figure 2C), which may be attributable in part to the age differences in the respective patient groups.[15]

In SF, we observed no change in whole CD3+ T cell frequency compared with blood, however there was a reduction in CD4+ T cells and an elevation of CD8+ T cells in AS SFMC versus PBMC (figure 2B). The most striking change in the composition of T cells in blood compared with SF was the enrichment of CD45RO in both CD4+ and CD8+ T cells irrespective of disease type (figure 2C). Given this final observation, and the fact that AS is an MHCI-associated disease,[1] we focused our studies on mature CD8+ T cells in the SF.

AS SF is characterised by a distinct population of CD103+CD49a+ mature CD8+ T cells

The enrichment of mature CD8+ T cells in the inflamed joint prompted a clustering analysis using the unsupervised viSNE algorithm that provides readouts at a single cell resolution. This revealed a subpopulation of mature CD8+ T cells that expressed CD103 in AS SF (figure 3A). In addition, other markers were co-expressed on CD103+CD8 T cells in SF, such as β7, CD49a, CD29 and CXCR6. These cells are likely not MAIT cells as they did not express CCR6 and CD161 (figure 3A). The enrichment of CD103+CD8+ T cells in AS SF was validated by conventional flow cytometry in an independent cohort (online supplementary figure S5A).

Closer examination of this novel CD8+ T cells subpopulation revealed that CD103 and β7 are tightly coexpressed in both blood and SF (figure 3B). There was an enrichment of CD103+β7+ mature CD8+ T cells in AS SF versus blood (mean 17.7±2.9% vs 11.3±3.3%), and which was higher in AS SF than RA SF (online supplementary figure S5B). CD103-β7- mature CD8 T cells were reduced in AS SF compared with blood (mean 76.0±3.1% vs 84.1±3.1%) and were much lower

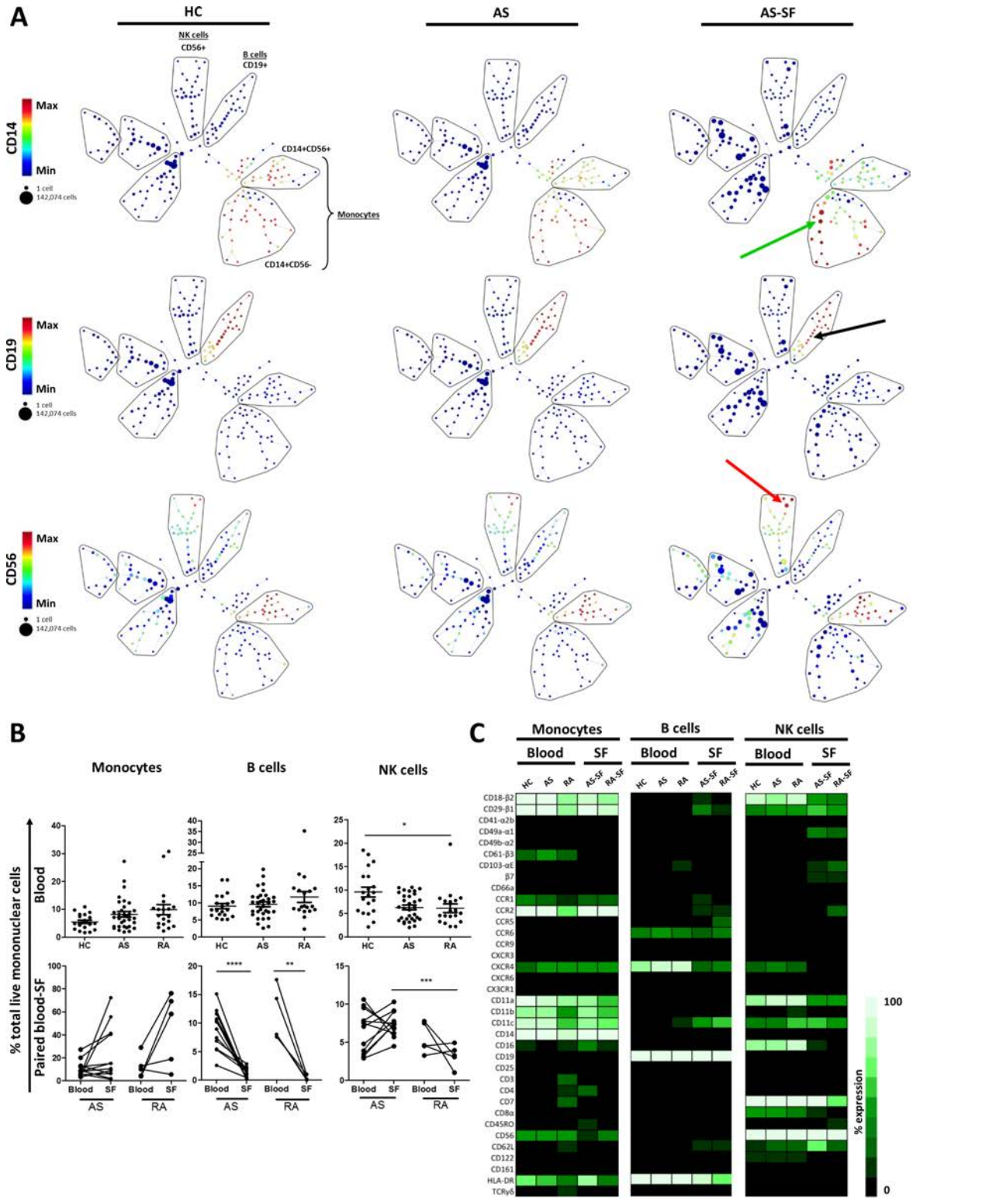

Figure 1 Overview of major non-T cell lineages in blood and synovial fluid using high-dimensional analyses. (A) Live single cells from HC PBMC (n=20) and AS PBMC (n=32) and AS SFMC (n=12) concatenated by group and analysed by SPADE. Pregating strategy given in online supplementary figure S1. Common lineage markers used to identify indicated cell populations (lassoed for illustrative purposes), with CD14, CD19 and CD56 expression given as examples. Arrows indicate populations highlighted in text. (B) Cell subsets were identified as follows in FlowJo: monocytes (CD14+CD19-), B cells (CD14-CD19+) and NK cells (CD14-CD19-CD3-HLADR-CD56+). HC, AS and RA blood (PBMC) data analysed by Kruskal-Wallis test with Dunns post-test. Monocytes paired blood-SF samples analysed by Wilcoxon matched pairs test, while B cells and NK cells paired blood-SF samples analysed by paired T test. AS-SF and RA-SF analysed by Mann-Whitney test. ****p<0.0001, ***p<0.001, **p<0.01, *p<0.05, n.s. p>0.05 (non-significant results not highlighted on graphs, unless indicated). (C) Monocytes, B and NK cells were gated in each sample and concatenated into a single file for each sample group using FlowJo. The expression (%+) of each marker was measured in the concatenated files, representing the mean expression per group, and were converted to a heatmap. HC PBMC (n=20), AS PBMC (n=32), RA PBMC (n=19), AS SFMC (n=12), RA SFMC (n=5). AS, ankylosing spondylitis; PBMC, peripheral blood mononuclear cell; RA, rheumatoid arthritis; SF, synovial fluid; SFMC, synovial fluid mononuclear cell.

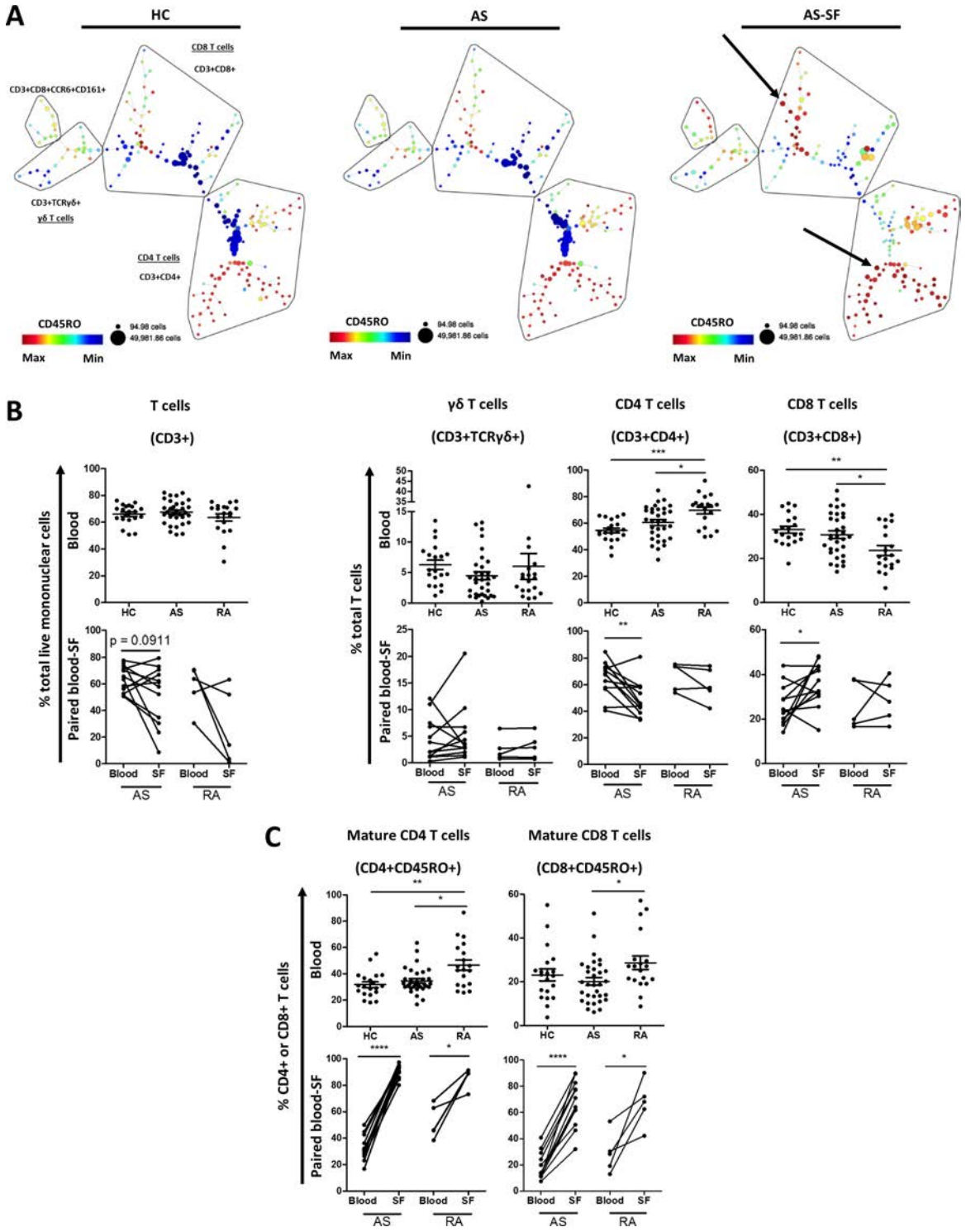

Figure 2 Overview of major blood and synovial fluid T cell populations using high-dimensional analyses. (A) Single file representations of HC (n=20), AS PBMC (n=32) and AS SFMC (n=12) were entered into Cytobank, and gated CD3+ cells were then entered into SPADE programme. T cell subpopulations were identified on the basis of common markers, which were lassoed for illustrative purposes. Median CD45RO expression intensities per node displayed from HC and AS (PBMC) and AS-SF. Expanded populations indicated using arrows. (B) Cell subsets were identified as follows using FlowJo: T cells (CD14-CD19-CD3+HLADR-), γδ T cells (CD14-CD19-HLADR-CD3+TCRγδ+), CD4+ T cells (CD14-CD19-HLADR-CD3+CD4+) and CD8+ T cells (CD14-CD19-HLADR-CD3+CD8+). Graphs depicting T cells and γδ T cells blood samples analysed by Kruskal-Wallis test with Dunns post-test. T cells paired blood-SF samples analysed by paired T test, while γδ T cells paired samples analysed by Wilcoxon matched pairs test. CD4 and CD8 T cells blood samples analysed by one-way ANOVA with Bonferroni correction, while paired samples analysed by paired T test. AS-SF and RA-SF analysed by Mann-Whitney test. ****p<0.0001, ***p<0.001, **p<0.01, *p<0.05, n.s. p>0.05 (non-significant results not highlighted on graphs, unless indicated). (C) Mature CD4 or CD8 cells identified as CD4+CD45RO+ or CD8+CD45RO+ using FlowJo. Blood samples analysed by Kruskal-Wallis test with Dunns post-test, while paired samples analysed by paired T test. AS-SF and RA-SF analysed by Mann-Whitney test. ***p<0.001, **p<0.01, *p<0.05, n.s. p>0.05 (non-significant results not highlighted on graphs, unless indicated). PBMC, peripheral blood mononuclear cell; RA, rheumatoid arthritis; SF, synovial fluid; SFMC, synovial fluid mononuclear cell.

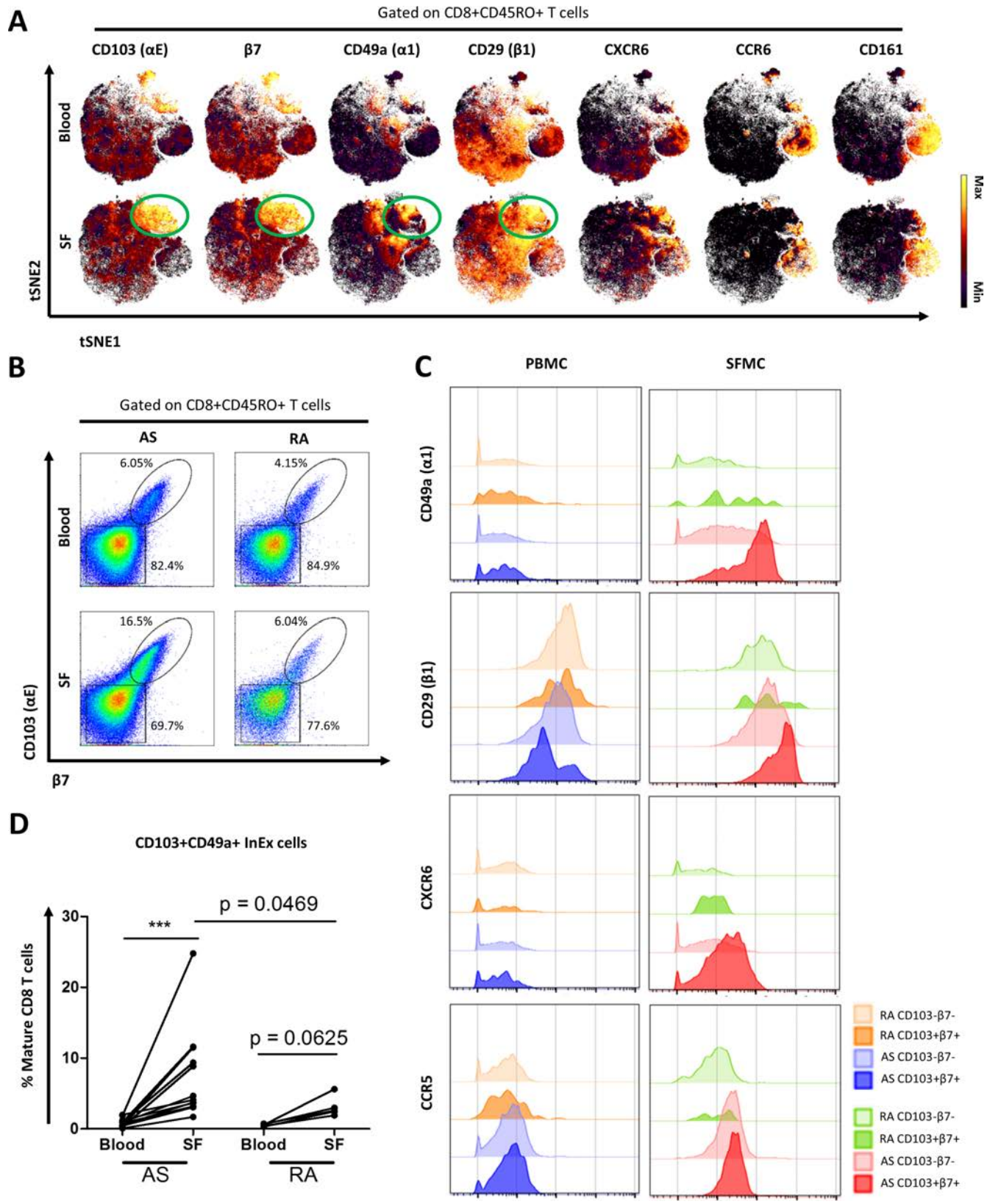

Figure 3 A population of CD103+CD49a+ (InEx) CD8 T cells is enriched in AS synovial fluid. (A) Mature CD8+ T cells from single file representations of AS PBMC (n=32) and AS SFMC (n=12) were entered into viSNE algorithm for high-dimensional analysis of trafficking molecules in as blood and SF. Trafficking molecules were selected to display their expression levels on mature CD8+ T cells. (B) Plots shown of merged single file representations of PBMC and SFMC obtained from AS and RA patients, to compare their CD103 (αE) and β7 expression patterns gated on mature CD8+ T cells. (C) Expression intensities of trafficking molecules as seen on CD103+β7+or CD103-β7- cells from mature CD8+ T cells from representative AS and RA paired PBMC-SFMC plots. (D) CD103+CD49a+ integrin expressing ('InEx') cells from paired blood-SF of AS and RA patients, gated using FlowJo. Paired samples analysed by Wilcoxon matched pairs test. AS SF and RA SF analysed by Mann-Whitney test. ***p<0.001. InEx, integrin-expressing; PBMC, peripheral blood mononuclear cell; RA, rheumatoid arthritis; SF, synovial fluid; SFMC, synovial fluid mononuclear cell.

than RA SF (online supplementary figure S5B). Further examination of these cells revealed disease-specific trafficking marker profiles: CD49a, CD29 and CXCR6 were found to be preferentially upregulated in CD103-αE+β7+ mature CD8 T cells from AS SFMC (figure 3C) whereas CXCR4 was downregulated in this cell population (online supplementary figure S5C). Trafficking markers such as CCR5, CD11a and CD18 showed no difference in expression on either CD103+β7+ or CD103-β7- mature CD8 T cells from AS or RA samples (figure 3C; online supplementary figure S5C). In addition to CD103+β7+ expression, mature CD8+ T cells from AS SF were also enriched in CD49a+CD29+ (VLA-1) expression versus blood (mean 27.5±4.4% vs 6.4±1.6%) (online supplementary figure S5D).

We designate the particular subpopulation of mature CD8+ T cells expressing CD103+β7+CD49a+CD29+ integrins as integrin-expressing (InEx) cells. Since CD103+β7+ and CD49a+CD29+ are often coexpressed we used CD103 and CD49a expression to simplify the identification of InEx cells. Indeed, using this definition, InEx cells were significantly enriched in AS SF, but barely detectable in the AS blood (mean 7.5±1.9% vs 0.9±0.2%; figure 3D). This effect was not seen between RA blood and SF paired samples. InEx cell frequency was also higher in AS SF than RA SF (mean 7.5±1.9% vs 3.1±0.6%). As a control cell population, CD103-CD49a- mature CD8+ T cells were classified as non-InEx cells and were noticeably decreased in SF versus blood of both AS and RA patients (online supplementary figure S5E). We used these definitions for subsequent studies.

As some AS and RA patients in this study were being prescribed anti-TNF biologics (Table S1), we sought to determine the influence of biological treatment on the InEx cell percentage using multiple regression analysis. In SFMC and PBMC, we discovered that biological status only predicted only 6.2% and 4.7% of the variability in the InEx cell percentages, respectively. Thus biological treatment was not a significant confounder in the immune profiling results (online supplementary figure S6A and S6B).

InEx cells possess a dual cytotoxic and regulatory transcriptome

To examine the InEx cells, their resting transcriptome was analysed using bulk RNA sequencing on fluorescence activated cell sorting (FACS)-sorted SFMC cells. Non-InEx and naïve CD8+ T cells from AS SFMC were used as comparators (online supplementary figure S7A). After filtering based on defined cut-offs (fold change greater than or less than 2 and $p<0.05$), there were 1124 differentially expressed genes (DEGs) (1450 including splice variants; online supplementary figure S7B) among the three groups. PCA analysis of these three populations, using the 1124 unique genes, revealed distinct transcriptomic profiles (online supplementary figure S7C). Overall, 197 genes were uniquely expressed in InEx versus non-InEx, out of which 80 were elevated and 117 downregulated (figure 4A). Specifically, InEx cells were defined by *GZMB*, *IRF4*, *ITGAE*, *CCL5*, *RPTOR*, *TNFAIP3*, *BAX*, *PRF1* and *IL-10*, non-InEx cells by *IRF7*, *CX3CR1*, *DUSP2*, *IFNAR1* and *IFNAR2* and naïve CD8 T cells by *GNLY*, *IL-17RC*, *S1PR1*, *IFNG*, *STAT3* and *CTNNB1* (figure 4B). These observations were all significant, with considerable fold change (online supplementary figure S7D).

We performed pathway analysis to infer function of InEx cells based on their gene expression profile. The 80 genes were involved in apoptotic signalling and in turn, lymphocyte homeostasis (*BAX, ETFDH, PPP3CB, TNFAIP3*), T cell receptor signalling (*CD3D, LCP2, PPP3CB*), ubiquitinyl hydrolase/protease activity (*ALG13*, *OTUD1*, *TNFAIP3*) and TNFα signalling (*BAX, TNFAIP3*) (figure 4C). Genes with functions such as cytokine binding (*A2M*, *CX3CR1, FZD4, IFNAR1, IFNAR2*), type I interferon activity (*IFNAR1*, *IFNAR2*) and calcium signalling pathway (*CTNNB1, FZD4, ITPR2*) were uniquely reduced in InEx versus non-InEx cells (online supplementary figure S7E). Pathway enrichment analysis revealed IL-10 and NFκB signalling networks as enriched in all 1124 genes, while granzyme A-mediated apoptosis pathway was enriched in InEx versus naïve (figure 4D).

To further phenotype InEx cells, we performed TCR stimulation and assessed gene expression. Compared with non-InEx cells, there was an elevation of transcripts from *KLRC1, KLRC2, GNLY, CCL20, CCR6, CCRL2* and *SLAMF1*. Genes such as *GZMK*, *PECAM1* and *HLA-DPA1* were lower in InEx cells (figure 5).

A protein–protein interaction network analysis was conducted in order to visualise the design of interactions between the 1124 DEGs comprehensively. Interestingly, some of the genes that define InEx cells, such as *IRF4*, *CCL5*, *TNFAIP3*, *BAX* and *IL-10*, encode proteins that appear to interact with each other (figure 6). Specifically, TNFAIP3 protein also seems to interact with LCP2, whose phosphorylated form acts as a substrate for ZAP-70 in early TCR-mediated signaling[16] (online supplementary figure S8A). Granzyme B (GZMB) and perforin (PRF1) proteins interact with CD2, a costimulatory molecule[17] (online supplementary figure S8B).

InEx cell phenotype was further explored by assessing secreted factors during resting and stimulated states. InEx cells produced IL-17A, PRF1, granzyme A and GZMB, even under resting conditions. This was congruent with our transcriptomic data. While *IL-10* and *TNFAIP3* transcripts were observed in InEx cells, production of IL-10 and TNFα at the protein level was limited by unstimulated InEx cells, although they were elevated when stimulated. Additionally, despite limited transcripts, InEx cells produced some IFNγ and granulysin when unstimulated (online supplementary figure S9).

In summary, InEx cells represent a distinctive population of joint-resident CD8+ T cells in AS patients. These cells have a distinct transcriptional profile under both resting and stimulated conditions compared with other joint-derived CD8+ T cells.

DISCUSSION

This study demonstrates how next generation technologies empower examination of the immune system at a previously unattainable level. This approach will be central to understanding AS. Using hierarchal clustering and dimensionality reduction algorithms, we were able to observe differences in immune cell composition and expression of trafficking molecules, most notably in the SF of AS patients.

We identified the InEx mature CD8+ T cell population, with characteristic markers—CD103, β7, CD29, CD49a. These markers were found to be unique to InEx cells among T cell populations, implicating a role in AS pathogenesis. CD103+β7+ expression defines gastrointestinal intraepithelial lymphocytes (IELs)[18] with CD49a defining a cytotoxic subset.[19] The CD49a+CD29+ integrin heterodimer, known as VLA-1, binds type IV collagen and is commonly found on gut-resident CD8+ T cells.[20] Prior studies blocking VLA-1 in immune-mediated inflammation models have confirmed amelioration of gut and joint immune infiltration, synovitis, cartilage damage and clinical disease.[21]

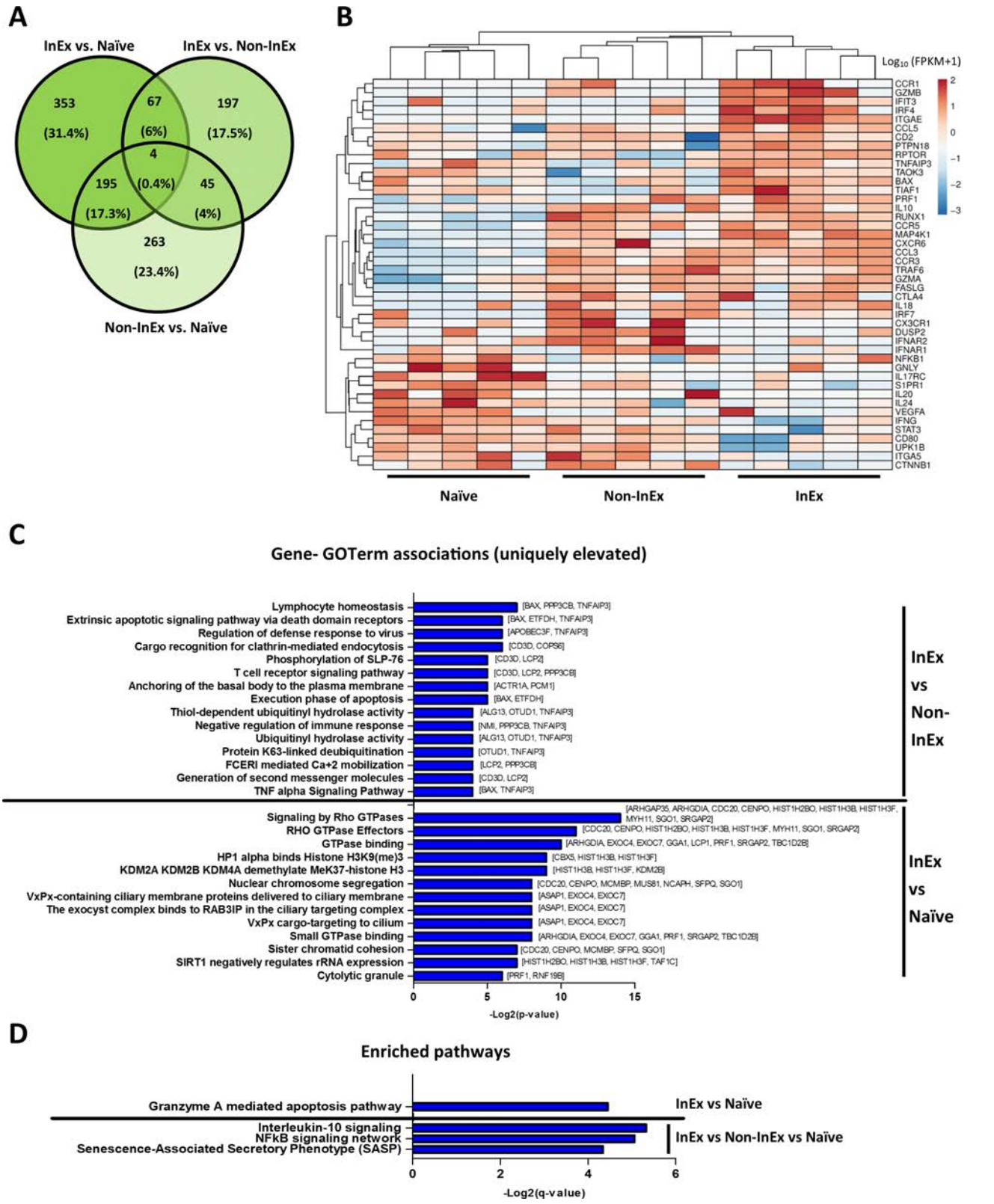

Figure 4 Transcriptomic analysis of 'integrin-expressing' (InEx) cell population. InEx, non-InEx and naïve CD8+ T cells were FACS purified from AS SF for RNAseq analysis (n=5 patients). (A) Resulting transcript fragments per kilobase of transcript per million (FPKM) values were filtered according to fold change greater than or less than 2 and p<0.05 cut-off and converted into Venn diagram to identify differentially expressed genes unique to or shared between each population (InEx, non-InEx and naïve). (B) Using aforementioned cut-offs, a heatmap displaying expression levels of selected genes in the three groups was plotted. Unit variance scaling was applied to rows. Rows clustered using correlation distance and average linkage. Columns clustered using Euclidean distance and average linkage. (C) 197 genes were differentially regulated in InEx versus non-InEx cells and 353 genes differentially regulated in InEx versus naïve cells. Out of these, 80 genes and 150 genes were uniquely elevated in InEx cells, respectively, and were fed into Cytoscape with ClueGo programme selected. All pathways/ontologies were selected, with network specificity 'medium' and p<0.05. Resulting data were converted into a graph to display pathway analysis of elevated genes. SF, synovial fluid.

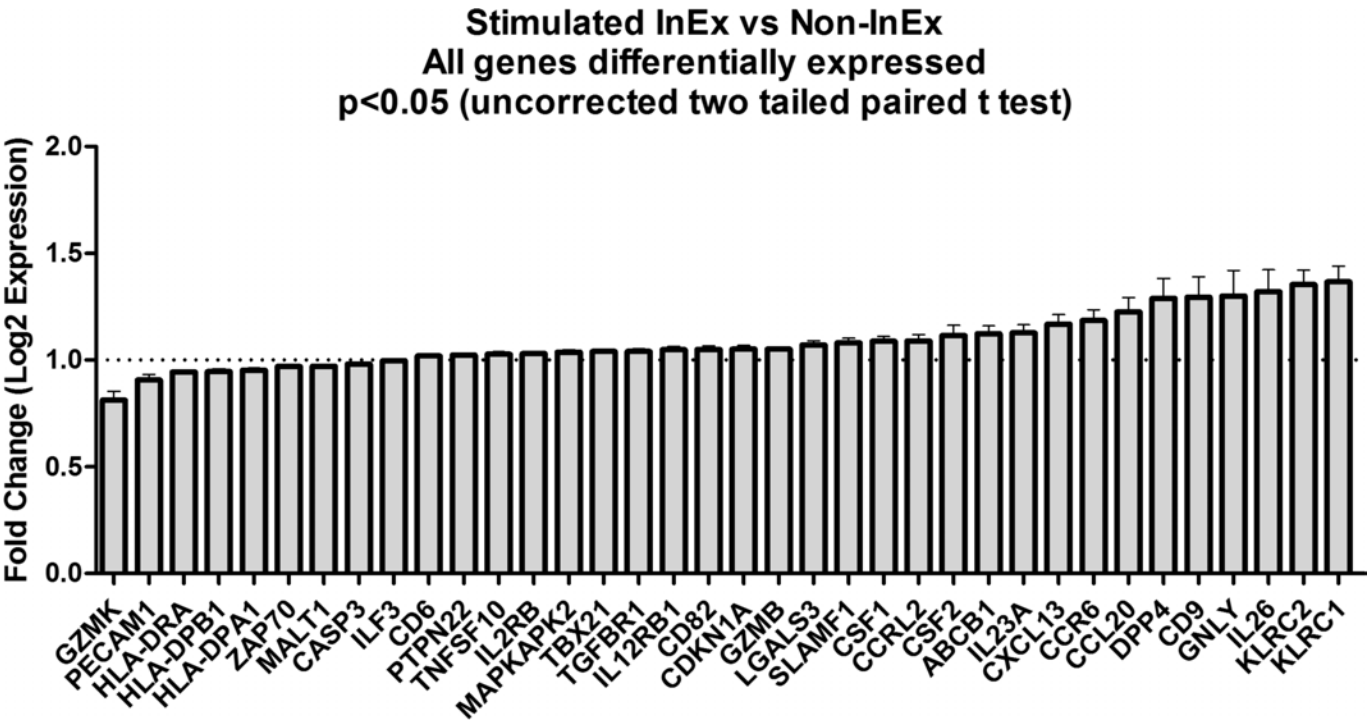

Figure 5 NanoString analysis of stimulated InEx and non-InEx cell populations. FACS-sorted InEx and non-InEx cells from AS SFMCs (n=6) were stimulated with anti-CD2/CD3/CD28 T cell activation beads and 500 U/mL IL-2 for 72 hours/37°C. RNA was isolated and processed using the nCounter mRNA assay. Log_2 normalised RNA transcripts were converted into fold change (FC) values (InEx/non-InEx), analysed using paired T test ($p<0.05$) and graphed. Dotted line indicates FC=1 (NO change). Uncorrected p values used for analysis owing to low sample numbers per group. InEx, integrin-expressing; SFMCs, synovial fluid mononuclear cells.

It can be speculated that InEx cells are involved with apoptosis via ubiquitynation and a complex signalling pathway. In fact, besides restricting NF-κB signalling downstream of tumour necrosis factor receptor 1, A20 (*TNFAIP3*) regulates multiple ubiquitin-dependent signalling by interacting with E3 enzymes like *TRAF6*.[22] This may be particularly important since *TNFAIP3* contains single-nucleotide polymorphisms linked to diseases like RA, psoriasis and IBD.[22] A20 also functions as a negative regulator of the inflammasome, which may protect against arthritis.[23] Apoptosis could also occur by the granzyme B/perforin cytotoxic pathway[24] or granulysin-mediated mechanisms.[25] Our expression profiles indicate that InEx cells may reflect a balancing act of cytotoxicity and cytokine release by way of inhibitory (NKG2A, encoded by *KLRC1*) and activating (NKG2C, encoded by *KLRC2*) receptors.[26] NKG2A has been thought to counterbalance the action of stimulatory NKG2C. In fact, polymorphisms in NKG2A and NKG2C have been reported to be associated with RA.[26]

InEx cells have the capacity to produce multiple cytokines. Upregulation of *IRF4* could suggest a mechanism by which IL-17 and IL-21 production could be observed in InEx cells.[27] *IRF4* is also known to promote expression and function of Blimp1 and T-bet, transcription factors required for CD8+ T cell differentiation. In fact, impaired *IRF4* in peripheral CD8+ T cells severely impacts their antiviral ability, viral clearance and host recovery from influenza infection. Furthermore, *IRF4* expression is regulated by TCR signalling through mammalian target of rapamycin (mTOR).[28] This is notable in light of our observation of enhanced *RPTOR* transcripts in InEx cells. Another informative observation was the *IL-10* elevation in InEx cells. An anti-inflammatory cytokine, IL-10 has been implicated in RA pathogenesis, particularly due to its increased mRNA levels in RA SFMC.[29]

It appears that upregulation of *CCL5* transcripts could potentially allow InEx cells to recruit myeloid cells such as macrophages, neutrophils and potentially dendritic cells[30] to the inflamed synovial microenvironment.

The InEx core transcriptional and phenotypic signature is reminiscent of human tissue-resident memory T cells (TRMs), in their expression of CD103, CD49a, CXCR6 and IL-10, and their lack of CD62L, S1PR1 and CX3CR1.[31] Future studies are required to understand InEx cells' function, TCR clonality and presence in gut as well as joint, which would enable insights into the gut–joint pathway implicated in AS and may lend support to the arthritogenic peptide hypothesis. In fact, CD8+ TRMs have been reported to express high-affinity TCRs,[32] an observation which may support our InEx-resident memory theory. Although we have not yet had the opportunity to incorporate gut biopsies in our study, the InEx cells also appear to be quite similar to IELs. This alternative theory might suggest that these cells have a mucosal origin and have potentially trafficked to the joint, which supports the hypothesis of the gut being an initiator of inflammation in AS.

In summary, through unbiased multidimensional analyses, we identified a distinctive trafficking molecule expression pattern in AS joint inflammation. We have also highlighted tissue and disease-preference pattern of marker expression. It is notable that in general CD8+ T cells are heterogeneous and include both cytotoxic and anti-inflammatory features,[33] however this has not been previously studied in AS via multiparameter analysis. Our analyses support the concept that immune heterogeneity in T cell populations in AS is far greater than previously appreciated and thus calls for further study. Most importantly, we have identified a novel and potentially pathogenic CD8+CD45RO+ subset, CD103+β7+CD29+CD49a+, that may contribute to AS pathogenesis. The functional capabilities of the InEx versus

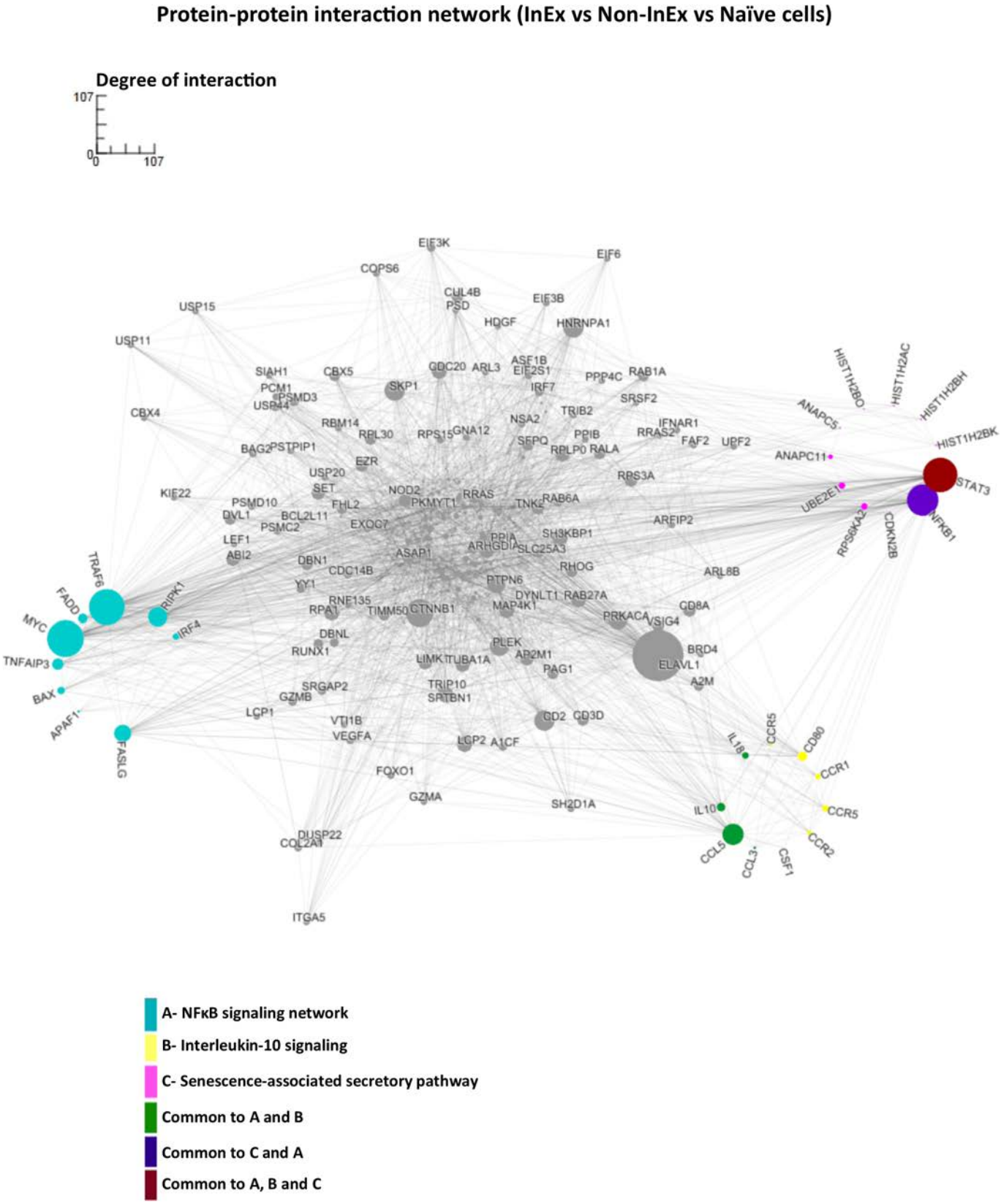

Figure 6 Protein–protein interaction network. The 1124 DEGs were submitted to the IID platform, from which corresponding protein-protein interactions (PPIs) and degree of interaction for each gene was obtained. These were then input into the navigator programme, and only those proteins with degree of interactions ≥15 were selected for ease of visualisation. The size of the node indicates degree of interaction with other proteins among the 1124 DEGs. Enriched pathways from pathDIP programme highlighted in colour.

the NK cells appear distinct, that is, cytotoxic versus cytokine-producing, respectively, even though both share similar trafficking molecule expression. Analysis of target tissues will be informative in future studies, as reflected in the recent finding of elevated CD103, β7, CD49a and CD29 in the colon of human CD8+ T cells.[34] Therapeutic approaches using integrin blockers

have been successful in IBD, but perhaps need to be better tailored to AS to prove efficacy, given inconsistent results using current antagonists.[8 10 11] Current cytokine-blocking agents in AS targeting TNFα or IL-17A have achieved symptomatic improvement but have not reliably prevented radiographic progression in AS nor have proven to be curative.

We acknowledge that our study is limited by sample size and a lack of gut biopsy samples; however, further work involving large sample sizes and tissue-specific data would address these limitations. Additionally, there are some discrepancies in our transcriptomic versus protein analyses, and this may be attributed to a low sample size (which could interfere with statistical analyses) or post-transcriptional modification processes. Notwithstanding, such exploratory approaches and genetic evaluations of TCR sequences and oligoclonality will help pave the way towards understanding of trafficking markers extensively in AS and unravel the complex relationship between the gut and joint axis of inflammation. These findings could prove beneficial for future blocking studies using murine AS models, through which researchers could conclusively determine the role of the identified subpopulation markers. This would establish a causal link and could lay the groundwork for innovative immunotherapy for AS in the future.

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Contributors ZQ, EG and RDI were involved with study conception and design. ZQ and YY processed the blood and synovial fluid samples, while ZQ and EG performed the remainder of the experiments. ZQ, EG and RDI analysed and interpreted the data. ZQ, EG and RDI wrote the manuscript. All authors agreed to publish the data and reviewed the manuscript.

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Patient consent for publication Obtained.

Ethics approval Venous blood and knee synovial fluid extraction obtained under the institutional approval.

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REFERENCES

1 Braun J, Sieper J. Ankylosing spondylitis. *Lancet* 2007;369:1379–90.
2 Rusman T, van Vollenhoven RF, van der Horst-Bruinsma IE. Gender differences in axial spondyloarthritis: women are not so lucky. *Curr Rheumatol Rep* 2018;20:35.
3 Gracey E, Dumas E, Yerushalmi M, *et al*. The ties that bind: skin, gut and spondyloarthritis. *Curr Opin Rheumatol* 2019;31:62–9.
4 Salmi M, Jalkanen S. Human leukocyte subpopulations from inflamed gut bind to joint vasculature using distinct sets of adhesion molecules. *J Immunol* 2001;166:4650–7.
5 Elewaut D, De Keyser F, Van Den Bosch F, *et al*. Enrichment of T cells carrying beta7 integrins in inflamed synovial tissue from patients with early spondyloarthropathy, compared to rheumatoid arthritis. *J Rheumatol* 1998;25:1932–7.
6 Ley K, Rivera-Nieves J, Sandborn WJ, *et al*. Integrin-Based therapeutics: biological basis, clinical use and new drugs. *Nat Rev Drug Discov* 2016;15:173–83.
7 Salmi M, Andrew DP, Butcher EC, *et al*. Dual binding capacity of mucosal immunoblasts to mucosal and synovial endothelium in humans: dissection of the molecular mechanisms. *J Exp Med* 1995;181:137–49.
8 Ciccia F, Rizzo A, Guggino G, *et al*. Clinical efficacy of α4 integrin block with natalizumab in ankylosing spondylitis. *Ann Rheum Dis* 2016;75:2053–4.
9 Sandborn WJ, Feagan BG, Rutgeerts P, *et al*. Vedolizumab as induction and maintenance therapy for Crohn's disease. *N Engl J Med* 2013;369:711–21.
10 Varkas G, Thevissen K, De Brabanter G, *et al*. An induction or flare of arthritis and/or sacroiliitis by vedolizumab in inflammatory bowel disease: a case series. *Ann Rheum Dis* 2017;76:878–81.
11 Orlando A, Orlando R, Ciccia F, *et al*. Clinical benefit of vedolizumab on articular manifestations in patients with active spondyloarthritis associated with inflammatory bowel disease. *Ann Rheum Dis* 2017;76:e31.
12 Vermeire S, O'Byrne S, Keir M, *et al*. Etrolizumab as induction therapy for ulcerative colitis: a randomised, controlled, phase 2 trial. *Lancet* 2014;384:309–18.
13 Melsen JE, Lugthart G, Lankester AC, *et al*. Human Circulating and Tissue-Resident CD56$^{(bright)}$ Natural Killer Cell Populations. *Front Immunol* 2016;7:262.
14 Poli A, Michel T, Thérésine M, *et al*. CD56^{bright} natural killer (NK) cells: an important NK cell subset. *Immunology* 2009;126:458–65.
15 Lefebvre JS, Haynes L. Aging of the CD4 T cell compartment. *Open Longev Sci* 2012;6:83–91.
16 Chylek LA, Akimov V, Dengjel J, *et al*. Phosphorylation site dynamics of early T-cell receptor signaling. *PLoS One* 2014;9:e104240.
17 Skånland SS, Moltu K, Berge T, *et al*. T-Cell co-stimulation through the CD2 and CD28 co-receptors induces distinct signalling responses. *Biochem J* 2014;460:399–410.
18 Parker CM, Cepek KL, Russell GJ, *et al*. A family of beta 7 integrins on human mucosal lymphocytes. *Proc Natl Acad Sci U S A* 1992;89:1924–8.
19 Cheuk S, Schlums H, Gallais Sérézal I, *et al*. CD49a Expression Defines Tissue-Resident CD8^{+} T Cells Poised for Cytotoxic Function in Human Skin. *Immunity* 2017;46:287–300.
20 Purwar R, Campbell J, Murphy G, *et al*. Resident memory T cells ($T_{(RM)}$) are abundant in human lung: diversity, function, and antigen specificity. *PLoS One* 2011;6:e16245.
21 Ben-Horin S, Bank I. The role of very late antigen-1 in immune-mediated inflammation. *Clin Immunol* 2004;113:119–29.
22 Ma A, Malynn BA. A20: linking a complex regulator of ubiquitylation to immunity and human disease. *Nat Rev Immunol* 2012;12:774–85.
23 Matmati M, Jacques P, Maelfait J, *et al*. A20 (TNFAIP3) deficiency in myeloid cells triggers erosive polyarthritis resembling rheumatoid arthritis. *Nat Genet* 2011;43:908–12.
24 Hiebert PR, Boivin WA, Zhao H, *et al*. Perforin and granzyme B have separate and distinct roles during atherosclerotic plaque development in apolipoprotein E knockout mice. *PLoS One* 2013;8:e78939.
25 Krensky AM, Clayberger C. Biology and clinical relevance of granulysin. *Tissue Antigens* 2009;73:193–8.
26 Park KS, Park JH, Song YW. Inhibitory NKG2A and activating NKG2D and NKG2C natural killer cell receptor genes: susceptibility for rheumatoid arthritis. *Tissue Antigens* 2008;72:342–6.
27 Biswas PS, Bhagat G, Pernis AB. Irf4 and its regulators: evolving insights into the pathogenesis of inflammatory arthritis? *Immunol Rev* 2010;233:79–96.
28 Yao S, Buzo BF, Pham D, *et al*. Interferon regulatory factor 4 sustains CD8(+) T cell expansion and effector differentiation. *Immunity* 2013;39:833–45.
29 Alanärä T, Karstila K, Moilanen T, *et al*. Expression of IL-10 family cytokines in rheumatoid arthritis: elevated levels of IL-19 in the joints. *Scand J Rheumatol* 2010;39:118–26.
30 Crawford A, Angelosanto JM, Nadwodny KL, *et al*. A role for the chemokine RANTES in regulating CD8 T cell responses during chronic viral infection. *PLoS Pathog* 2011;7:e1002098.
31 Kumar BV, Ma W, Miron M, *et al*. Human tissue-resident memory T cells are defined by core transcriptional and functional signatures in lymphoid and mucosal sites. *Cell Rep* 2017;20:2921–34.
32 Frost EL, Kersh AE, Evavold BD, *et al*. Cutting edge: resident memory CD8 T cells express high-affinity TCRs. *J Immunol* 2015;195:3520–4.
33 Petrelli A, van Wijk F. CD8(+) T cells in human autoimmune arthritis: the unusual suspects. *Nat Rev Rheumatol* 2016;12:421–8.
34 Wong MT, Ong DEH, Lim FSH, *et al*. A high-dimensional atlas of human T cell diversity reveals tissue-specific trafficking and cytokine signatures. *Immunity* 2016;45:442–56.

CLINICAL SCIENCE

Vasodilators and low-dose acetylsalicylic acid are associated with a lower incidence of distinct primary myocardial disease manifestations in systemic sclerosis: results of the DeSScipher inception cohort study

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ABSTRACT

Objectives To investigate the influence of vasodilator drugs on the occurrence of features depending on myocardial ischaemia/fibrosis (ventricular arrhythmias, Q waves, cardiac blocks, pacemaker implantation, left ventricular ejection fraction (LVEF) <55%, and/or congestive heart failure and sudden cardiac death) in systemic sclerosis (SSc).

Methods 601 patients with SSc were enrolled from 1 December 2012 to 30 November 2015 and had a second visit 0.5–4 years apart. 153 received no vasodilators; 448 received vasodilator therapy (ie, calcium channel blockers and/or ACE inhibitors or angiotensin II receptor blockers or combinations of them), 89 of them being also treated with either endothelin receptor antagonists or PDE5 inhibitors or prostanoids. Associations between the occurrence of myocardial disease manifestations and any demographic, disease and therapeutic aspect were investigated by Cox regression analysis. A Cox frailty survival model with centre of enrolment as random effect was performed.

Results During 914 follow-up patient-years, 12 ventricular arrhythmias, 5 Q waves, 40 cardiac blocks, 6 pacemaker implantations and 19 reduced LVEF and/or congestive heart failure (CHF) occurred. In multivariate Cox regression analysis, vasodilator therapy was associated with a lower incidence of ventricular arrhythmias (p=0.03); low-dose acetylsalicylic acid (ASA) with a lower incidence of cardiac blocks and/or Q waves and/or pacemaker implantation (p=0.02); active disease with a higher incidence of LVEF <55% and/or CHF and cardiac blocks and/or Q waves and/or pacemaker implantation (p=0.05).

Conclusions The present study might suggest a preventative effect on the occurrence of distinct myocardial manifestations by vasodilator therapy and low-dose ASA.

Key messages

What is already known about this subject?

- Short-term studies have underlined a beneficial effect of calcium channel blockers and other vasodilators including ACE inhibitors on cardiac vascularization and function in systemic sclerosis (SSc).
- However, the role of vasodilative agents in the prevention of primary myocardial disease has not yet been defined.

What does this study add?

- This is the first observational, long-term study to investigate the association between vasodilator use and the occurrence of disease manifestations probably or potentially related to myocardial fibrosis.
- Associations between vasodilators and low-dose acetylsalicylic acid (ASA) use and a decrease in the incidence of distinct manifestations have emerged.

How might this impact on clinical practice or future developments?

- Our study could prompt clinicians to consider adding a vasodilator agent and low-dose ASA to the therapeutic strategy of any patient with SSc.

INTRODUCTION

Myocardial disease occurring in patients with systemic sclerosis (SSc) is classically subdivided into primary and secondary, depending the

absence or, respectively, coexistence of pulmonary and/or renal involvement.[1–3]

Primary myocardial disease is morphologically characterised by vasculopathy of small arteries and biventricular patchy myocardial fibrosis which presents a strong association with contraction band necrosis, suggesting the implication of ischaemia–reperfusion events, i.e., a myocardial Raynaud's phenomenon (RP).[4] In this regard, short-term trials and retrospective observational studies have underlined a beneficial effect of calcium channel blockers (CCBs) and ACE inhibitors (ACEinh) on cardiac vascularization and function.[5–11]

By now, the role of vasodilator agents in the prevention of primary myocardial disease in SSc has not yet been clarified. In order to define the management of SSc, a project named DeSScipher (to decipher the optimal treatment of SSc) was submitted to and funded by the European Community (FP7-HEALTH no. 305495). Here, we report the results of the subproject devoted to investigate the influence of vasodilator drugs on the occurrence of primary myocardial complications, specifically those associated with a poor prognosis, i.e. ventricular arrhythmias, Q waves, cardiac blocks, pacemaker implantation, reduced left ventricular ejection fraction (LVEF), congestive heart failure (CHF) and sudden cardiac death.[1–3 12–14]

METHODS

Patients and study design

Patients fulfilling the American College of Rheumatology/European League Against Rheumatism criteria for SSc,[15] consecutively admitted to 20 DeSScipher-EUSTAR centres from 1 December 2012 to 30 November 2015, were enrolled, according to local ethical requirements.

Patients with the following characteristics were excluded: significant pulmonary parenchymal (FVC and/or diffusing lung capacity for CO <70%) or vascular involvement (estimated systolic pulmonary arterial pressure >40 mm Hg), intestinal involvement (malabsorption syndrome or paralytic ileus) or renal involvement (serum creatinine level >1.2 mg/dL and/or dialysis or previous scleroderma renal crisis (SRC)), or any sign/symptom/ECG finding of myocardial disease, basal pulmonary rales and/or leg oedema indicative of CHF.

Patients enrolled in the study were investigated according to the DeSScipher protocol, shared by all participating centres. In particular, they were assessed for the items listed in the European Scleroderma Trials and Research group (EUSTAR) protocol,[16] including European Scleroderma Study Group (EScSG) activity criteria.[17] Moreover, as far as myocardial disease is concerned, each patient was examined at baseline by means of medical history, clinical examination, ECG, Holter ECG and B-mode echocardiography at baseline, and was reassessed every 3 months with respect to medical history, clinical examination and ECG, and every 6 months by Holter ECG and B-mode echocardiography until the end of each follow-up year. According to local policies, patients had to undergo either standard vasodilator therapy, (CCB such as nifedipine up to 60 mg/qd or comparable doses of other drugs of the same class and/or ACEinh such as captopril up to 100 mg/qd) or no vasodilator therapy. Two hundred fifty patients per arm had to be enrolled. Despite the strictly defined entry criteria, two major protocol deviations occurred. As far as myocardial disease is concerned, some patients with baseline myocardial disease were enrolled. As far as treatment is concerned, 63 patients undergoing AgIIrb±CCB treatment were enrolled. Because of the influence on the same pathophysiological pathway, they were considered in the same class of ACEinh and included in the arm of those treated with CCB and/or ACEinh, with the whole group being referred to as standard vasodilator therapy. Moreover, some patients treated with targeted vasodilator drugs (ie, prostanoids or endothelin receptor antagonists or phosphodiesterase type 5 inhibitors) were enrolled. Out of them, those undergoing standard vasodilator therapy were included in the same arm which was referred to as vasodilator therapy; those treated with targeted vasodilator drugs only were excluded because of the intermittent drug regimen in most of them. The role of other features potentially influencing the occurrence of cardiac disease during follow-up was also investigated, i.e. diffuse subset, disease activity, digital ulcers, traditional risk factors such as sex, cigarette smoking, systemic arterial hypertension, hypercholesterolemia and drugs including ongoing corticosteroids±immunosuppressive therapy and low-dose acetylsalicylic acid (ASA) (≤325 mg daily).[1–3 18–21]

Follow-up and outcome measures

The new occurrence of ventricular arrhythmias as manifestations indicative of myocardial ischaemia, that of Q waves and/or cardiac blocks and/or pacemaker implantation as manifestations indicative of myocardial fibrosis or a therapeutic intervention promoted by it, and that of LVEF <55% and/or CHF, as manifestations of evolved disease, were investigated.[1–4]

Finally, the incidence of withdrawal from treatment was used as safety endpoint.

Statistical analysis

StataMP V.13, IBM SPSS V.24.0 and MedCalc V.11.3 for Windows software were used for statistical analyses. Continuous data were expressed as means and SD and compared by Student's t-test. The predictivity of myocardial disease occurrence by each distinct feature was assessed by Cox proportional hazard regression models. The number of covariates to be included in the multivariate model was defined by using a ratio of cases per covariate in the size of 10.[22] Moreover, in order to address the potential influence of different therapeutic strategies by clinician from different centres, we carried out a Cox frailty survival model with centre of enrolment as random effect.[23] Statistical significance was set at p value <0.05.

RESULTS

Patients

From 1 December 2012 to 30 November 2015, a total of 654 patients with SSc, with a mean age of 56±13 years and a disease duration from the first non-RP manifestation ranging from 0.5 to 61 years (mean 10±9 SD), were enrolled in the study and followed up for at least 6 months.

One hundred fifty-three patients did not undergo any vasodilator; 448 were prescribed vasodilators including 89 treated with either prostanoids and/or endothelin receptor antagonists and/or phosphodiesterase inhibitors. The 43 patients treated only with targeted vasodilators were excluded.

Table 1 shows the demographic, clinical, serological and therapeutic features as assessed at enrolment and during follow-up as far as the drug regimen is concerned, in the remaining 601 patients subdivided according to the therapeutic subgroup. Given the presence of missed items, the prevalence of each feature has been calculated among patients in whom it had been underlined. Hypercholesterolemia was noticed in few patients; no data were available for statin use.

With respect to patients undergoing no vasodilators, those treated with vasodilator therapy resulted to be more frequently

Table 1 Demographic, clinical, serological and therapeutic features of the 601 patients with SSc subdivided according to the treatment subgroup

FEATURES	No vasodilators (n=153)	Vasodilator therapy (n=448)	P values
Female Sex	134/153 (87%)	395/448 (88%)	0.88
Age (mean±SD) years	55±14	57±13	0.21
Age≥50 years	95/153 (62%)	332/448 (74%)	**0.005**
Early disease	53/145 (36%)	148/428 (35%)	0.69
Clinical subset			
Limited cutaneous	124 (81%)	348 (78%)	0.42
Diffuse cutaneous	29 (19%)	100 (22%)	0.42
Serological subset			
Antinuclear antibodies (ANA) positive	134/137 (98%)	400/410 (98%)	0.99
Anti-centromere (ACA) positive	64/137 (47%)	163/410 (42%)	0.16
Anti-Scl-70 positive	39/130 (30%)	136/388 (35%)	0.33
Further aspects			
Baseline Myocardial Disease	18/123 (15%)	56/353 (16%)	0.27
Digital ulcers (ever)	50/149 (33%)	168/437 (38%)	0.33
Tendon friction rubs	7/148 (5%)	20/432 (5%)	0.99
Arthritis	18/153 (12%)	52/442 (12%)	0.99
EScSG activity index≥3	13/153 (8%)	41/448 (9%)	0.87
Systemic arterial hypertension	0/153	139/448 (31%)	**<0.001**
Cigarette smoking ever	39/127 (31%)	88/350 (25%)	0.24
Hypercholesterolemia	0/7	0/23	–
Ongoing corticosteroids±immunosuppressors	44/145 (30%)	215/408 (53%)	**<0.001**
Ongoing low dose acetylsalicylic acid	28/146 (19%)	205/377 (54%)	**<0.001**

EScSG, European Scleroderma Study Group; SSc, systemic sclerosis.

aged ≥50 years (p=0.005), affected by systemic arterial hypertension (p<0.001) and to be undergoing in a greater percentage corticosteroids±immunosuppressors (p<0.001) and low-dose ASA (p<0.001), i.e. they presented a greater prevalence of disease features potentially associated with a worse cardiovascular outcome.

Occurrence of myocardial disease features during follow-up

During 914 follow-up patient-years, ventricular arrhythmias developed in 12 patients; Q waves developed in 5, cardiac blocks in 40 and a pacemaker was implanted in 6; 15 developed a LVEF <55% and/or a CHF. No patient underwent a sudden cardiac death. In univariate analysis, vasodilator therapy resulted to be associated with a nearly significant lower occurrence of ventricular arrhythmias (7/285 events (2%) occurring during 709 patient-years as compared with 5/97 (5%) during 206 patient-years in those not treated with any vasodilator) (HR 0.33, 95% CI 0.10 to 104; p=0.060); low-dose ASA with a reduced incidence of Q waves and/or cardiac blocks and/or pacemaker implantation (17/161 events (10%) occurring during 434 patient-years as compared with 29/182 (16%) during 383 patient-years in those not treated with ASA) (HR 0.41, 95% CI 1.98 to 16.56; p=0.004). On the contrary, male sex (HR 5.73, 95% CI 1.98 to 16.56; p=0.002) and an EScSG activity index ≥3 at the enrolment into the study (HR 4.83, 95% CI 1.52 to 15.34; p=0.008) were found to predict the development of a LVEF <55% and/or CHF.

In order to perform the multivariate Cox regression analysis, five covariates were selected because of their potential value in influencing the occurrence of cardiac events over time. Several tentatives were performed by selecting, according to the number of events that occurred, all the five covariates that were considered for cardiac blocks and/or Q waves and/or pacemaker implantation; two covariates for ventricular arrhythmias; two covariates for LVEF <55% and or CHF. Table 2 shows the results of this approach: vasodilator therapy resulted to be associated with a lower incidence of ventricular arrhythmias (HR 0.28, 95% CI 0.09 to 0.90; p=0.03); low-dose ASA with a lower incidence of cardiac blocks and/or Q waves and/or pacemaker implantation (HR 0.46, 95% CI 0.24 to 0.87; p=0.02); an EScSG activity index ≥3 with a higher occurrence of a LVEF <55% and/or CHF (HR 3.71, 95% CI 1.02 to 13.42; p=0.05) and cardiac blocks and/or Q waves and/or pacemaker implantation (HR 2.15, 95% CI 1.00 to 4.63; p=0.05). Moreover, an unfavourable role of male sex emerged.

Finally, since therapeutic strategies can differ among distinct centres, a Cox frailty survival model with centre of enrolment as random effect was performed (table 3). The associations of vasodilators, low-dose ASA and an EScSG activity index ≥3 were confirmed.

Withdrawal from vasodilator therapy and low-dose ASA

Ninety-three out of the 448 patients undergoing vasodilator therapy withdrew from treatment: 15 treated with CCB alone, 3 treated with ACEi or AngIIrb alone, none with CCB+ACEi or AngIIrb reaching an incidence of 2.1/100 patient-years, 31 treated with endothelin receptor antagonists, 19 treated with phosphodiesterase type 5 inhibitors and 25 treated with prostanoids reaching an incidence of 32/100 patient-years. Moreover, 16 of the 230 patients undergoing ASA withdrew from treatment reaching an incidence rate of 3/100 patient-years.

DISCUSSION

To the best of our knowledge, this is the first observational, prospective, long-term study to investigate the association

Table 2 Associations detected for each outcome measure by multivariate Cox regression analysis

COVARIATES	Cardiac Blocks and/or Q waves and/or Pacemaker Implantation n.events=49* HR; 95% CI; p value	Ventricular Arrhytmias n.events=12 HR; 95% CI; p value	LVEF≤55% and/or CHF n.events=19 HR; 95% CI; p value
Male sex			5.70; 2.20–18.9;<0.001
Age≥50			
EScSG activity index≥3	2.15; 1.00–4.63; 0.05		3.71; 1.02–13.42; 0.05
Low dose ASA	0.46; 0.24–0.87; 0.02		
Vasodilators		0.28; 0.09–0.90; 0.03	

*Two patients developed 2 events (1 cardiac block and pacemaker implantation; 1 cardiac block and Q wave).
ASA, acetylsalicylic acid; CHF, congestive heart failure; EScSG, European Scleroderma Study Group; LVEF, left ventricular ejection fraction.

Table 3 Associations detected for each outcome measure by Cox frailty analysis

COVARIATES	Cardiac Blocks and/or Q waves and/or Pacemaker Implantation n.events=49* HR; 95% CI; p value	Ventricular Arrhytmias n.events=12 HR; 95% CI; p value	LVEF≤55% and/or CHF n.events=19 HR; 95% CI; p value
EScSG activity index≥3	2.12; 0.98–4.57; 0.06		3.79; 1.04–13.82; 0.04
Low dose ASA	0.53; 0.26–1.08; 0.08		
Vasodilators		0.32; 0.10–1.02; 0.05	

*Two patients developed 2 events (1 cardiac block and pacemaker implantation; 1 cardiac block and Q wave).
ASA, acetylsalicylic acid; CHF, congestive heart failure; EScSG, European Scleroderma Study Group; LVEF, left ventricular ejection fraction.

between vasodilator therapy and the occurrence of disease manifestations probably or potentially related to myocardial ischaemia (ventricular arrhythmias), fibrosis (Q waves and/or cardiac blocks and/or pacemaker implantation) or both (reduced LVEF, CHF and sudden cardiac death). Actually, as far as the influence of vasodilator therapy on myocardial disease is concerned, Kazzam *et al*[24] only investigated diastolic and systolic function in 22 patients with SSc receiving captopril treatment (1.3 mg/kg daily) for 11–15 months. These authors found an increase in LVEF and a decrease in isovolumic relaxation time, indicating an improved left ventricular filling, but did not consider any of the features assessed in our study.

In order to address the aim of the study, we also investigated the association between the occurrence of the investigated manifestations and demographic, disease and different therapeutic aspects potentially involved in SSc cardiac disease.[1–3 18–21 25 26] After excluding any bias deriving from potential differences in the treatment policies among the distinct centres involved in the study, vasodilators were found to be associated with a lower incidence of ventricular arrhythmias; low-dose ASA with a nearly significant, lower incidence of cardiac blocks and/or Q waves and/or pacemaker implantation; and active disease, as defined by an EScSG activity index ≥3 at enrolment, with a higher incidence of a reduced LVEF and/or CHF.

We undertook our prospective study because of the commonly shared opinion on the implication of ischaemia/reperfusion events in the induction of myocardial fibrosis in SSc,[1–4] as well as the evidence emerged by short-term trials and retrospective observational studies suggesting a beneficial effect of vasodilators on cardiac vascularization and function in the disease.[5–11] We could not confirm the retrospectively detected association between vasodilator use and a preserved LVEF,[10] and neither did we detect any association between vasodilators and a reduced incidence of cardiac blocks and/or Q waves and/or pacemaker implantation, which are distinct manifestations of myocardial fibrosis or of a therapeutic intervention promoted by its consequences.[12] Nevertheless, we pointed out an association between vasodilators and a lower incidence of ventricular arrhythmias, which likely depend on ischaemic processes.[13 14] This result deserves to be underlined since ventricular arrhythmias have long been known to be associated with a poor prognosis in SSc.[13 14 21]

Investigating different aspects potentially associated with the incidence of cardiac events, we happened to point out an unexpected protective role of low-dose ASA and an unfavourable prognostic role of the EScSG activity index. Low-dose ASA is currently prescribed to patients with a high risk of coronary artery disease.[26] Moreover, it has been recently reported to be associated with a decrease in the occurrence of major cardiovascular events (ie, myocardial infarction and stroke) in patients with systemic lupus erythematosus[27 28] and rheumatoid arthritis.[29] It might, therefore, be hypothesised that the associations detected between the reduction in the occurrence of distinct cardiac events and low-dose ASA do not depend on a potential protective effect on small intramyocardial coronary artery disease. Nevertheless, platelet activation has been reported to play a role of both vascular and fibrotic manifestations of SSc.[30] Moreover, markers of platelet activation have long been known to be responsive to antiplatelet therapy.[31] As far as EScSG activity index, Nevskaya *et al*[19] have recently reported a predictive role of the severity of heart disease accrual by its adjusted mean over 3 years. Our results seem to indicate that even a single evaluation might have a

prognostic meaning. This result prospects that achieving an EScSG activity index ≥3 might be a target at least in clinical practice.

In the original design of our study, we had envisaged three treatment arms, that is, CCB, ACEinh and CCB+ACEinh. Actually, we had not considered the possibility of a patient with SSc who is not prescribed any vasodilator drug. This does not appear to be the case, our data on prospectively enrolled patients from 20 EUSTAR centres confirming those reported by the German SSc network highlighting the high percentage of patients with SSc who do not receive any vasoactive therapy.[32]

The observational nature of the study does not allow to prospect any cause/effect relationship. Well-designed randomised controlled trials (RCTs) are needed to either support or refuse any therapeutic role of vasodilators and low-dose ASA in the prevention of myocardial disease in patients with SSc. In addition, the variable, non-standardised length of follow-up represents a limitation that, however, appears to be balanced by the long cumulative duration of follow-up (914 patient-years) and its median time (2.4 years).

Vascular disease has long been considered a pathological hallmark of SSc.[33] The low incidence of withdrawals from vasodilator therapy and low-dose ASA in our study, even if waiting for the results of properly designed RCTs, might suggest to consider adding low-dose ASA and a vasodilator agent to the therapeutic strategy of any patient with SSc. In that regard, given the apparent protective role of CCB for SRC on one side,[34] and the increased risk of death associated with previous exposure to ACEinh in patients developing a SRC,[35] it appears advisable to start with a CCB and to add an ACEinh in patients with diastolic dysfunction for the known effect of the latter on ventricular filling.[24]

In conclusion, our prospective, observational study suggests a protective role of vasodilators and low-dose ASA on distinct manifestations of SSc myocardial disease and prospects the opportunity to conduct well-designed RCTs on both therapeutic strategies.

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REFERENCES

1. Kahan A, Allanore Y. Primary myocardial involvement in systemic sclerosis. *Rheumatology* 2006;45(Suppl 4):iv14–17.
2. Kahan A, Coghlan G, McLaughlin V. Cardiac complications of systemic sclerosis. *Rheumatology* 2006;48(Suppl 3):iii45–8.
3. Parks JL, Taylor MH, Parks LP, *et al*. Systemic sclerosis and the heart. *Rheum Dis Clin North Am* 2014;40:87–102.
4. Follansbee WP, Miller TR, Curtiss EI, *et al*. A controlled clinicopathologic study of myocardial fibrosis in systemic sclerosis (scleroderma). *J Rheumatol* 1990;17:656–62.
5. Kahan A, Devaux JY, Amor B, *et al*. Nifedipine and thallium-201 myocardial perfusion in progressive systemic sclerosis. *N Engl J Med* 1986;314:1397–402.
6. Kahan A, Devaux JY, Amor B, *et al*. Nicardipine improves myocardial perfusion in systemic sclerosis. *J Rheumatol* 1988;15:1395–400.
7. Kahan A, Devaux JY, Amor B, *et al*. Pharmacodynamic effect of nicardipine on left ventricular function in systemic sclerosis. *J Cardiovasc Pharmacol* 1990;15:249–53.
8. Kahan A, Devaux JY, Amor B, *et al*. The effect of captopril on thallium 201 myocardial perfusion in systemic sclerosis. *Clin Pharmacol Ther* 1990;47:483–9.
9. Duboc D, Kahan A, Maziere B, *et al*. The effect of nifedipine on myocardial perfusion and metabolism in systemic sclerosis. A positron emission tomographic study. *Arthritis & Rheumatism* 1991;34:198–203.
10. Allanore Y, Meune C, Vonk MC, *et al*. Prevalence and factors associated with left ventricular dysfunction in the EULAR Scleroderma Trial and Research group (EUSTAR) database of patients with systemic sclerosis. *Ann Rheum Dis* 2010;69:218–21.
11. Lee S-W, Choi E-Y, Jung SY, *et al*. E/E' ratio is more sensitive than E/A ratio for detection of left ventricular diastolic dysfunction in patients with systemic sclerosis. *Clin Exp Rheumatol* 2010;28(Suppl 58):S12–17.
12. Follansbee WP, Curtiss EI, Rahko PS, *et al*. The electrocardiogram in systemic sclerosis (scleroderma). Study of 102 consecutive cases with functional correlations and review of the literature. *Am J Med* 1985;79:183–92.
13. Kostis JB, Seibold JR, Turkevich D, *et al*. Prognostic importance of cardiac arrhythmias in systemic sclerosis. *Am J Med* 1988;84:1007–15.
14. Vacca A, Meune C, Gordon J, *et al*. Scleroderma Clinical Trial Consortium Cardiac Subcommittee. Cardiac arrhythmias and conduction defects in systemic sclerosis. *Rheumatology* 2014;53:1172–7.
15. Van den Hoogen F, Khanna D, Fransen J, *et al*. Classification criteria for systemic sclerosis: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis Rheum* 2013;2013:2737–47.

16. Walker UA, Tyndall A, Czirják L, *et al*. Clinical risk assessment of organ manifestations in systemic sclerosis: a report from the EULAR Scleroderma Trials and Research group database. *Ann Rheum Dis* 2007;66:754–63.
17. Valentini G, Bencivelli W, Bombardieri S, *et al*. European Scleroderma Study Group to define disease activity criteria for systemic sclerosis. III. Assessment of the construct validity of the preliminary activity criteria. *Ann Rheum Dis* 2003;62:901–3.
18. Steen VD, Medsger TA. Severe organ involvement in systemic sclerosis with diffuse scleroderma. *Arthritis Rheum* 2000;43:2437–44.
19. Nevskaya T, Baron M, Pope JE. Canadian Scleroderma Research Group. Predictive value of European Scleroderma Group Activity Index in an early scleroderma cohort. *Rheumatology* 2017;56:1111–22.
20. Mihai C, Landewé R, van der Heijde D, *et al*. Digital ulcers predict a worse disease course in patients with systemic sclerosis. *Ann Rheum Dis* 2016;75:681–6.
21. Tyndall AJ, Bannert B, Vonk M, *et al*. Causes and risk factors for death in systemic sclerosis: a study from the EULAR Scleroderma Trials and Research (EUSTAR) database. *Ann Rheum Dis* 2010;69:1809–15.
22. Lydersen S. Statistical review: frequently given comments. *Ann Rheum Dis* 2015;74:323–5.
23. Karagrigoriou A. Frailty models in survival analysis. *J Appl Stat* 2011;38:2988–9.
24. Kazzam E, CAIDAHL K, HÄLLGREN R, *et al*. Non-Invasive evaluation of long-term cardiac effects of captopril in systemic sclerosis. *J Intern Med* 1991;230:203–12.
25. Elhai M, Meune C, Boubaya M, *et al*. Mapping and predicting mortality from systemic sclerosis. *Ann Rheum Dis* 2017;76:1897–905.
26. Piepoli MF, Hoes AW, Agewall S, *et al*. 2016 European Guidelines on cardiovascular disease prevention in clinical practice: The Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts) developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). *Eur Heart J* 2016;37:2315–81.
27. Iudici M, Fasano S, Gabriele Falcone L, *et al*. Low-dose aspirin as primary prophylaxis for cardiovascular events in systemic lupus erythematosus: a long-term retrospective cohort study. *Rheumatology* 2016;55:1623–30.
28. Fasano S, Pierro L, Pantano I, *et al*. Longterm hydroxychloroquine therapy and low-dose aspirin may have an additive effectiveness in the primary prevention of cardiovascular events in patients with systemic lupus erythematosus. *J Rheumatol* 2017;44:1032–8.
29. Iacono D, Fasano S, Pantano I, *et al*. Low-dose aspirin as primary prophylaxis for cardiovascular events in rheumatoid arthritis: an Italian multicentre retrospective study. *Cardiol Res Pract* 2019;2748035.
30. Ntelis K, Solomou EE, Sakkas L, *et al*. The role of platelets in autoimmunity, vasculopathy, and fibrosis: implications for systemic sclerosis. *Semin Arthritis Rheum* 2017;47:409–17.
31. Kahaleh MB, Osborn I, LeRoy EC. Elevated levels of circulating platelet aggregates and beta-thromboglobulin in scleroderma. *Ann Intern Med* 1982;96:610–3.
32. Moinzadeh P, Riemekasten G, Siegert E, *et al*. German Network for Systemic Scleroderma. Vasoactive therapy in systemic sclerosis: real-life therapeutic practice in more than 3000 patients. *J Rheumatol* 2016;43:66–74.
33. Matucci-Cerinic M, Kahaleh B, Wigley FM. Review: Evidence that systemic sclerosis is a vascular disease. *Arthritis Rheum* 2013;65:1953–62.
34. Montanelli G, Beretta L, Santaniello A, *et al*. Effect of dihydropyridine calcium channel blockers and glucocorticoids on the prevention and development of scleroderma renal crisis in an Italian case series. *Clin Exp Rheumatol* 2013;31(Suppl 76):135–9.
35. Hudson M, Baron M, Tatibouet S, *et al*. Exposure to ACE inhibitors prior to the onset of scleroderma renal crisis—results from the International Scleroderma Renal Crisis Survey. *Semin Arthritis Rheum* 2014;43:666–72.

TRANSLATIONAL SCIENCE

Interferon regulatory factor 7 (IRF7) represents a link between inflammation and fibrosis in the pathogenesis of systemic sclerosis

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ABSTRACT

Objectives There is considerable evidence that implicates dysregulation of type I interferon signalling (or type I IFN signature) in the pathogenesis of systemic sclerosis (SSc). Interferon regulatory factor 7 (IRF7) has been recognised as a master regulator of type I IFN signalling. The objective of this study was to elucidate the role of IRF7 in dermal fibrosis and SSc pathogenesis.

Methods SSc and healthy control skin biopsies were investigated to determine IRF7 expression and activation. The role of IRF7 in fibrosis was investigated using IRF7 knockout (KO) mice in the bleomycin-induced and TSK/+mouse models. In vitro experiments with dermal fibroblasts from patients with SSc and healthy controls were performed.

Results IRF7 expression was significantly upregulated and activated in SSc skin tissue and explanted SSc dermal fibroblasts compared with unaffected, matched controls. Moreover, IRF7 expression was stimulated by IFN-α in dermal fibroblasts. Importantly, IRF7 co-immunoprecipitated with Smad3, a key mediator of transforming growth factor (TGF)-β signalling, and IRF7 knockdown reduced profibrotic factors in SSc fibroblasts. IRF7 KO mice demonstrated attenuated dermal fibrosis and inflammation compared with wild-type mice in response to bleomycin. Specifically, hydroxyproline content, dermal thickness as well as Col1a2, ACTA2 and interleukin-6 mRNA levels were significantly attenuated in IRF7 KO mice skin tissue. Furthermore, IRF7 KO in TSK/+mice attenuated hydroxyproline content, subcutaneous hypodermal thickness, Col1a2 mRNA as well as α-smooth muscle actin and fibronectin expression.

Conclusions IRF7 is upregulated in SSc skin, interacts with Smad3 and potentiates TGF-β-mediated fibrosis, and therefore may represent a promising therapeutic target in SSc.

Key messages

What is already known about this subject?
- Patients with systemic sclerosis (SSc) have a prominent interferon (IFN) signature.
- Interferon regulatory factor 7 (IRF7) is predicted to be a top regulator of SSc skin gene transcript signature based on previous global gene expression studies.

What does this study add?
- IRF7 is overexpressed in SSc fibrotic skin and interact with Smad3, a prominent member of transforming growth factor-β (TGF-β) signalling pathway in dermal fibroblasts.
- IRF7 knockdown in SSc dermal fibroblasts abrogated TGF-β-induced profibrotic gene expression.
- Knockout of IRF7 ameliorates experimental fibrosis in bleomycin-induced skin fibrosis and TSK/+congenic murine models of SSc.

How might this impact on clinical practice or future developments?
- Our studies indicate that IRF7 can potentiate the TGF-β-mediated fibrosis in dermal fibroblasts and might provide a link between the prominent IFN signature and fibrosis in SSc.

INTRODUCTION

Systemic sclerosis (scleroderma, SSc) is a multisystem autoimmune disease characterised by vasculopathy, fibrosis and immune system dysregulation. The aetiology of SSc is unknown. There are currently no targeted treatment options for the fibrotic complications of SSc, and disease-related mortality remains high.[1] Type I interferons (IFNs) are key regulators of the innate immune system. They modulate immune cell differentiation, proliferation and cytokine production. IFN excess is evident in the blood and skin of a large percentage of patients with SSc.[2 3] The development of SSc has been reported in patients undergoing IFN treatment.[4] Furthermore, a randomised, placebo-controlled trial of subcutaneous IFN-α injection in patients with early SSc showed that treatment with IFN-α resulted in worsening lung function and less improvement in skin fibrosis scores, suggesting a pathologic effect.[5] We first described a prominent transcript pattern of upregulated type I IFN-inducible genes, that is, the IFN signature, in peripheral blood cells from patients with SSc, a finding that has been confirmed by several other groups.[6–8] A more recent study showed that type I IFN-producing plasmacytoid dendritic cells (pDC) and toll-like receptor 8 (TLR8) are involved in the pathogenesis of SSc.[9] However, the exact roles of type I IFN signalling in SSc pathogenesis, including

the mechanisms by which dysregulated type I IFN signalling contribute to fibrosis are unclear.

TGF-β has long been recognised as a key mediator of fibrosis in SSc.[10] In a previous study, IFN-α2b increased TGF-β1 production and secretion in hepatocytes, suggesting a positive cross-talk between IFN and TGF-β signalling through smad2/3 activation.[11] Among IFN signalling pathways, interferon regulatory factors (IRFs) are best characterised as transcriptional regulators of type I IFNs and IFN-inducible genes and play a pivotal role in the regulation of many facets of the innate and adaptive immune response.[12] This family comprises nine members: IRF1, IRF2, IRF3, IRF4 (also known as LSIRF, PIP or ICSAT), IRF5, IRF6, IRF7, IRF8 (also known as ICSBP) and IRF9 (also known as ISGF3γ).[13 14] As transcription factors, IRFs recognise the IFN-stimulated response element (ISRE) in the promoter region of target inflammatory genes including IFN-α, IL-6 and TNF-α.[15–17] Specifically, IRF7 has been recognised as a master regulator of type I IFN signalling. Once phosphorylated, activated IRF7 moves into the nucleus, where it binds to the ISRE site of target genes and leads to secretion of type I IFN and other cytokines.[18] IRF7 also has been identified as a Smad3 interacting protein, a key component of TGF-β signalling for collagen production.[19] Furthermore, we and others have reported that single nucleotide polymorphisms in the *IRF7* gene, including the functional non-synonymous variant rs1131665, are associated with SSc.[20 21] Upregulation of *IRF7* gene expression in peripheral blood cells from patients with SSc has been reported.[6] More importantly, in our large-scale, unbiased, global skin and blood gene expression studies, we found that IRF7 was the top predicted transcription factor responsible for the dysregulated gene expression observed in patients with SSc.[22] Altogether, these data implicate an important role for IRF7 in SSc pathogenesis. However, the specific mechanistic role of IRF7 in the fibrotic complications of SSc is unknown.

In the present study, we investigated the role of IRF7 in SSc skin fibroblasts and in two different murine models, the bleomycin-induced skin fibrosis model and TSK/+congenic skin fibrosis model.

METHODS

Patients with SSc and controls

Patients with SSc and unaffected controls were recruited from the Rheumatology Division at the University of Texas Health Science Center at Houston. Three mm punch biopsies were obtained from the extensor forearm using standard methods. Formalin-fixed paraffin-embedded skin tissue was processed for histology and immunohistochemistry (additional details are included in the online supplementary methods section). Fibroblasts from skin tissue were cultured in Dulbecco's Modified Eagle's Medium with 10% fetal bovine serum and four to six passage fibroblasts were used for experiments.[23] All patients with SSc fulfilled the 2013 classification criteria for systemic sclerosis.[24] Online supplementary tables S1 and S2 show the patient and control characteristics. All study subjects provided written informed consent.

Bleomycin-induced skin fibrosis mouse model

IRF7 knockout (KO) mice congenic on the C57BL/6J background were acquired from Riken Japan (Tokyo, Japan). Female C57BL/6J wild-type mice aged 8 weeks were purchased from Jackson Laboratory (Bar Harbor, Maine, USA). Mice were housed in autoclaved cages and fed sterile food and water. Bleomycin (0.02 units/day) (Teva Parenteral Medicines, Irvine, California, USA) dissolved in phosphate-buffered saline (PBS) or PBS alone was administered by subcutaneous injection to the back skin of mice aged 8 weeks 6 days per week daily for 4 weeks. On day 7 or 28, mice were sacrificed and lesional skin was harvested for hydroxyproline, total RNA, histology and immunohistochemistry.

Statistical analysis

Experimental groups were compared by t-test or Mann-Whitney U test depending on the distribution of residuals. Data are presented as mean±SD or SE of the mean (SEM),[25] when t-test was used. Dot plot with median, as well as IQR was shown if Mann-Whitney U test was used. Two-tailed p values <0.05 were considered to be statistically significant.

More information on material and methods is provided in the online supplementary materials.

RESULTS

Upregulation and activation of IRF7 in patients with SSc

Because previous studies demonstrated that type I IFN signalling was the most prominent signature in peripheral blood cells from SSc,[7] we hypothesised that IRF7 might be upregulated in skin tissue of patient with SSc. Immunohistochemistry confirmed that IRF7 expression was significantly upregulated in SSc skin tissue compared with healthy controls (n=10 per group) (see online supplementary table S1 for demographic and clinical characteristics). Importantly, increased IRF7 phosphorylation was observed in SSc skin compared with healthy controls, indicating increased IRF7 activation (figure 1A). Next, we determined the IRF7-positive fibroblasts and inflammatory cell numbers by morphology (excluding nerve and endothelial cells) in the dermis. IRF7 and phospho-IRF7-positive cell numbers were increased in patients with SSc compared with healthy controls (figure 1A, lower panel). Furthermore, IRF7 and phospho-IRF7 were highly expressed in α-smooth muscle actin (α-SMA)-positive myofibroblasts and CD68-positive macrophages from SSc skin tissues, as evidenced by double immunofluorescent staining (figure 1B). These results demonstrate that IRF7 is upregulated and activated in macrophages and myofibroblasts in the skin of patients with SSc.

IRF7 blockade attenuated fibrotic responses to TGF-β and IFN-α in SSc skin fibroblasts in vitro

Considering the prominent IRF7 protein expression and activation in SSc dermal fibroblasts, and the specific role of fibroblasts in the production of extracellular matrix proteins, we next examined IRF7 expression in cultured fibroblasts from patients with SSc (see online supplementary table S2 for demographic and clinical characteristics). Both IRF7 mRNA and protein levels were significantly upregulated in fibroblasts from patients with SSc compared with age-matched and gender-matched healthy controls by quantitative PCR (qPCR) (figure 2A) and western blot analysis, respectively (figure 2B). To characterise the potential upstream regulators of IRF7 in dermal fibroblasts, cultured fibroblasts were stimulated with IFN-α, as well as nine prominent T helper (Th)1/Th2/Th17 cytokines (IFN-γ, TNF-α, TGF-β1, IL-6, CCL-2, IL-10, IL-13, IL-17A, IL-22), followed by measurement of IRF7 mRNA induction by real-time-qPCR. Of the cytokines tested, IFN-α stimulation resulted in the highest IRF7 expression levels (figure 2C).

Next, we assessed whether IRF7 impacts the TGF-β-induced or IFN-α-induced fibrotic responses in cultured fibroblasts. We pretreated fibroblasts with IRF7 siRNA for 24 hours followed

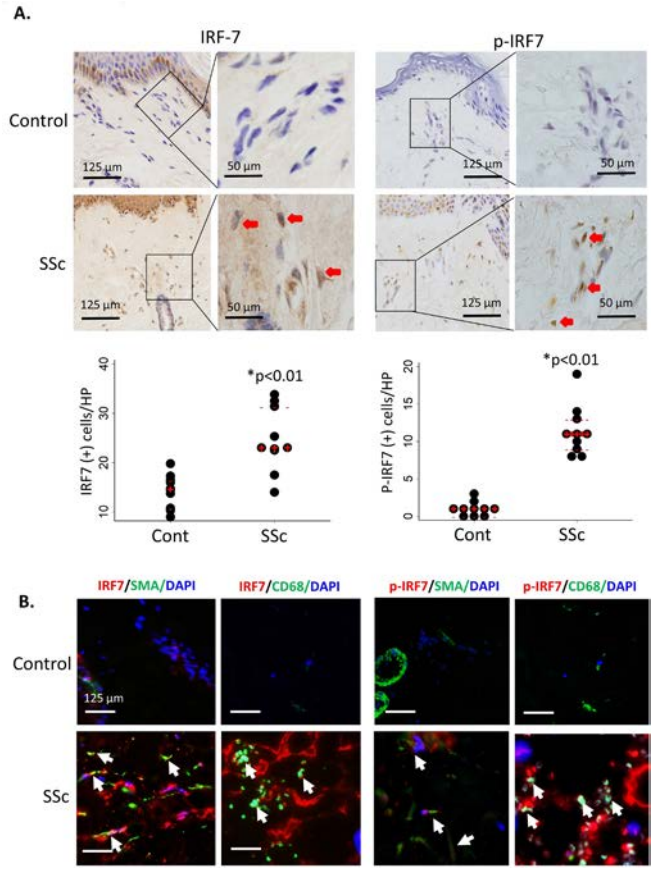

Figure 1 Upregulation and activation of IRF7 in SSc skin tissue. Five-micron thick skin tissue was stained with anti-IRF7 and p-IRF7 antibodies and immunostaining-positive cells (excluding nerve and endothelial cells) number in the dermis was counted in high power field. (A) Representative images. Dot plots with median (red +) and IQR (red –) shows IRF7-positive and p-IRF7-positive cell number in the per high power field. Red arrows: IRF7 or p-IRF7 staining positive cells. n=10 per group; healthy control vs SSc; *p<0.01 (analysed by Mann- Whitney U test). (B) Immunofluorescence analysis using anti-IRF7 or p-IRF7 and α-SMA antibodies for IRF7-positive or p-IRF7-positive myofibroblast; anti-IRF7 or p-IRF7 and CD68 antibodies for IRF7-positive or p-IRF7-positive macrophages in the SSc and normal control skin. Representative images. Original magnifications: x400 magnifications. Scale bar: 125 µm. n=10. Arrows: IRF7 or p-IRF7 with α-SMA, or IRF7 or p-IRF7 with CD68 double staining-positive cells. IRF7, interferon regulatory factor 7; α-SMA, α-smooth muscle actin; SSc, systemic sclerosis.

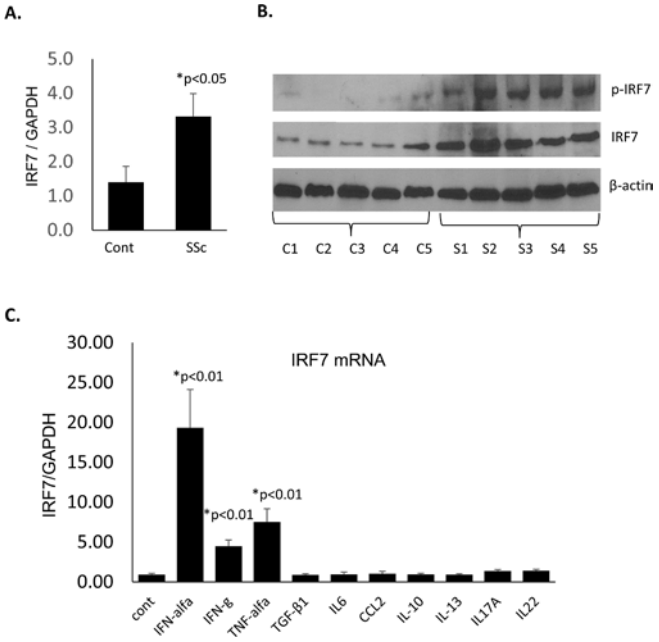

Figure 2 Upregulation and activation of IRF7 in cultured fibroblasts from patients with SSc compared with controls. (A) Early passage (four to six passages) of confluent fibroblasts were harvested and total RNA was isolated for real-time qPCR. IRF7 expression was normalised against GAPDH expression. The bars represent the mean±SD n=10. *P<0.05 (analysed by t-test). (B) Confluent fibroblasts (four to six passages) were harvested and total protein was isolated for western blot analysis, n=5. (C) Healthy control fibroblasts (four to six passages) were treated with the indicated cytokines for 24 hours. Total RNA was extracted and IRF7 mRNA expression was examined by real-time quantitative PCR. The results were normalised with GAPDH mRNA, and IRF7 expression was compared with that in untreated fibroblasts (lane 1); the bars represent the mean±SEM, n=5. *P<0.05 (analysed by t-test). IRF7, interferon regulatory factor 7; SSc, systemic sclerosis.

by stimulation with TGF-β for 24 hours or IFN-α for 72 hours. The 72 hours stimulation was selected for IFN-α treatment to replicate the effects of long-term exposure to IFN-α as present in SSc. First, IRF7 gene knockdown was confirmed by IRF7 mRNA expression (figure 3A). IRF7 siRNA treatment attenuated TGF-β1-induced Col1a1, Col1a2 and FN1 mRNA upregulation, and type I collagen and fibronectin expression in SSc fibroblasts (figure 3A–C). On the other hand, IRF7 siRNA pretreatment did not attenuate TGF-β1-induced Col1a1, Col1a2 and FN1 mRNA expression and type I collagen expression in healthy control fibroblasts (figure 3B,C and see online supplementary figure S1). Furthermore, IRF7 siRNA pretreatment abrogated Col1a2 and FN1 mRNA upregulation induced by long-term IFN-α treatment in healthy control fibroblasts (figure 3D). Cumulatively, these data indicate that in a high IFN-α milieu (control fibroblasts stimulated with IFN-α or SSc fibroblasts), IRF7 knockdown decreases the fibrotic response to TGF-β in fibroblasts.

Next, we investigated the interaction of IRF7 with Smad3, a prominent transcription factor in the TGF-β canonical pathway. IRF7 and Smad3 strongly co-localised in SSc fibroblasts, but this strong co-localisation was not observed in unstimulated control fibroblasts, perhaps in part due to lower expression levels of IRF7 and Smad3 (see online supplementary figure S2). Smad3 and IRF7 co-immunoprecipitated in cultured control and SSc fibroblasts, an effect that was enhanced by treatment with IFN-α or the combination of TGF-β plus IFN-α in SSc fibroblasts (figure 3E). Altogether, these results suggest that IRF7 plays a role in potentiating TGF-β-induced fibrosis, possibly through an interaction with Smad3 in fibroblasts.

IRF7 was upregulated in the skin of bleomycin-treated mice and mediated the fibrotic response to bleomycin

To further understand the role of IRF7 in SSc pathogenesis, we used the bleomycin-treated mouse model. C57BL/6 wild-type mice received subcutaneous injections of bleomycin (or PBS as a control) for 6 days a week for 7 or 28 days, then lesional skin tissue was harvested for analysis. IRF7 expression was significantly upregulated in the skin of bleomycin-treated mice compared with control mice (figure 4A). Furthermore, IRF7 activation, as indicated by phosphorylation, was also increased in the skin of bleomycin-injected mice (figure 4A). IRF7 and

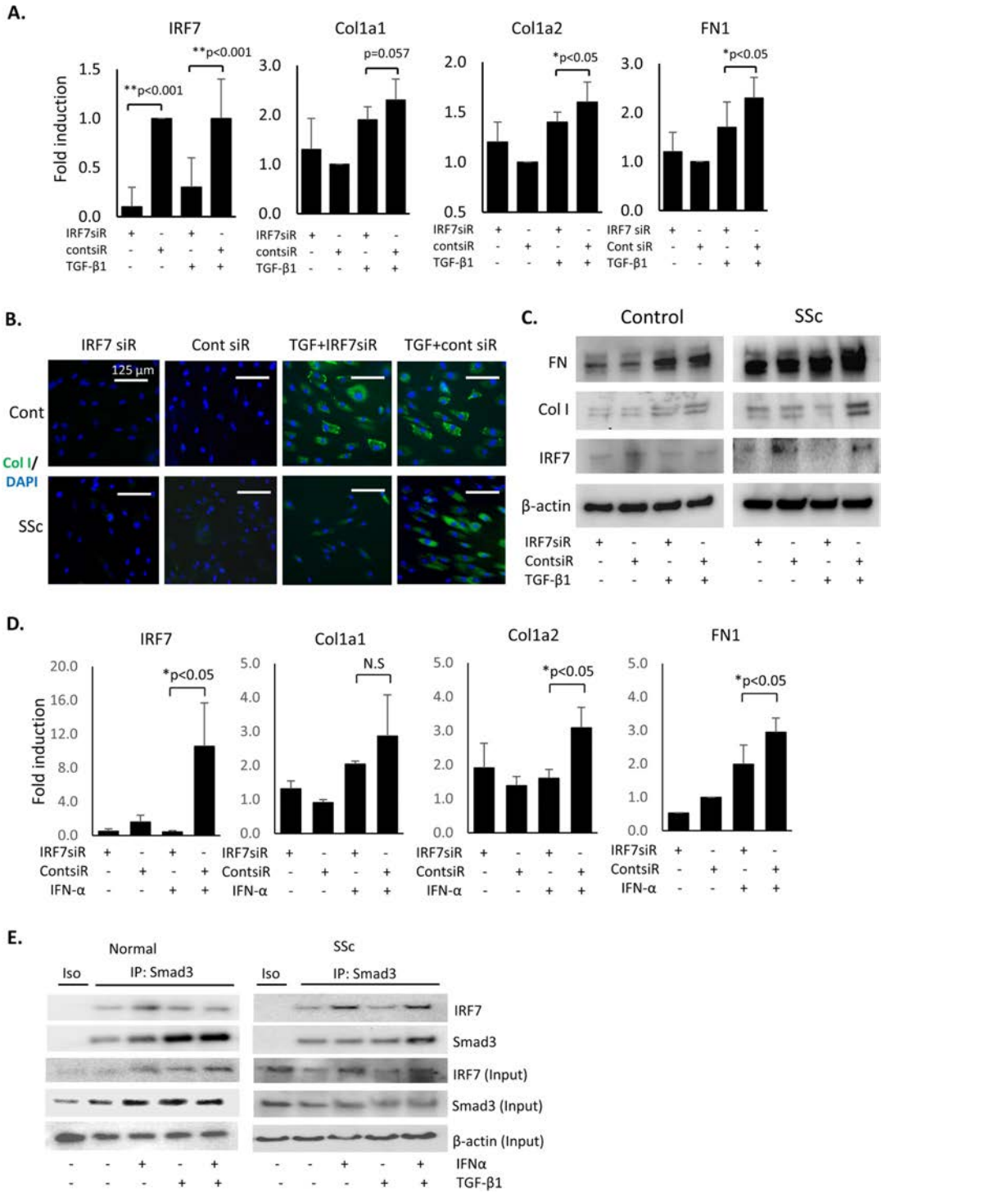

Figure 3 IRF7 siRNA pretreatment blocked Col1a1, Col1a2 and FN1 (encoding fibronectin protein) mRNA expression in SSc dermal fibroblasts. (A) Passages four to six of SSc fibroblasts were pretreated with IRF7 siRNA (20 pmol/mL) for 24 hours followed by treatment with 10 ng/mL of TGF-β1 for 24 hours. Total RNA was extracted and Col1a1, Col1a2, FN1 and IRF7 mRNA expression was examined by real-time quantitative PCR. The results were normalised with GAPDH mRNA; the bars represent the mean±SEM. n=5. **P<0.001; *p<0.05 (analysed by t-test). (B) Immunofluorescence analysis. SSc and healthy control fibroblasts were pretreated with IRF7 or control siRNA (20 pmol/mL) for 24 hours, followed by TGF-β1 treatment (10 ng/mL) for 24 hours, and processed for immunofluorescence staining for type I collagen expression. Representative images. n=5. (C) Passages four to six of healthy control and SSc fibroblasts were pretreated with IRF7 siRNA for 24 hours followed by treatment with 10 ng/mL of TGF-β1 for 24 hours. Fibroblasts were harvested and total protein was isolated for western blot analysis. (D) Early passage (four to six passages) of confluent healthy control fibroblasts were pretreated with IRF7 or control siRNA for 24 hours, followed by treatment with IFN-α (100 ng/mL) for 72 hours. Total RNA was extracted and Col1a1, Col1a2 FN1 (encoding fibronectin) and IRF7 mRNA expression was examined by real-time quantitative PCR. The results, normalised with GAPDH mRNA, represent the means±SEM. n=5. *P<0.05 (analysed by t-test). (E) Control or SSc fibroblasts were cultured with IFN-α (100 ng/mL), TGF-β1 (10 ng/mL) or IFN-α+TGF-β1 for 24 hours. Total protein was isolated and after immunoprecipitation with Smad3 protein, immunoblotting of IRF7 was performed. Representative images. n=3. IRF7, interferon regulatory factor 7; SSc, systemic sclerosis; TGF, transforming growth factor.

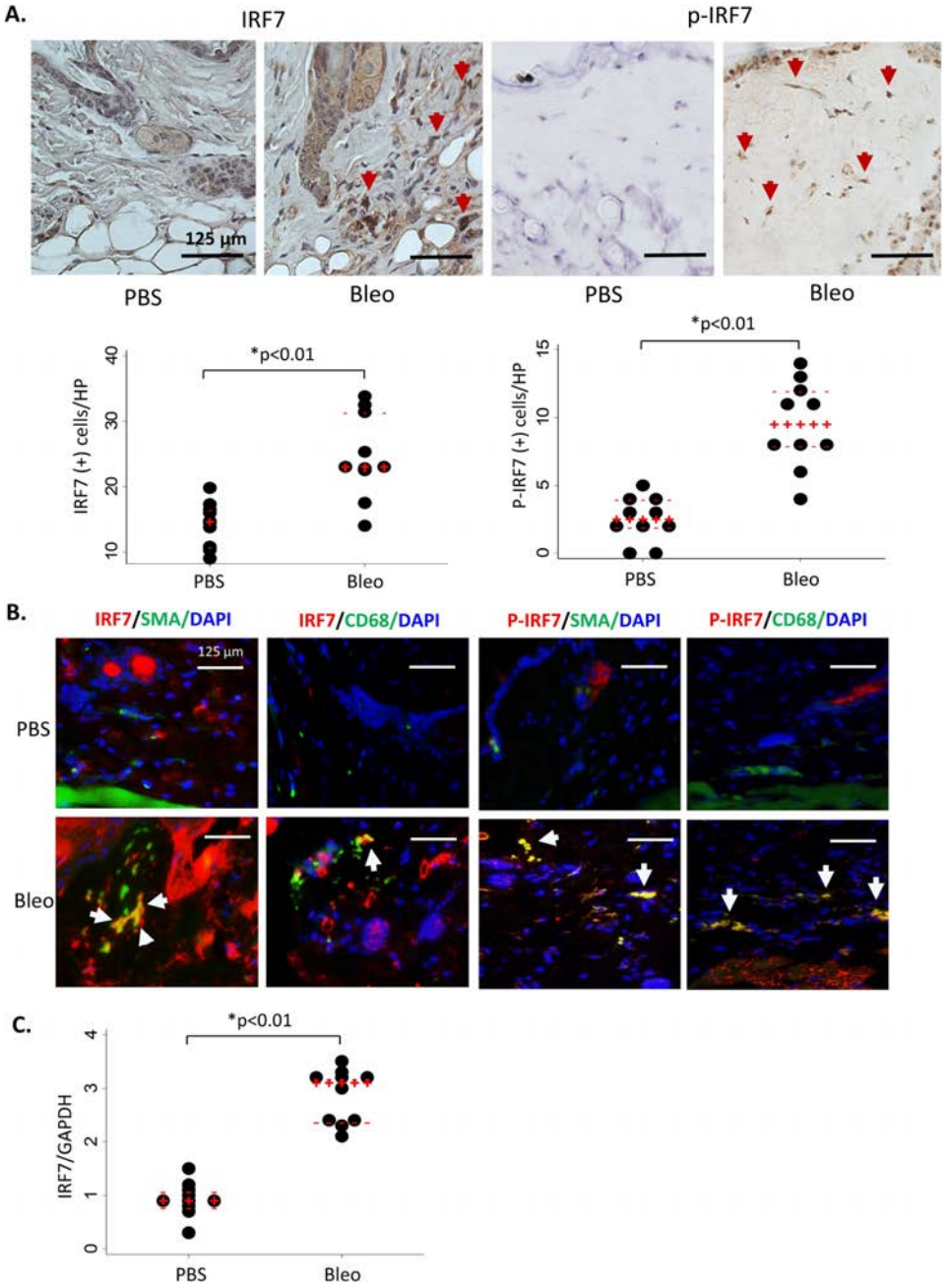

Figure 4 Upregulation and activation of IRF7 in the Bleo-induced skin fibrotic tissues. (A) C57BL/6 wild-type mice received subcutaneous injections of Bleo (6 days per week for 4 weeks) and lesional skin tissue was stained with IRF7 and p-IRF7 antibodies. IRF7-positive or p-IRF7-positive cell (excluding endothelial cells and hair follicle cells) number in the dermis was counted per high power field and analysed. Representative images. Red arrows: IRF7-positive or p-IRF7-positive cells. n=10. Scale bar: 125 µm; PBS vs Bleo; dot plots with median (red +) and IQR (red –) shows IRF7-positive and p-IRF7-positive cell number in the per high power fields.*P<0.01 (analysed by Mann-Whitney U test).(B) Immunofluorescence analysis using anti-IRF7 or p-IRF7 and α-SMA antibodies for IRF7-positive or p-IRF7-positive myofibroblast; anti-IRF7 or p-IRF7 and CD68 antibodies for IRF7-positive or p-IRF7-positive macrophages in the PBS and Bleo-treated lesional skin. Representative images. Original magnifications: x400 magnifications. n=10. Arrows: IRF7 or p-IRF7 with α-SMA, or IRF7 or p-IRF7 with CD68 double staining positive cells. (C) Total RNA from lesional skin was examined for IRF7 mRNA expression by real-time quantitative PCR analysis. The results were normalised with GAPDH mRNA. The results presented by dot plots with median and IQR. n=10. *P<0.01 (analysed by Mann-Whitney U test). Bleo, bleomycin; IRF7, interferon regulatory factor 7; α-SMA, α-smooth muscle actin; PBS, phosphate-buffered saline; SSc, systemic sclerosis.

phospho-IRF7 were observed in α-SMA-positive myofibroblasts and CD68-positive macrophages in the bleomycin-treated fibrotic skin (figure 4B). IRF7 mRNA was also significantly up-regulated in bleomycin-injected lesional skin compare with PBS-injected skin (figure 4C). Thus, bleomycin-induced IRF7 upregulation was similar to that observed in affected skin of patients with SSc.

To further understand the role of IRF7 in the fibrotic response to bleomycin, we compared the response of bleomycin injections in IRF7 KO mice versus control mice. Subcutaneous injection of bleomycin for 7 days induced IL-6, TGF-β1 cytokines along with Col1a2 mRNA upregulation in the wild-type mice skin tissue, and these changes were significantly attenuated in the IRF7 KO mice (see online supplementary figure S3). At 28

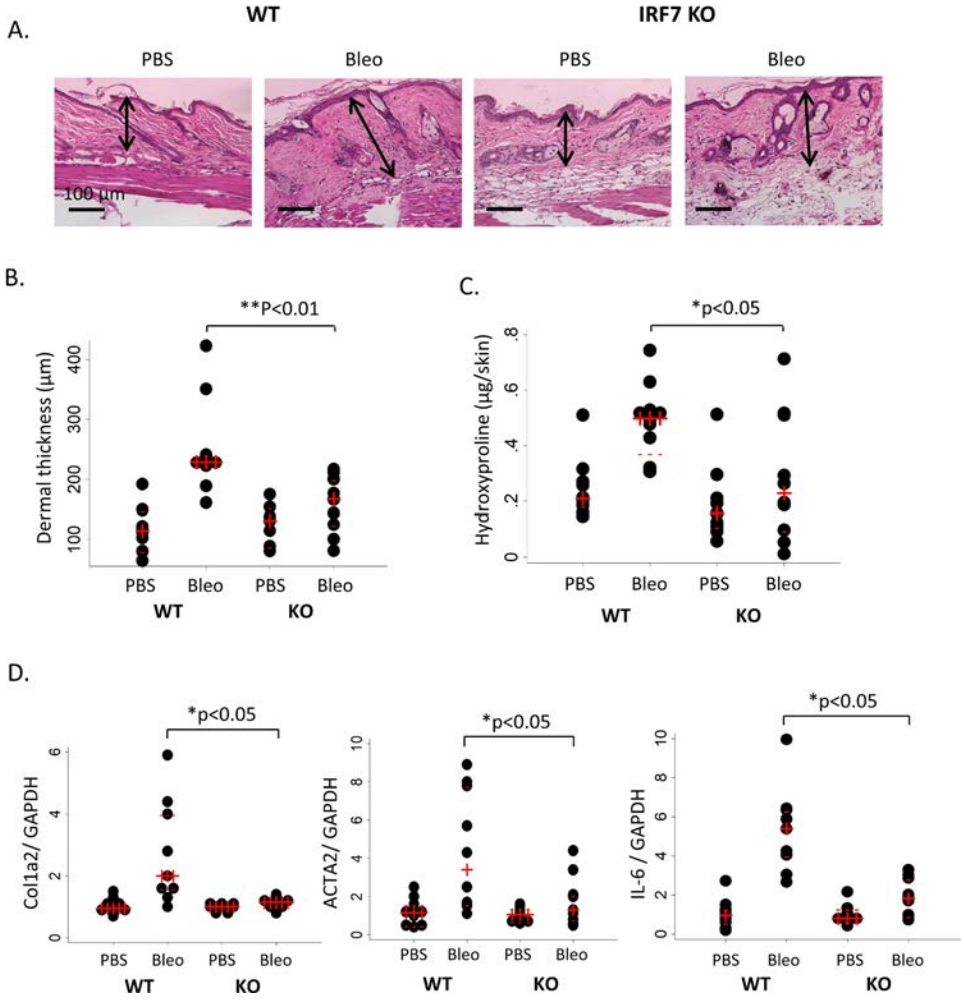

Figure 5 Attenuated skin fibrosis in IRF7 KO mice lesional skin in the Bleo animal model. IRF7 KO and WT mice received daily subcutaneous injections of PBS or Bleo for 6 days per week for 4 weeks, and lesional skin was analysed. (A) Representative images with H&E stain. Original magnification: x100. n=10. Black arrows represent the distance measurement. Scale bar: 100 µm. (B) Quantitation of dermal thickness (n=10) and (C) hydroxyproline content (n=10) demonstrating decreased dermal thickness in IRF7 KO mice injected with Bleo compared with WT mice. The results are shown as dot plots with median (red +) and IQR (red –). *P<0.05, **p<0.01 (analysed by Mann-Whitney U test). (D) Real-time quantitative PCR analysis in the lesional skin from WT and IRF7 KO mice treated with Bleo for 6 days per week for 4 weeks. Results were normalised with GAPDH mRNA and presented by dot plots with median (red +) and IQR (red –) from 10 mice per group. *P<0.05 (analysed by Mann-Whitney U test). IRF7, interferon regulatory factor 7; KO, knockout; PBS, phosphate-buffered saline; WT, wild-type.

days of injection, bleomycin-induced expected fibrotic features including inflammation, increased dermal thickness and collagen accumulation in the wild-type mice skin. These effects were significantly attenuated in IRF7 KO mice (figure 5A). IRF7 KO mice demonstrated attenuated dermal thickness and hydroxyproline content in the lesional skin tissue (figure 5B,C). Bleomycin-induced Col1a2, ACTA2 and IL-6 mRNA expression were attenuated in IRF7 KO mice (figure 5D). TGF-β-stimulated Col1a2 mRNA expression was significantly abrogated in the cultured IRF7 KO mouse fibroblasts but not the Col1a1 mRNA expression (see online supplementary figure S4). These results indicate that lacking of IRF7 led to reduced inflammatory cytokines and fibrotic response in the bleomycin-induced dermal fibrosis animal model.

Attenuated fibrosis development in TSK/IRF7 KO congenic mice

To further understand the role of IRF7 in fibrosis, we examined the TSK/+mice as a second animal model, which resembles the fibrotic features of SSc but does not have prominent inflammatory features. We crossbred TSK/+ and IRF7 KO mice to generate a TSK/IRF7 KO double congenic mouse model. All animals used in the experiments were confirmed by genotyping for the *fibrillin* and *IRF7* gene variation (see online supplementary figure S5). Compared with TSK/mice skin tissue, TSK/IRF7 KO double congenic mice showed significantly reduced hypodermal fibrosis (figure 6A). Hypodermal thickness in TSK+/IRF7 KO mice was significantly reduced compared with TSK/+mice (figure 6B). Collagen hydroxyproline content and Col1a2 mRNA levels in the skin were also significantly reduced in TSK/IRF7 KO double congenic mice compared with TSK/+mice (figure 6C,D). Furthermore, α-SMA and fibronectin expression were significantly attenuated in TSK/IRF7 KO mice compared with TSK/+mice (figure 6E). To investigate whether IRF7 and Smad3 interaction was involved in the mechanism of fibrosis in the TSK/+mouse model, we performed immunofluorescence using skin tissue from the TSK/+mouse. The results showed strong co-localisation of Smad3 and IRF7 in TSK/+mice skin tissue. As expected, this was not observed in the TSK+/IRF7 KO double congenic mice (see online supplementary figure S6).

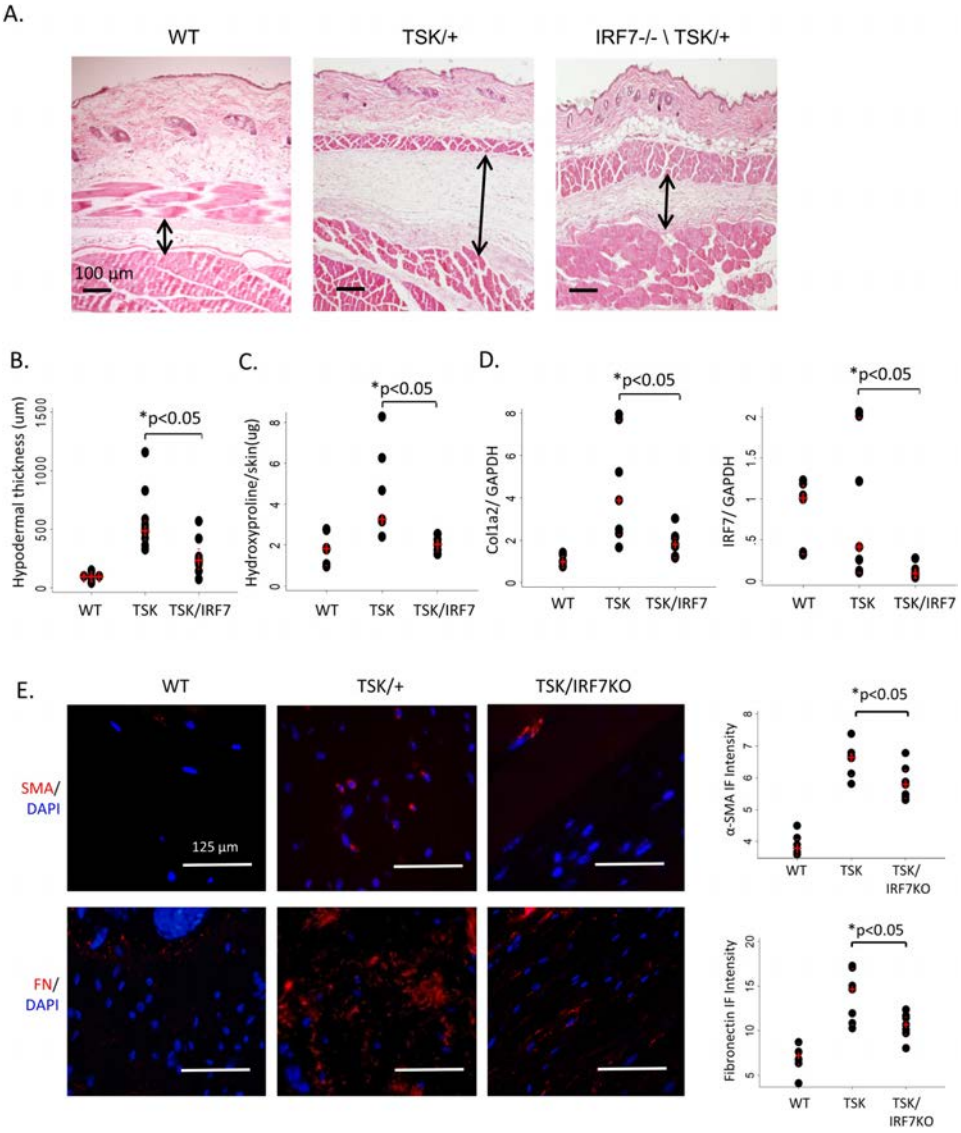

Figure 6 Attenuated skin fibrosis in TSK/IRF7 KO double congenic mice skin. (A) Representative images with H&E stain. Hypodermal thickness was reduced (arrows) in TSK/IRF7 KO mice compared with TSK/+mice. Original magnification: x100. n=7. Black arrows represent the distance measurement. (B) Quantitation of hypodermal thickness demonstrating attenuated hypodermal thickness in TSK/IRF7 KO mice compared with TSK/+mice. The results were given as dot plots with median (red +) and IQR (red –). n=7. *P<0.05 (analysed by Mann-Whitney U test). (C) Hydroxyproline content demonstrating reduced collagen accumulation in TSK/IRF7 KO mice skin compared with TSK/+mice. The results are shown as dot plots with median (red +) and IQR (red –). n=7. *P<0.05 (analysed by Mann-Whitney U test). (D) Total RNA was extracted from back skin of mice and Col1a2 and, IRF7 mRNA expression was determined by real-time quantitative PCR. The results were normalised with GAPDH mRNA. The results presented by dot plots with median (red +) and IQR (red –). n=7. *P<0.05 (analysed by Mann-Whitney U test). (E) Immunofluorescence analysis using anti-FN and α-SMA antibodies in the TSK/IRF7 KO double transgenic mice skin. Representative images (left panels) and intensity analysis of the α-SMA and FN-positive cells in the skin tissue (right panels). The results presented by dot plots with median (red +) and IQR (red –). n=7. *P<0.05 (analysed by Mann-Whitney U test). Original magnifications: x400 magnifications. Scale bar: 125 µm. FN, fibronectin; IRF7, interferon regulatory factor 7; KO, knockout; α-SMA, α-smooth muscle actin; WT, wild-type.

These results suggest an interaction between IRF7 and Smad3 in the TSK/+mouse model of skin fibrosis.

DISCUSSION

In the current study, we demonstrated that IRF7 mRNA and protein levels were significantly upregulated in the skin and cultured fibroblasts of patients with SSc compared with healthy controls. IRF7 appeared to be expressed in myofibroblasts and macrophages in affected skin tissue. IRF7 knockdown abrogated IFN-α-induced profibrotic gene expression, and IRF7/Smad3 co-localisation was observed in dermal fibroblasts. Moreover, in the bleomycin-induced dermal fibrosis and TSK/+mouse models, IRF7 KO mice had decreased dermal and hypodermal thickness and dermal fibrosis, with associated attenuation in the expression of hydroxyproline and profibrotic genes. These results demonstrate that IRF7 mediates the profibrotic effects of bleomycin, which is thought to induce fibrosis secondary to an inflammatory response, and in TSK/+mice, which is thought to directly induce fibrosis independent of inflammation.

In our previously published skin gene expression study, the skin of patients with SSc had 2754 differentially expressed genes compared with controls. Pathway analysis suggested that IRF7

is the most activated upstream transcription factor inducing the observed aberrant gene expression.[22] The top upstream cytokines/growth factors in this analysis were TGF-β1, IFN-α, IFN-γ and IRF5, another transcription factor of the IFN signalling pathway in which SSc-associated polymorphisms have been identified.[26] IRF5 was previously shown to mediate the fibrotic response in bleomycin-treated mice through multiple mechanisms, including fibroblast activation, inflammatory cell infiltration, endothelial-to-mesenchymal transition, vascular destabilisation, Th2/Th17 skewed immune polarisation and B-cell activation in this mouse model.[27] These data support that type I IFN signalling and its downstream pathway factors like IRF5 and IRF7 are important mediators of dermal fibrosis in animal models of SSc and suggest that IRF7 may play a pathogenic role in SSc.

IRF7, previously known as lymphoid-specific factor, is expressed in fibroblasts, B cells, lymphocytes, pDCs and monocytes. Its expression can be induced by type I IFNs, TNF-α, IL-1β and viral infections.[28–32] Inactive IRF7 resides in the cytoplasm. At the early 'priming' stage of virus infection, the low level of endogenous IRF7 is phosphorylated and activated by signalling triggered through pathogen recognition receptors and TLRs, and forms a transcriptional complex with nuclear factor-κB or IRF3. This transcriptional complex binds to the virus-response elements in the IFN-α and IFN-β promoters and induces small amounts of type I IFNs.[33–35] Through positive feedback, secreted type I IFNs induce synthesis of more IRF7. Later, the newly synthesised IRF7 is activated, accumulates and further upregulates IFN expression in a positive feedback loop. In cultured IRF7 KO mouse embryonic fibroblasts, the viral induction of IFN-α/IFN-β was severely impaired and markedly decreased serum IFN-α levels were observed in the IRF7 KO mice.[36] Furthermore, IRF7 induces the pro-inflammatory cytokine IL-6 in pDCs and monocytes.[37 38] Importantly, a recent study demonstrated that depletion of pDCs, a major source of IFN-α, in animal models significantly decreased IRF7 mRNA expression and led to attenuated skin fibrosis even when fibrosis was already established.[9] These observations demonstrate the importance of IRF7-dependent systemic inflammatory responses.

TGF-β has long been recognised as a key mediator of fibrosis in SSc.[10] The C-terminal regions of IRFs, including IRF7, show homology to the C-terminal domains of the Smad family, which may mediate the response to TGF-β.[39] Smad3, a key component of TGF-β signalling for stimulation of collagen production, has been identified as an IRF7 interacting protein. Smad3 physically and functionally interacts with IRF7, and TGF-β/Smad3 signalling regulates the transcriptional activity of IRF7.[19] On activation, IRF7 forms a transcriptional complex together with other IRFs such as IRF3 or IRF5.[33 35] In this study, we further confirmed that IRF7 forms complexes with Smad3. Our in vitro experiments also indicated that IRF7 knockdown abrogated TGF-β-induced fibrosis in SSc fibroblasts regardless of IFN-α stimulation. Taken together, our results suggest a fibroblast-specific function of IRF7, namely in potentiating the TGF-β-mediated fibrotic response (see online supplementary figure S7). However, IRF7 knockdown did not attenuate TGF-β-stimulated collagen accumulation in cultured healthy control skin fibroblasts. This may be explained by considering that TGF-β itself does not stimulate IRF7 accumulation in healthy control fibroblasts (figure 2C, lane 5). On the other hand, IRF7 KO in the non-inflammatory dermal fibrosis model, TSK/+mouse, abrogated the fibrotic response. This finding is contrary to other inflammatory targets such as STAT4 and OX40L whose blockade show an abrogated fibrotic response in the inflammatory bleomycin-induced dermal fibrosis model but not in the TSK/+mouse.[40 41] In the present study, we primarily focused on the role of IRF7 in fibroblasts and its potential interplay with the TGF-β pathway. However, a direct link between TGF-β and IFN-α was not tested in the bleomycin-induced skin fibrosis mouse experiments. The possible link is suggested by the ability of IFN-α to upregulate IRF7 expression, the interaction between IRF7 and Smad3 in vitro and reduced fibrosis seen in the IRF7 KO mouse. As an extension of the present study, we plan to investigate the role of IRF7 upregulation in the inflammatory cells and its impact on the exaggerated fibrosis in SSc including experiments with cell-specific conditional KO mouse models in our future studies. Altogether, our data suggest that the upregulation and activation of IRF7 in SSc skin plays an important role in the observed exaggerated inflammation as well as fibrosis, and might provide a link between the prominent IFN signature and activation of TGF-β in SSc. IRF7 may therefore represent a promising novel therapeutic target in SSc.

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Competing interests None declared.

Ethics approval The study was approved by the institutional review board of the University of Texas Health Science Center at Houston. The animal protocols were institutionally approved by the University of Texas Health Science Center at Houston Animal Care and Use Committee.

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REFERENCES

1 Elhai M, Meune C, Avouac J, *et al*. Trends in mortality in patients with systemic sclerosis over 40 years: a systematic review and meta-analysis of cohort studies. *Rheumatology* 2012;51:1017–26.
2 Skaug B, Assassi S. Type I interferon dysregulation in systemic sclerosis. *Cytokine* 2019.
3 Wu M, Assassi S. The role of type 1 interferon in systemic sclerosis. *Front Immunol* 2013;4:266.
4 Solans R, Bosch JA, Esteban I, *et al*. Systemic sclerosis developing in association with the use of interferon alpha therapy for chronic viral hepatitis. *Clin Exp Rheumatol* 2004;22:625–8.
5 Black CM, Silman AJ, Herrick AI, *et al*. Interferon-? does not improve outcome at one year in patients with diffuse cutaneous scleroderma: results of a randomized, double-blind, placebo-controlled trial. *Arthritis Rheum* 1999;42:299–305.
6 Tan FK, Zhou X, Mayes MD, *et al*. Signatures of differentially regulated interferon gene expression and vasculotrophism in the peripheral blood cells of systemic sclerosis patients. *Rheumatology* 2006;45:694–702.
7 York MR, Nagai T, Mangini AJ, *et al*. A macrophage marker, siglec-1, is increased on circulating monocytes in patients with systemic sclerosis and induced by type I interferons and Toll-like receptor agonists. *Arthritis Rheum* 2007;56:1010–20.
8 Farina GA, York MR, Di Marzio M, *et al*. Poly(I:C) drives type I IFN- and TGFβ-mediated inflammation and dermal fibrosis simulating altered gene expression in systemic sclerosis. *J Invest Dermatol* 2010;130:2583–93.
9 Ah Kioon MD, Tripodo C, Fernandez D, *et al*. Plasmacytoid dendritic cells promote systemic sclerosis with a key role for TLR8. *Sci Transl Med* 2018;10:eaam8458.
10 Lafyatis R. Transforming growth factor β—at the centre of systemic sclerosis. *Nat Rev Rheumatol* 2014;10:706–19.

11 De Luján Alvarez* M, Quiroga* AD, Parody JP, *et al*. Cross-talk between IFN-α and TGF-β_1 signaling pathways in preneoplastic rat liver. *Growth Factors* 2009;27:1–11.
12 Tamura T, Yanai H, Savitsky D, *et al*. The IRF family transcription factors in immunity and oncogenesis. *Annu Rev Immunol* 2008;26:535–84.
13 Mamane Y, Heylbroeck C, Génin P, *et al*. Interferon regulatory factors: the next generation. *Gene* 1999;237:1–14.
14 Taniguchi T, Ogasawara K, Takaoka A, *et al*. IRF family of transcription factors as regulators of host defense. *Annu Rev Immunol* 2001;19:623–55.
15 Taniguchi T, Takaoka A. A weak signal for strong responses: interferon-alpha/beta revisited. *Nat Rev Mol Cell Biol* 2001;2:378–86.
16 Platanias LC. Mechanisms of type-I- and type-II-interferon-mediated signalling. *Nat Rev Immunol* 2005;5:375–86.
17 Decker T, Müller M, Stockinger S. The yin and yang of type I interferon activity in bacterial infection. *Nat Rev Immunol* 2005;5:675–87.
18 Dietrich N, Lienenklaus S, Weiss S, *et al*. Murine toll-like receptor 2 activation induces type I interferon responses from endolysosomal compartments. *PLoS One* 2010;5:e10250.
19 Qing J, Liu C, Choy L, *et al*. Transforming growth factor beta/Smad3 signaling regulates IRF-7 function and transcriptional activation of the beta interferon promoter. *Mol Cell Biol* 2004;24:1411–25.
20 Carmona FD, Gutala R, Simeón CP, *et al*. Novel identification of the *IRF7* region as an anticentromere autoantibody propensity locus in systemic sclerosis. *Ann Rheum Dis* 2012;71:114–9.
21 Fu Q, Zhao J, Qian X, *et al*. Association of a functional IRF7 variant with systemic lupus erythematosus. *Arthritis Rheum* 2011;63:749–54.
22 Assassi S, Swindell WR, Wu M, *et al*. Dissecting the heterogeneity of skin gene expression patterns in systemic sclerosis. *Arthritis Rheumatol* 2015;67:3016–26.
23 Vangipuram M, Ting D, Kim S, *et al*. Skin punch biopsy explant culture for derivation of primary human fibroblasts. *J Vis Exp* 2013;77:e3779.
24 van den Hoogen F, Khanna D, Fransen J, *et al*. 2013 classification criteria for systemic sclerosis: an American College of rheumatology/European League against rheumatism collaborative initiative. *Ann Rheum Dis* 2013;72:1747–55.
25 Lydersen S. Statistical review: frequently given comments. *Ann Rheum Dis* 2015;74:323–5.
26 Radstake TRDJ, Gorlova O, Rueda B, *et al*. Genome-wide association study of systemic sclerosis identifies CD247 as a new susceptibility locus. *Nat Genet* 2010;42:426–9.
27 Saigusa R, Asano Y, Taniguchi T, *et al*. Multifaceted contribution of the TLR4-activated IRF5 transcription factor in systemic sclerosis. *Proc Natl Acad Sci U S A* 2015;112:15136–41.
28 Zhang L, Pagano JS. IRF-7, a new interferon regulatory factor associated with Epstein-Barr virus latency. *Mol Cell Biol* 1997;17:5748–57.
29 WC A, Moore PA, LaFleur DW, *et al*. Characterization of the interferon regulatory factor-7 and its potential role in the transcription activation of interferon A genes. *J Biol Chem* 1998;273:29210–7.
30 Rivieccio MA, John GR, Song X, *et al*. The cytokine IL-1beta activates IFN response factor 3 in human fetal astrocytes in culture. *J Immunol* 2005;174:3719–26.
31 Sato M, Suemori H, Hata N, *et al*. Distinct and essential roles of transcription factors IRF-3 and IRF-7 in response to viruses for IFN-α/β gene induction. *Immunity* 2000;13:539–48.
32 Farina A, Cirone M, York M, *et al*. Epstein–Barr virus infection induces aberrant TLR activation pathway and fibroblast-myofibroblast conversion in scleroderma. *J Invest Dermatol* 2014;134:954–64.
33 Wathelet MG, Lin CH, Parekh BS, *et al*. Virus infection induces the assembly of coordinately activated transcription factors on the IFN-β enhancer in vivo. *Mol Cell* 1998;1:507–18.
34 Yang H, Lin CH, Ma G, *et al*. Interferon regulatory factor-7 synergizes with other transcription factors through multiple interactions with p300/CBP coactivators. *J. Biol. Chem.* 2003;278:15495–504.
35 Barnes BJ, Field AE, Pitha-Rowe PM. Virus-induced Heterodimer Formation between *IRF-5* and *IRF-7* Modulates Assembly of the *IFNA* Enhanceosome *in Vivo* and Transcriptional Activity of *IFNA* Genes. *J. Biol. Chem.* 2003;278:16630–41.
36 Honda K, Yanai H, Negishi H, *et al*. IRF-7 is the master regulator of type-I interferon-dependent immune responses. *Nature* 2005;434:772–7.
37 Sweeney SE. Targeting interferon regulatory factors to inhibit activation of the type I IFN response: implications for treatment of autoimmune disorders. *Cell Immunol* 2011;271:342–9.
38 Yasuda K, Richez C, Maciaszek JW, *et al*. Murine dendritic cell type I IFN production induced by human IgG-RNA immune complexes is IFN regulatory factor (IRF)5 and IRF7 dependent and is required for IL-6 production. *J Immunol* 2007;178:6876–85.
39 Eroshkin A, Mushegian A. Conserved transactivation domain shared by interferon regulatory factors and Smad morphogens. *J Mol Med* 1999;77:403–5.
40 Avouac J, Fürnrohr BG, Tomcik M, *et al*. Inactivation of the transcription factor STAT-4 prevents inflammation-driven fibrosis in animal models of systemic sclerosis. *Arthritis Rheum* 2011;63:800–9.
41 Elhai M, Avouac J, Hoffmann-Vold AM, *et al*. OX40L blockade protects against inflammation-driven fibrosis. *Proc Natl Acad Sci U S A* 2016;113:E3901–E3910.

CLINICAL SCIENCE

Gout, Hyperuricaemia and Crystal-Associated Disease Network (G-CAN) consensus statement regarding labels and definitions of disease states of gout

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ABSTRACT

Objective There is a lack of standardisation in the terminology used to describe gout. The aim of this project was to develop a consensus statement describing the recommended nomenclature for disease states of gout.

Methods A content analysis of gout-related articles from rheumatology and general internal medicine journals published over a 5-year period identified potential disease states and the labels commonly assigned to them. Based on these findings, experts in gout were invited to participate in a Delphi exercise and face-to-face consensus meeting to reach agreement on disease state labels and definitions.

Results The content analysis identified 13 unique disease states and a total of 63 unique labels. The Delphi exercise (n=76 respondents) and face-to-face meeting (n=35 attendees) established consensus agreement for eight disease state labels and definitions. The agreed labels were as follows: 'asymptomatic hyperuricaemia', 'asymptomatic monosodium urate crystal deposition', 'asymptomatic hyperuricaemia with monosodium urate crystal deposition', 'gout', 'tophaceous gout', 'erosive gout', 'first gout flare' and 'recurrent gout flares'. There was consensus agreement that the label 'gout' should be restricted to current or prior clinically evident disease caused by monosodium urate crystal deposition (gout flare, chronic gouty arthritis or subcutaneous tophus).

Conclusion Consensus agreement has been established for the labels and definitions of eight gout disease states, including 'gout' itself. The Gout, Hyperuricaemia and Crystal-Associated Disease Network recommends the use of these labels when describing disease states of gout in research and clinical practice.

INTRODUCTION

The language used to describe gout is characterised by a lack of consistent terminology and definitions.[1 2] In particular, many different terms are used interchangeably to describe different disease states and their constituent features. This lack of agreement and clarity has implications for how disease-related concepts are communicated in both clinical and research settings.[3–5] Notably, there is no universally accepted definition of 'gout' itself.[6]

The Gout, Hyperuricaemia and Crystal-Associated Disease Network (G-CAN) is an international, multidisciplinary network for collaborative research, committed to advancing all aspects of the crystal deposition-associated disorders. G-CAN has

Key messages

What is already known about this subject?

- The language used to describe gout is characterised by a lack of consistent terminology and definitions.

What does this study add?

- Consensus agreement has been reached about the labels and definitions of disease states of gout.
- The agreed labels are as follows: 'asymptomatic hyperuricaemia', 'asymptomatic monosodium urate crystal deposition', 'asymptomatic hyperuricaemia with monosodium urate crystal deposition', 'gout', 'tophaceous gout', 'erosive gout', 'first gout flare' and 'recurrent gout flares'.
- The label 'gout' should be restricted to current or prior clinically evident disease caused by monosodium urate crystal deposition.

How might this impact on clinical practice or future developments?

- The Gout, Hyperuricaemia and Crystal-Associated Disease Network recommends the use of these labels when communicating in the scientific literature and in professional practice.

supported a project to establish consensus agreement on the nomenclature of hyperuricaemia and gout, its primary objective being the promotion of accurate, well defined terms that facilitate understanding of disease-related concepts. The intended audience is healthcare professionals and non-physician scientists in clinical and research settings.

In the first stage of the G-CAN gout nomenclature project, consensus agreement was reached on the labels and definitions of the disease elements of gout. The content analysis of the literature and subsequent G-CAN-endorsed consensus statement have been published, with the results of the latter summarised in table 1.[1 7] This initial work provided labels and definitions for clinical elements including gout flare, chronic gouty arthritis and subcutaneous tophus, as well as imaging elements such as gouty bone erosion. Using these results as a framework, the objective of this second stage of the G-CAN gout nomenclature project was to reach agreement on the nomenclature of disease states of gout. For the purpose of this project, a disease state was defined as 'a clinically meaningful cluster of the presence, or absence, of two or more disease elements'. Here, we describe the process and outcomes of this project addressing the labels and definitions of the disease states of gout.

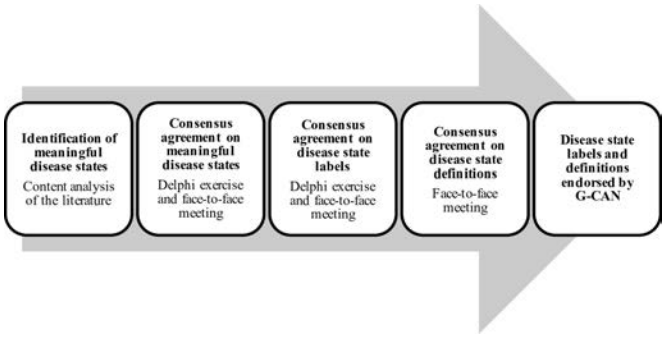

Figure 1 Outline of the project to develop the G-CAN consensus statement regarding the labels and definitions of disease states of gout. G-CAN, Gout, Hyperuricaemia and Crystal-Associated Disease Network.

METHODS

This work consisted of three components: a content analysis of the literature, a Delphi exercise and a face-to-face consensus meeting. The content analysis of the literature was performed to identify the language currently used to represent disease states of gout. The results of this analysis were then used as the basis for two group consensus exercises—a Delphi exercise and a face-to-face meeting—with the overall objective of reaching agreement on a nomenclature for disease states of gout. A schematic representation of these project components is shown in figure 1.

Content analysis of the literature

This component of the project had two aims: first, to establish the range of disease states described in the contemporary gout-related and hyperuricaemia-related literature; and second, to identify the labels currently used to denote these disease states. Articles were extracted from the 10 highest-ranked general rheumatology journals, and the five highest-ranked general internal medicine journals (according to Impact Factor, 2016 Thomson-Reuters Journal Citation Reports) published between first January 2013 and 31 January 2018. These journals are shown

Table 1 G-CAN endorsed labels and definitions of the disease elements of gout[7]

	Consensus label	Consensus definition
Chemical elements	1. MSU crystals	The pathogenic crystals in gout (chemical formula: $C_5H_4N_4NaO_3$)
	2. Urate	The circulating form of the final enzymatic product generated by xanthine oxidase in purine metabolism in humans (chemical formula: $C_5H_3N_4O_3^-$)
	3. Hyperuricaemia*	Elevated blood urate concentration over the saturation threshold
Clinical elements	4. Gout flare	A clinically evident episode of acute inflammation induced by MSU crystals
	5. Intercritical gout	The asymptomatic period after or between gout flares, despite the persistence of MSU crystals
	6. Chronic gouty arthritis	Persistent joint inflammation induced by MSU crystals
	6a. G-CAN recommendation	The label 'chronic gout' should be avoided
	7. Tophus	An ordered structure of MSU crystals and the associated host tissue response
	8. Subcutaneous tophus	A tophus that is detectable by physical examination
	9. Podagra	A gout flare at the first metatarsophalangeal joint
Imaging elements	10. Imaging evidence of MSU crystal deposition	Findings that are highly suggestive of MSU crystals on an imaging test
	11. Gouty bone erosion	Evidence of a cortical break in bone suggestive of gout (overhanging edge with sclerotic margin)

*In British English, hyperuricaemia.
G-CAN, Gout, Hyperuricaemia and Crystal-Associated Disease Network; MSU, monosodium urate.

in supplementary table S1. Relevant articles within each journal were identified through MEDLINE using the search terms 'gout' or 'urate' or 'hyperuricaemia' without exclusion criteria. This methodology was used to provide a suitably large representation of contemporary literature for the extraction of disease states and their labels, with the intention of reflecting the current language of gout and hyperuricaemia, rather than its progression over time.

For the purpose of this project, a disease state was defined as a 'clinically meaningful cluster of the presence, or absence, of two or more disease elements'. The G-CAN-endorsed labels and definitions for the disease elements of gout are summarised in table 1. A cluster was considered 'meaningful' if the co-occurrence of these disease elements had the potential to impact either disease prognosis or management. Articles were manually searched for passages of text referring to the collective presence, or absence, of two or more disease elements. Labels for each identified disease state were extracted to determine the range and frequency of unique labels. Disease state labels were taken verbatim from the examined text, except where the labels for component disease elements were modified to comply with existing G-CAN consensus statement for disease elements (as shown in table 1). Labels were considered 'unique' if they used different words or phrases to describe a disease state. For each article, the use of a unique label was recorded only once. All articles were analysed by a single investigator (DB). To ensure the accuracy of the disease state and label identification, the first 10 articles examined were jointly reviewed by a second investigator (ND) with 98% agreement on identified disease element clusters.

Delphi exercise

The Delphi exercise was conducted as a series of three web-based surveys using Survey Monkey™ software (SurveyMonkey, San Mateo, CA, USA). Physicians and non-physician scientists with expertise in gout were identified through their membership of G-CAN and invited by email to participate in the first round of the survey. Subsequent rounds were only made available to those who had engaged in the previous surveys. In each survey, respondents were presented with disease states identified by the content analysis of the literature, represented by the disease element clusters. Respondents were first asked if each proposed disease state was meaningful for disease prognosis or management. Next, respondents were asked to select and rank their preferred labels for each disease state from a list of options derived from the content analysis of the literature; labels were included if present in at least two of the articles analysed, with the frequency with which they occurred in the literature also shown. In the first round, respondents were also able to nominate their own preferred disease states or labels that had not already been presented; these were included as voting options in the second round of the Delphi if nominated by at least two respondents. Respondents were given the option to comment on disease states or labels that they felt either strongly for or against; a thematic summary of these comments was provided as group feedback in subsequent rounds according to Delphi principles. Disease state label options were refined as the Delphi rounds progressed. Voting on whether a disease state was meaningful, and for its preferred label, ceased once consensus agreement was achieved, defined as at least 80% agreement.

Face-to-face meeting

The face-to-face meeting took place on 20 October 2018 in Chicago, IL. All G-CAN members were invited to attend irrespective of their involvement in the Delphi exercise. There were two main objectives for this meeting. The first objective was to address those disease states for which consensus agreement was not met at the conclusion of the Delphi exercises, either for whether they were meaningful, or for the preferred label. The second objective was to agree on a definition for each disease state included in the final consensus statement. Attendees were provided pre-reading that included a summary of the content analysis of the literature, results of the Delphi exercise and draft definitions of the disease states as a starting point for discussion. The meeting was conducted as a facilitated discussion, moderated by two investigators (DB and ND). Key points raised by attendees were summarised, refined by group discussion and then brought forward for voting by show of hands. Consensus agreement was defined as at least 80% agreement by those present at the time of voting.

The group was first asked to consider which of the proposed disease states should be included in the nomenclature based on the results of the Delphi exercise. It was agreed that only those disease states that had achieved consensus agreement as being meaningful following the three rounds of the Delphi exercise would be included. Next, disease state labels for which consensus agreement had not been reached during the Delphi exercise were discussed and voted on. Finally, the definitions for each disease state were developed and iteratively modified until consensus agreement was reached.

G-CAN endorsement

The results of the project and consensus nomenclature statement have been reviewed and endorsed by the G-CAN Board of Directors.

RESULTS

Content analysis of the literature

A total of 539 articles were extracted using the search criteria. Analysis of these articles identified 13 disease states that were categorised into pre-clinical states, clinical states and states describing the disease course of gout (table 2). In total, there were 63 unique labels identified for these 13 disease states. A detailed description of these results is shown in the supplementary material.

Delphi exercise

In all, 76 G-CAN members responded to the first round of the survey; of these, 72 (95%) completed all three rounds. The respondents included 34 members from Europe (45%), 24 from North America (32%), 13 from the Asia-Pacific region (17%) and five from Latin America (7%). The majority of respondents were rheumatologists (n=67, 88%); other physician specialists (n=4, 5%) and non-physician scientists (n=5, 7%) also participated.

Of the 13 disease states identified from the content analysis of the literature, nine were deemed to be meaningful by consensus agreement (table 3). Of these nine disease states deemed to be meaningful, seven disease states reached consensus agreement on their preferred label: 'asymptomatic hyperuricaemia', 'asymptomatic monosodium urate crystal deposition', 'severe gout', 'tophaceous gout', 'erosive gout', 'first gout flare' and 'recurrent gout flares' (table 4). A detailed description of the Delphi exercise results regarding whether disease states were meaningful and preferred labels is shown in the supplementary material.

Table 2 Results of the content analysis of 539 gout-related and hyperuricaemia-related articles: disease element clusters identified as potentially meaningful disease states of gout and characteristics of their labels

Disease states represented by disease element clusters		Number of articles labelling disease state (% of total articles)	Number of unique labels identified	Most frequently used labels (% of articles referencing disease state)
Pre-clinical states	Hyperuricaemia* **without** clinical disease elements† of gout	79 (14.5%)	1	Asymptomatic hyperuricaemia* (100%)
	Imaging evidence of MSU crystal deposition **without** clinical disease elements† of gout	32 (5.9%)	8	Asymptomatic MSU crystal deposition (43.8%)
	Hyperuricaemia* **with** imaging evidence of MSU crystal deposition and **without** clinical disease elements† of gout	32 (5.9%)	4	Asymptomatic hyperuricaemia* with MSU‡ crystal deposition (90.6%)
Clinical states	Presence of MSU crystals **with** clinical disease elements† of gout	61 (11.3%)	14	Symptomatic gout (50.8%)
	Presence of MSU crystals **with** any of the following: frequent recurrent gout flares, chronic gouty arthritis, subcutaneous tophi or imaging disease elements§ of gout	72 (13.4%)	6	Severe gout (81.9%)
	Presence of MSU crystals **with** at least one subcutaneous tophus	106 (19.7%)	3	Tophaceous gout (81.1%)
	Chronic gouty arthritis **with** at least one subcutaneous tophus	10 (1.9%)	4	Chronic tophaceous gouty arthropathy (40.0%)
	Presence of MSU crystals **with** any of the following: gout flare, chronic gouty arthritis and **without** subcutaneous tophi	10 (1.9%)	3	Non-tophaceous gout (80%)
	Presence of MSU crystals **with** clinical disease elements† of gout and **with** at least one gouty bone erosion	6 (1.1%)	1	Erosive gout (100%)
Disease course states	The first episode of gout flare **without** preceding intercritical gout	73 (13.5%)	5	Incident gout (75.3%)
	More than one episode of gout flare **with** intercritical gout	79 (14.7%)	8	Recurrent gout flares (94.9%)
	Presence of MSU crystals **with** clinical disease elements† of gout **and** early in the course of disease natural history	19 (3.5%)	4	Early gout (68.4%)
	Presence of MSU crystals **with** clinical disease elements† of gout **and** late in the course of disease natural history	9 (1.7%)	2	Long-standing gout (66.7%)

*In British English, hyperuricaemia.
†Clinical disease elements: gout flare, intercritical gout, chronic gouty arthritis, subcutaneous tophus.
‡Imaging disease elements: imaging evidence of MSU crystal deposition, gouty bone erosion.
MSU, monosodium urate.

Face-to-face meeting

A total of 35 G-CAN members attended the face-to-face meeting, the majority of whom were rheumatologists (n=33, 94%). Of those attending, 32 (91%) had also participated in all three rounds of the Delphi exercise. The panel included 18 members from Europe (51%), 11 from North America (31%), 4 from the Asia-Pacific region (11%) and 2 from Latin America (6%). The number of attendees participating in voting activities during the meeting varied from 28 to 35.

Agreement about which disease states are meaningful

The first item raised was the proposal that only disease states reaching consensus agreement as being meaningful during the Delphi exercise should be included within the final disease state consensus statement. This proposal was unanimously agreed on (35 of 35 voting in favour), reducing the total number of disease states for consideration to nine; this was further reduced to eight when it was unanimously agreed to eliminate the disease state 'the presence of monosodium urate crystals *with* any of the following: frequent recurrent gout flares, chronic gouty arthritis, subcutaneous tophi or imaging disease elements of gout'. This disease state, labelled 'severe gout' through the Delphi exercise, was thought to be a broad, non-specific state that would be difficult to define in clinical and research settings. It was also considered to be potentially misleading for gout treatment; for example, it might imply that patients not fulfilling this definition have 'non-severe gout' and that urate lowering therapy is not warranted in this case. For the cluster of disease elements, 'hyperuricaemia *with* imaging evidence of monosodium urate crystal deposition but *without* clinical disease elements of gout', consensus agreement on this state being meaningful was achieved through the Delphi exercise. However, a number of respondents commented that this state was similar to the disease state, 'asymptomatic monosodium urate crystal deposition', and therefore may be redundant. After being put to vote, it was unanimously agreed (35/35 in favour) that this represented a unique and meaningful disease state, distinct from 'asymptomatic monosodium urate crystal deposition' which could represent a state of asymptomatic crystal deposition irrespective of serum urate concentration. The final eight disease states deemed meaningful by consensus agreement at the conclusion of both the Delphi exercise and face-to-face meeting are shown in table 5.

Disease state labels

Consensus agreement was achieved on two disease state labels that remained unresolved after the Delphi exercise. These consensus labels were: 'asymptomatic hyperuricaemia with monosodium urate crystal deposition' and 'gout' (table 4). Further details on voting results are shown in supplementary table S2.

For the disease state referring to 'hyperuricaemia *with* imaging evidence of monosodium urate crystal deposition but *without* clinical disease elements of gout', the label 'asymptomatic hyperuricaemia with monosodium urate crystal deposition' was very close to reaching consensus following the Delphi exercise with

Table 3 Results of the Delphi exercise for agreement about whether the proposed gout disease states are meaningful*

Disease states represented by disease element clusters		Delphi exercise	
		Consensus achieved† (round)	Agreement (%)
Pre-clinical states	Hyperuricaemia‡ **without** clinical disease elements§ of gout	Yes (1)	84%
	Imaging evidence of MSU¶ crystal deposition **without** clinical disease elements§ of gout	Yes (1)	89%
	Hyperuricaemia† **with** imaging evidence of MSU¶ crystal deposition **without** clinical disease elements§ of gout	Yes (1)	86%
Clinical states	Presence of MSU¶ crystals **with** clinical disease elements§ of gout	Yes (1)	97%
	Presence of MSU¶ crystals **with** any of the following: frequent recurrent gout flares, chronic gouty arthritis, subcutaneous tophi or imaging disease elements** of gout	Yes (1)	93%
	Presence of MSU¶ crystals **with** subcutaneous tophi	Yes (1)	89%
	Chronic gouty arthritis **with** at least one subcutaneous tophus	No	74% after round 3
	Presence of MSU¶ crystals **with** any of the following: gout flare, chronic gouty arthritis; **without** subcutaneous tophi	No	74% after round 3
	Presence of MSU¶ crystals **with** clinical disease elements§ of gout and **with** at least one gouty bone erosion	Yes (1)	85%
Disease course states	The first episode of gout flare **without** preceding intercritical gout	Yes (1)	92%
	More than one episode of gout flare **with** intercritical gout	Yes (1)	88%
	Presence of MSU¶ crystals **with** clinical disease elements§ of gout **and** early in the course of disease natural history	No	67% after round 3
	Presence of MSU crystals **with** clinical disease elements§ of gout **and** late in the course of disease natural history	No	69% after round 3

*'Meaningful' defined as 'having important implications for disease management and/or prognosis'.
†Consensus defined as ≥80% agreement on preferred label.
‡In British English, hyperuricaemia.
§Clinical disease elements: gout flare, intercritical gout, chronic gouty arthritis, subcutaneous tophus.
¶Imaging disease elements: imaging evidence of MSU crystal deposition, gouty bone erosion.
MSU, monosodium urate.

79% agreement; after being put to vote, consensus agreement was reached with 33 of 35 (94%) in favour of this label.

The second disease state label that remain unresolved following the Delphi exercise concerned the disease state 'the presence of monosodium urate crystals *with* clinical disease elements of gout'. The two most preferred labels for this disease state following the Delphi exercise were 'gout' (56% agreement) and 'symptomatic gout' (43% agreement). This situation raised the fundamental question of whether 'gout' refers to the underlying pathophysiological process of monosodium urate crystal deposition or the clinically evident sequelae of crystal deposition. Consensus agreement for the label 'gout' to describe the disease state 'the presence of monosodium urate crystals *with* clinical disease elements of gout' was achieved with 34 of 34 (100%, one abstention) voting in favour. Thus, consensus was reached that the label 'gout' should be reserved for clinically evident disease.

Disease state definitions

Consensus agreement was achieved for the definitions of all eight disease states of gout (table 5). Relevant issues arising from group discussions on the composition of these definitions are outlined here. Further details on voting results are shown in supplementary table S2.

When considering the definition of the disease state of gout, it was considered important to include reference to 'a disease caused by monosodium urate crystal deposition' resulting in clinical disease elements. Therefore 'gout', according to this definition, requires current or prior clinically evident symptoms or signs resulting from monosodium urate crystal deposition. The issue was also raised as to whether 'monosodium urate crystal-proven' should be used as a modifier for the label 'gout'. Although use of this descriptor is popular in clinical practice, it strictly refers to method of diagnosis, which can be achieved through a number of modalities, including synovial fluid analysis, ultrasound or dual-energy CT (DECT). As this does not represent a separate disease state, it was not included in the recommended nomenclature.

Disease state labels not specifically addressed by the nomenclature

Throughout discussions, it was acknowledged that disease states are not necessarily mutually exclusive and that the potential for overlap exists. It was also recognised that a consensus nomenclature cannot formally address all combinations of disease elements of gout. This led to the suggestion of a hierarchical approach to address those disease states that are not formally included in the agreed nomenclature. Specifically, the following recommendation was proposed: 'Where there is more than one disease state present, these can be combined (eg, 'tophaceous and erosive gout'). Where there are additional elements present, not recognised as disease states, these will be labelled as the recognised disease state with or without additional disease elements (eg, 'tophaceous gout with chronic gouty arthritis')'. This proposal was unanimously agreed on with 27 of 27 voting in favour (100%, one abstention).

Table 4 Results of the Delphi exercise and face-to-face consensus meeting for agreement on the labels for the disease states of gout

Disease states represented by disease element clusters		Delphi exercise		Face-to-face meeting		
		Consensus achieved* (round)	Agreement (%)	Consensus achieved†	Agreement (%)	Agreed label
Pre-clinical states	Hyperuricaemia‡ **without** clinical disease elements† of gout	Yes (3)	85%	–	–	Asymptomatic hyperuricaemia‡
	Imaging evidence of MSU crystal deposition **without** clinical disease elements of gout	Yes (3)	86%	–	–	Asymptomatic MSU crystal deposition
	Hyperuricaemia‡ **with** imaging evidence of MSU crystal deposition **without** clinical disease elements† of gout	No	–	Yes	100%	Asymptomatic hyperuricaemia‡ with MSU crystal deposition
Clinical states	Presence of MSU crystals **with** clinical disease elements† of gout	No	–	Yes	97%	Gout
	Presence of MSU crystals **with** any of the following: frequent recurrent gout flares, chronic gouty arthritis, subcutaneous tophi or imaging disease elements¶ of gout	Yes (2)	82%	–	–	Severe gout**
	Presence of MSU crystals **with** subcutaneous tophi	Yes (1)	89%	–	–	Tophaceous gout
	Presence of MSU crystals **with** clinical disease elements† of gout and **with** at least one gouty bone erosion	Yes (1)	82%	–	–	Erosive gout
Disease course states	The first episode of gout flare **without** preceding intercritical gout	Yes (3)	83%	–	–	First gout flare
	More than one episode of gout flare **with** intercritical gout	Yes (3)	89%	–	–	Recurrent gout flares

*Consensus defined as ≥80% agreement on preferred label.
†Clinical disease elements: gout flare, intercritical gout, chronic gouty arthritis, subcutaneous tophus.
‡In British English, hyperuricaemia.
§Imaging disease elements: imaging evidence of MSU crystal deposition, gouty bone erosion.
¶The disease state 'severe gout' was subsequently determined **not** to be clinically meaningful by consensus agreement[2] during the face-to-face consensus meeting.
MSU, monosodium urate.

DISCUSSION

In this project, we have achieved consensus agreement on the labels and definitions for disease states of gout. This project builds on the G-CAN-endorsed nomenclature for the disease elements of gout,[7] which provided a foundation for both the extraction of disease element clusters in the content analysis of the literature, and for the formulation of disease state terminology. The G-CAN endorsed labels for disease elements and for disease states should be used concurrently where appropriate. These technical language labels and definitions for disease states which have been endorsed by G-CAN have been developed for use by healthcare professionals and non-physician scientists in clinical and research settings.

Our content analysis of the literature demonstrated that the existing terminology of the disease states of gout is deficient in a number of key areas. Disease states were, in general, infrequently mentioned, poorly defined or inconsistently labelled in the large body of contemporary gout-related literature that was analysed. With the exception of 'asymptomatic hyperuricaemia', little mention was made of pre-clinical disease states defined by the presence of monosodium urate crystal deposition on imaging and the absence of clinical disease elements of gout. Increasing availability of advanced imaging such as ultrasound and DECT will inevitably lead to increased detection of monosodium urate crystal deposition prior to the development of clinical disease. While further research is required regarding the sensitivity and specificity of these imaging modalities, and the implications of these findings for disease management, there is a need to consistently label and define these pre-clinical states. This project has provided consensus labels and definitions for two further pre-clinical disease states: 'asymptomatic monosodium urate crystal deposition' and 'asymptomatic hyperuricaemia with monosodium urate crystal deposition'.

One of the key outcomes of this project was defining the label 'gout'. There was much discussion about what constitutes 'gout', whether it is the presence of monosodium urate crystal deposition, or more specifically, the clinical manifestations resulting from this crystal deposition. In this consensus statement, we recommend the label 'gout' be used only when there are current or prior clinical symptoms or signs of monosodium

Table 5 G-CAN endorsed labels and definitions for the disease states of gout

	Consensus label	Consensus definition
Pre-clinical states	1. Asymptomatic hyperuricaemia*	Hyperuricaemia* in the absence of gout.
	2. Asymptomatic MSU crystal deposition	Evidence of MSU crystal deposition in the absence of gout. MSU crystal deposition may be demonstrated by imaging or microscopic analysis
	3. Asymptomatic hyperuricaemia* with MSU crystal deposition	Hyperuricaemia* with evidence of MSU crystal deposition in the absence of gout. MSU crystal deposition may be demonstrated by imaging or microscopic analysis
Clinical states	4. Gout	A disease caused by MSU crystal deposition with any of the following clinical presentations (current or prior): gout flare, chronic gouty arthritis, or subcutaneous tophus
	5. Tophaceous gout	Gout with at least one subcutaneous tophus
	6. Erosive gout	Gout with at least one gouty bone erosion
Disease course states	7. First gout flare	The first episode of gout flare
	8. Recurrent gout flares	More than one gout flare
Additional recommendation on disease states not addressed by the nomenclature		Where there is more than one disease state present, these can be combined (eg, tophaceous and erosive gout). Where there are additional elements present, not recognised as disease states, these will be labelled as the recognised disease state with or without additional disease elements (eg, tophaceous gout with chronic gouty arthritis)

*In British English, hyperuricaemia.
MSU, monosodium urate.

urate crystal deposition (including gout flare, chronic gouty arthritis or subcutaneous tophus). The prognostic significance of asymptomatic monosodium urate crystal deposition is currently uncertain and we recommend that the label 'gout' is not used in the absence of current or prior clinical symptoms or signs caused by monosodium urate crystal deposition. Another key outcome was the rejection of non-specific labels of the clinical features of gout, such as 'severe gout', which are, despite their ambiguity, present in a number of international gout management guidelines.[8–11] Where cluster of elements cannot be described using a single label, guidance has been provided for the use of consistent nomenclature.

In summary, this consensus statement presents recommended labels and definitions for disease states of gout. The G-CAN recommends the use of these labels when communicating in the scientific literature and in professional practice.

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Contributors ND (the guarantor) accepts full responsibility for the work and the conduct of the project, had access to the data and controlled the decision to publish. ND, DB, WJT and RT conceived of the project. DB and ND were responsible for devising the Delphi exercise surveys and the running of the face-to-face meeting, including the analysis of results. All authors participated in either or both of the Delphi exercise and face-to-face consensus meeting. DB and ND drafted the first version of the manuscript. All authors contributed to manuscript revisions and approved the final manuscript.

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REFERENCES

1 Bursill D, Taylor WJ, Terkeltaub R, *et al*. The nomenclature of the basic disease elements of gout: a content analysis of contemporary medical journals. *Semin Arthritis Rheum* 2018;48:456–61.
2 Lawrence Edwards N, Malouf R, Perez-Ruiz F, *et al*. Computational Lexical analysis of the language commonly used to describe gout. *Arthritis Care Res* 2016;68:763–8.
3 Liddle J, Roddy E, Mallen CD, *et al*. Mapping patients' experiences from initial symptoms to gout diagnosis: a qualitative exploration. *BMJ Open* 2015;5:e008323.
4 Vaccher S, Kannangara DRW, Baysari MT, *et al*. Barriers to care in gout: from prescriber to patient. *J Rheumatol* 2016;43:144–9.
5 Walsh CP, Prior JA, Chandratre P, *et al*. Illness perceptions of gout patients and the use of allopurinol in primary care: baseline findings from a prospective cohort study. *BMC Musculoskelet Disord* 2016;17:394.
6 Bardin T, Richette P. Definition of hyperuricemia and gouty conditions. *Curr Opin Rheumatol* 2014;26:186–91.
7 Bursill D, Taylor WJ, Terkeltaub R, *et al*. Gout, hyperuricemia, and Crystal-Associated disease network consensus statement regarding labels and definitions for disease elements in gout. *Arthritis Care Res* 2019;71:427–34.

8 Hui M, Carr A, Cameron S, *et al*. The British Society for Rheumatology guideline for the management of gout. *Rheumatology* 2017;56:e1–20.
9 Khanna D, Fitzgerald JD, Khanna PP, *et al*. 2012 American College of Rheumatology guidelines for management of gout. Part 1: systematic nonpharmacologic and pharmacologic therapeutic approaches to hyperuricemia. *Arthritis Care Res* 2012;64:1431–46.
10 Richette P, Doherty M, Pascual E, *et al*. 2016 updated EULAR evidence-based recommendations for the management of gout. *Ann Rheum Dis* 2017;76:29–42.
11 Sivera F, Andrés M, Carmona L, *et al*. Multinational evidence-based recommendations for the diagnosis and management of gout: integrating systematic literature review and expert opinion of a broad panel of rheumatologists in the 3E initiative. *Ann Rheum Dis* 2014;73:328–35.

EPIDEMIOLOGICAL SCIENCE

Risk of gout flares after vaccination: a prospective case cross-over study

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ABSTRACT

Objectives The recombinant zoster vaccine (RZV) containing a strong non-aluminium adjuvant is associated with increased risk of gout flares, presumably via NLRP3 inflammasome activation. We tested the possibility that other vaccines may also be associated with gout flares.

Methods We conducted an online case-crossover study of patients with gout to examine the association between vaccination and gout flares. We collected information through the Internet on exposures to potential risk factors, including vaccinations, during 2-day hazard periods prior to gout flare and 2-day control periods without a flare. Conditional logistic regression was used to adjust for covariates.

Results There were 517 participants with gout (mean age 55 years, 79% male) who experienced gout flares during follow-up. There were 28 vaccinations during 990 hazard periods and 21 vaccinations during 1407 control periods. Vaccination was associated with twofold higher odds of gout flare (adjusted OR 1.99; 95% CI 1.01 to 3.89).

Conclusion Our findings suggest vaccines other than RZV are associated with increased odds of gout flares, potentially through a shared pathogenetic mechanism like NLRP3 inflammasome. However, the absolute magnitude of increased odds of gout flares with vaccinations remains small and must be interpreted within the context of the overwhelming benefits of vaccinations.

Key messages

What is already known about this subject?
- The new recombinant shingles vaccine (RZV) was associated with increased risk of gout flares.
- Aluminium adjuvants contained in vaccines can activate the NLRP3 inflammasome *in vitro*.

What does this study add?
- This study showed that receiving a non-RZV vaccine was associated with a two-fold higher odds of developing a gout flare within 2 days of vaccination compared with periods when vaccination was not administered.

How might this impact on clinical practice or future developments?
- These findings warrant further investigation into whether temporary prophylactic use of anti-inflammatory medications may mitigate the risk of gout flares with vaccination without affecting vaccine efficacy.
- These findings must be interpreted within the context of overwhelming benefits of vaccines worldwide.

INTRODUCTION

The recombinant zoster vaccine (RZV) provides enhanced protection against shingles and postherpetic neuralgia and is now the preferred zoster vaccine endorsed by the Advisory Committee on Immunization Practices.[1] However, safety data from two phase III trials for RZV demonstrated a 3.6-fold higher risk of gout.[2–4] Whether this is unique to RZV or if other vaccines may also increase the risk of gout flares is unknown. Given gout's prevalence among the elderly[5 6] and those with multiple comorbidities[7] who benefit most from routine vaccinations, it is important to determine whether vaccines other than RZV similarly increase the risk of gout flares.

Studies have shown that monosodium urate-induced activation of the NLRP3 inflammasome, leading to maturation and release of interleukin-1 beta, is a key second signal triggering the inflammatory cascade responsible for gout flares.[8] The non-aluminium adjuvant used in RZV is hypothesised to activate the NLRP3 inflammasome.[4] Furthermore, aluminium adjuvants, which are contained in more than half of routinely administered adult vaccines,[9] have also been shown to activate the NLRP3 inflammasome.[10 11] This study tested the hypothesis that vaccines other than RZV may also be associated with increased acute gout flares using a prospective case-crossover study[12] that recorded both exposures and gout flares before the RZV vaccine was available.

METHODS

Study design and participants

We conducted a prospective, Internet-based, case-crossover study with the primary aim to investigate purported triggers for recurrent gout flares as previously described.[12] This study was conducted between 2003 and 2010, thus, before the introduction of the RZV vaccine.[1] The study design and timing of exposure measurements in relation to recurrent gout flares are depicted in figure 1.

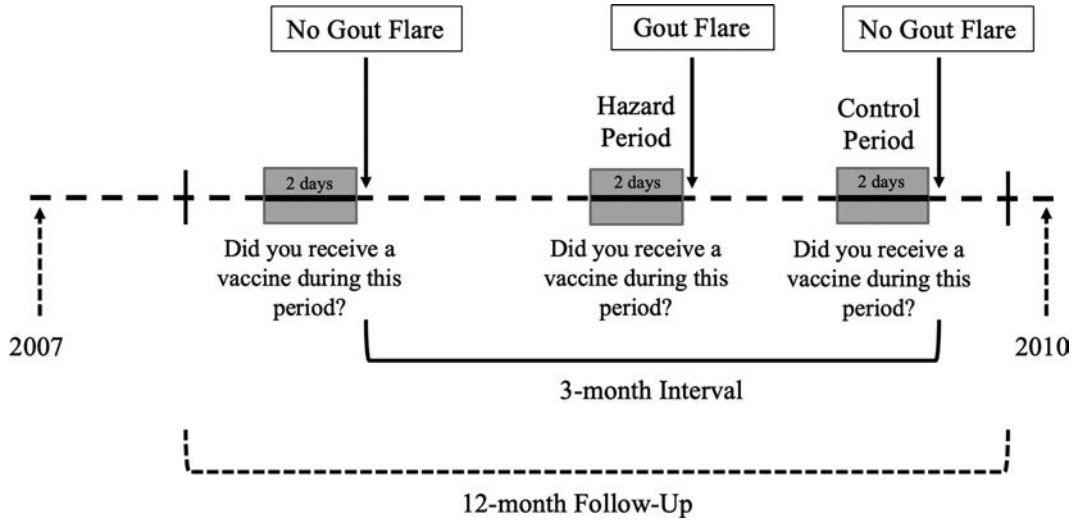

Figure 1 Study design and timing of exposure measurements.

Eligibility criteria included history of physician-diagnosed gout and at least one gout flare in the last year.[12] Medical records pertaining to gout diagnosis and treatment were collected so that the diagnosis could be adjudicated according to the 1977 American College of Rheumatology (ACR) Preliminary Gout Classification Criteria.[13] Each participant was asked to complete online questionnaires every 3 months over the 1-year follow-up and at the time of a gout flare. A question regarding vaccination was added in 2007. The case-crossover study design allows each participant to serve as his/her own control, thereby eliminating time-fixed confounding between participants.[12]

Patient and public involvement

There was no patient or public involvement in the design or conduct of this study.

Ascertainment of gout flares

Participants were asked to log into the study website at the time of gout flares. Consistent with the methods proposed by ACR/European League Against Rheumatism,[14] we collected data at the time of gout flare regarding date of onset, clinical signs and symptoms, and any medications used to treat the flare.

Ascertainment of exposure

Subjects completed questionnaires about exposures to purported risk factors such as purine intake, alcohol consumption and medication exposures during the 2-day period prior to the gout flare (ie, hazard period). To ascertain vaccination, subjects were asked: 'Did you receive an immunization, such as a flu shot, tetanus, travel vaccination?' These same questions regarding exposures to purported risk factors were also ascertained over a 2-day period when the participant was free from a gout flare (ie, control period) every 3 months over the 1-year follow-up.

Statistical analysis

Because every participant could contribute multiple hazard or control periods which were matched within a participant, we used a conditional logistic regression for M:N matched case-control study to examine the relation of vaccination to risk of gout flares, adjusting for alcohol consumption, diuretic use and purine intake.

RESULTS

There were 517 participants with gout who completed both hazard and control period questionnaires; their baseline characteristics are shown in table 1. The mean age was 55 years, and 79% were male. Most responders were Caucasian and college educated (89% and 59%, respectively). The participants reported a mean disease duration of 7.9 (range 0–55) years, and a mean of 1.9 gout attacks per person.

During the 1-year follow-up period, the participants completed 990 hazard period questionnaires, of which 28 reported receiving vaccinations in the 2-day period prior to flare onset (table 2). Of the 1407 control period questionnaires completed, 21 of them reported receiving vaccinations. Receiving a vaccination within the prior 2 days was associated with two-fold higher odds of developing a gout flare (adjusted OR 1.99; 95% CI 1.01 to 3.89). We pursued a parsimonious multivariable model, including only conventionally known triggers of gout (ie, alcohol, purine and diuretics), as our bivariate models did not suggest confounding by any covariate (see online Supplemental Table 1). For male participants, vaccination within the prior 2 days was associated with a 2.4-fold (adjusted OR 2.35, 95% CI 1.12 to 4.92) higher odds of developing a gout flare, whereas the small number of vaccinations did not allow for robust multivariable adjustment (see online supplemental table 1). The OR tended to be larger among participants ≤60 years, those on allopurinol, non-steroidal anti-inflammatory drugs and diuretics, and those with higher alcohol and purine consumption, although none reached

Table 1 Participant characteristics

Characteristics	N=517 participants
Age (mean, SD)	55 (13)
Male (n, %)	406 (79)
White (n, %)	460 (89)
Completed college (n, %)	305 (59)
BMI (kg/m^2, mean, SD)	32 (7)
Disease duration, years (mean, range)	7.9 (0–55)
Gout flares per person (n, mean)	1.9
Control periods per person (n, mean)	2.7

BMI, body mass index.

Table 2 Vaccination in the prior 48-hour period and risk of recurrent gout flares

Vaccination	Hazard periods (n)	Control periods (n)	Crude OR (95% CI)	Adjusted OR* (95% CI)
No	962	1386	1.0 (reference)	1.0 (reference)
Yes	28	21	2.16 (1.14 to 4.12)	1.99 (1.01 to 3.89)

*Adjusted for alcohol consumption, diuretic use and purine intake.

statistical significance (see online supplemental table 1) (all p values for interaction >0.05).

DISCUSSION

In this case-crossover study of patients with gout, vaccination in the prior 2 days was associated with a two-fold increased odds of gout flares compared with periods when vaccination was not administered. Because this study was conducted before the availability of RZV, our finding expands on what is currently known about vaccinations and risk of gout flares. In two phase III clinical trials for RZV, 27 patients (0.18%) who received RZV reported a gout flare within 30 days of vaccine administration compared with 8 patients (0.05%) who received placebo,[2 3] yielding a 3.6-fold higher risk of gout flare with RZV.[2–4] This has prompted the implementation of an enhanced postmarketing surveillance programme for gout flares.[4] Our findings suggest that this adverse effect is also applicable to other vaccines, although the effect size may be lower.

These findings collectively raise relevant pathogenic implications. The activation of the NLRP3 inflammasome is hypothesised to be the key mechanistic link between vaccinations and gout flares. The non-aluminium adjuvant contained in RZV triggers a local and transient activation of the innate immune system through MPL and QS-21 signalling, the latter of which is believed to involve the NLRP3 inflammasome.[15] Aluminium adjuvants, which are contained in half of all routine adult vaccines, including tetanus, diphtheria and pertussis (Tdap, Td), pneumococcal conjugate (PCV-13) and hepatitis B vaccines,[16] have also been shown to activate the NLRP3 inflammasome *in vitro*.[10 11] As such, aluminium adjuvants in vaccines can conceivably trigger gout flares through the same mechanism. Whether other adjuvants, live and killed organisms, and recombinant peptide constituents of vaccines also promote gout flares remains to be clarified.

Nevertheless, the clinical implications of our findings must be interpreted carefully, as vaccines decrease the morbidity and mortality associated with communicable illnesses.[17] Given the high prevalence of gout among patients most susceptible to adverse outcomes from infections, such as the elderly[5 6] and those with multiple comorbidities,[7] avoiding vaccinations due to the increased odds of gout flares is not advisable. There appears to be a low absolute frequency of gout flares in relation to vaccination, both in the RSV trials[2–4] and our current study. Studying the capacity of prophylactic use of anti-inflammatory medications to prevent such flares without hindering vaccine efficacy may be warranted.

Some strengths and weaknesses of this study warrant comment. The data were deliberately collected prospectively through the Internet to effectively capture real-life time-varying triggers of gout over a short timeframe (ie, 24–48 hours). As such, this study design is highly adaptable to assessing the effect of a transient exposure as a trigger for an acute event,[18] and self-matching of each participant minimises bias in control selection and removes the confounding effects of factors that are constant within a subject over the study period but differ between participants.[19] The participants in our study may not be representative of patients with gout in the general population given predominance of White and college-educated patients; however, the biological effects of vaccination on gout flares should be similar. Although this study relied on self-report of exposures, vaccination was not suspected of causing gout flares during the entirety of this study, leaving no ground for preferential recall. While we were unable to verify vaccine administration through the patients' medical records, vaccination is known to be one of the most accurately reported medical exposures,[20] thereby minimising potential for recall bias. Weaknesses include the small number of events not allowing for robust conclusions from our subgroup analyses and lacking information on specific vaccines administered which may have been associated with gout flares.

In conclusion, this study suggests that vaccination in the prior 2 days is associated with a two-fold higher odds of gout flares compared with periods when no vaccine was administered. However, these findings must be interpreted within the context of the low frequency of gout flares in relation to vaccinations and the overwhelming benefits of vaccinations.

Contributors TN, YZ, HC contributed to study design. CC, TN, CEC, YZ and HC contributed to study conduct. CY, NM, YZ and HC contributed to study analysis. All authors contributed to interpretation of results. CY and HC drafted the manuscript, and all authors reviewed and revised the manuscript for content.

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REFERENCES

1 Dooling KL, Guo A, Patel M, *et al*. Recommendations of the Advisory Committee on immunization practices for use of herpes zoster vaccines. *MMWR Morb Mortal Wkly Rep* 2018;67:103–8.
2 Cunningham AL, Lal H, Kovac M, *et al*. Efficacy of the herpes zoster subunit vaccine in adults 70 years of age or older. *N Engl J Med* 2016;375:1019–32.
3 Lal H, Cunningham AL, Godeaux O, *et al*. Efficacy of an adjuvanted herpes zoster subunit vaccine in older adults. *N Engl J Med* 2015;372:2087–96.
4 Paula EA, Rebecca Reindel MD. *Fda biologics license application clinical review memorandum: Shingrix*, 2017.
5 Kuo C-F, Grainge MJ, Zhang W, *et al*. Global epidemiology of gout: prevalence, incidence and risk factors. *Nat Rev Rheumatol* 2015;11:649–62.
6 Kuo C-F, Grainge MJ, Mallen C, *et al*. Rising burden of gout in the UK but continuing suboptimal management: a nationwide population study. *Ann Rheum Dis* 2015;74:661–7.
7 Zhu Y, Pandya BJ, Choi HK. Comorbidities of gout and hyperuricemia in the US general population: NHANES 2007-2008. *Am J Med* 2012;125:679–87.
8 Martinon F, Pétrilli V, Mayor A, *et al*. Gout-associated uric acid crystals activate the NALP3 inflammasome. *Nature* 2006;440:237–41.

9 Richette P, Clerson P, Périssin L, *et al*. Revisiting comorbidities in gout: a cluster analysis. *Ann Rheum Dis* 2015;74:142–7.
10 Eisenbarth SC, Colegio OR, O'Connor W, *et al*. Crucial role for the NALP3 inflammasome in the immunostimulatory properties of aluminium adjuvants. *Nature* 2008;453:1122–6.
11 Franchi L, Núñez G. The NLRP3 inflammasome is critical for aluminium hydroxide-mediated IL-1beta secretion but dispensable for adjuvant activity. *Eur J Immunol* 2008;38:2085–9.
12 Zhang Y, Chaisson CE, McAlindon T, *et al*. The online case-crossover study is a novel approach to study triggers for recurrent disease flares. *J Clin Epidemiol* 2007;60:50–5.
13 Wallace SL, Robinson H, Masi AT, *et al*. Preliminary criteria for the classification of the acute arthritis of primary gout. *Arthritis Rheum* 1977;20:895–900.
14 Gaffo AL, Dalbeth N, Saag KG, *et al*. Brief report: validation of a definition of flare in patients with established gout. *Arthritis Rheumatol* 2018;70:462–7.
15 Heineman TC, Cunningham A, Levin M. Understanding the immunology of Shingrix, a recombinant glycoprotein E adjuvanted herpes zoster vaccine. *Curr Opin Immunol* 2019;59:42–8.
16 CDC. Adjuvants help vaccines work better. Available: https://www.cdc.gov/vaccinesafety/concerns/adjuvants.html [Accessed 22 Oct 2018].
17 De Gregorio E, Rappuoli R. From empiricism to rational design: a personal perspective of the evolution of vaccine development. *Nat Rev Immunol* 2014;14:505–14.
18 Maclure M. The case-crossover design: a method for studying transient effects on the risk of acute events. *Am J Epidemiol* 1991;133:144–53.
19 McAlindon T, Formica M, Kabbara K, *et al*. Conducting clinical trials over the Internet: feasibility study. *BMJ* 2003;327:484–7.
20 Maclure M, Fireman B, Nelson JC, *et al*. When should case-only designs be used for safety monitoring of medical products? *Pharmacoepidemiol Drug Saf* 2012;21(Suppl 1):50–61.

Low specificity but high sensitivity of inflammatory back pain criteria in rheumatology settings in Europe: confirmation of findings from a German cohort study

Inflammatory back pain (IBP) is considered so essential in the axial spondyloarthritis (axSpA) diagnostic process that it is recommended as referral parameter in primary care.[1–3] However, axSpA patients without IBP do exist as well as patients with IBP that do not have an axSpA diagnosis, leaving IBP to be a strong and useful feature for recognising axSpA but not pathognomonic.[4 5] A recent study in German chronic back pain patients with a suspicion of axSpA report on the performance of, among others, the Assessment of Spondyloarthritis international Society (ASAS) IBP criteria and individual IBP parameters. Data showed high sensitivity of the criteria and individual IBP parameters, however specificity was low.[6] Important findings like low specificity indicate that in the German rheumatology setting diagnostic utility of IBP criteria was lower than expected. In addition, they commonly find IBP in patients in routine rheumatology care, suggesting that IBP is used as a reference parameter in primary care and that the diagnostic value is almost completely used up in that setting.

The performance of ASAS IBP criteria in other countries is less known. Therefore, this study reports on the diagnostic utility of IBP according to the ASAS criteria and the individual IBP parameters in several rheumatology settings throughout Europe.

Data of the multicentre, observational BelGian inflammatory arthritis and spondylitis (Be-Giant) and international SPondyloArthritis Cause Early (SPACE) cohorts were used. The Be-Giant cohort included ≥18 years old, newly diagnosed axSpA patients who were diagnosed by a team of expert rheumatologists and fulfilled the ASAS axSpA criteria. The SPACE cohort included patients, ≥16 years, with (almost) daily chronic back pain for ≥3 months and ≤2 years, with a symptom onset <45 years. All patients underwent a diagnostic workup according to a predefined protocol of the corresponding cohort, including data on the individual parameters of the ASAS IBP criteria.

AxSpA patients from the SPACE cohort diagnosed by a rheumatologist with a level of confidence (LOC) on the diagnosis of ≥7 on a 0–10 scale were included as 'axSpA'. All patients in SPACE without axSpA diagnosis (LOC ≥7 for no axSpA) were categorised as 'no axSpA'. For patients with a LOC <7, diagnosis was considered not sufficiently certain and these patients were excluded from primary analyses. Sensitivity, specificity and positive likelihood ratios (LR+) were calculated using axSpA patients from each cohort separately and no axSpA patients as control group.

Information on IBP parameters was available in 228 patients (Be-Giant) and 559 patients (SPACE) of whom 49.6% and 39.5% were male and mean age was 34.7 (SD 9.7) and 30.7 (SD 8.0) years, respectively. Few axSpA patients had <2 IBP

Table 1 Frequencies (1A) and performance (1B) of IBP criteria and the individual parameters in axSpA patients and patients with chronic back pain

	Be-Giant cohort	SPACE cohort	
1A	axSpA N=205	axSpA* N=307	No axSpA† N=252
IBP (ASAS criteria), n (%)	172 (83.9)	228 (74.3)	122 (48.4)
Age at onset <40 years	197 (96.1)	277 (90.2)	224 (88.9)
Insidious onset	185 (90.2)	261 (85.0)	212 (84.1)
Improvement with exercise	181 (88.3)	252 (82.1)	151 (59.9)
No improvement with rest	172 (83.9)	264 (86.0)	193 (76.6)
Pain at night	162 (79.0)	172 (56.0)	165 (65.5)
No of IBP parameters present, n (%)			
0	0	1 (0.3)	0
1	1 (0.5)	5 (1.6)	5 (2.0)
2	7 (3.4)	22 (7.2)	22 (8.7)
3	25 (12.2)	51 (16.6)	51 (20.2)
4	53 (25.9)	116 (37.8)	116 (46.0)
5	119 (58.1)	112 (36.5)	112 (44.4)

	Sensitivity (95% CI)		Specificity (95% CI)	LR+	
1B	Be-Giant cohort N=205	SPACE cohort N=307	SPACE cohort‡ N=252	Be-Giant cohort	SPACE cohort
IBP (ASAS criteria)	83.9% (78.0 to 88.5)	74.3% (68.9 to 79.0)	51.6% (45.2 to 57.9)	1.7	1.5
Age at onset <40 years	96.1% (92.2 to 98.2)	90.2% (86.2 to 93.2)	11.1% (7.6 to 12.9)	1.1	1.0
Insidious onset	90.2% (85.1 to 93.8)	85.0% (80.4 to 88.7)	15.9% (11.7 to 21.1)	1.1	1.0
Improvement with exercise	88.3% (82.9 to 92.2)	82.1% (77.2 to 86.1)	40.1% (34.0 to 46.4)	1.4	1.3
No improvement with rest	83.9% (78.0 to 88.5)	86.0% (81.5 to 89.6)	23.4% (18.4 to 29.2)	1.1	1.1
Pain at night	79.0% (72.7 to 84.3)	56.0% (50.3 to 61.6)	34.5% (28.7 to 40.8)	2.1	1.5

*axSpA diagnosis according to rheumatologist with a level of confidence of ≥7 on a 0–10 scale.
†No axSpA patients according to rheumatologist with a level of confidence of ≥7 on a 0–10 scale.
‡Only patients without axSpA diagnosis according to rheumatologist (level of confidence of <7 on a 0–10 scale) are used as control group to calculate specificity.
ASAS, Assessment of Spondyloarthritis international Society;Be-Giant, BelGian inflammatory arthritis and spondylitis
; IBP, inflammatory back pain; LR+, positive likelihood ratio; SPACE, SPondyloArthritis Cause Early; axSpA, axial spondyloarthritis.

parameters (table 1A; Be-Giant 3.9%, SPACE 9.1%). The individual IBP parameters show high sensitivity (>80%) but low specificity (<36%) with 'pain at night' as exception with slightly better specificity. LR+ of IBP were in both cohorts much lower (table 1B) than the LR+ for IBP of 3.1 previously reported as diagnostic estimate in routine practice.[7] When including patients with an uncertain diagnosis (LOC <7) as control group, we see similar low specificity as with the stricter diagnosis of no axSpA patients (LOC ≥7).

IBP and individual IBP parameters are commonly seen in patients with and without an axSpA diagnosis. Our data confirm results from Germany, in which similar sensitivity (73.9–88.9) and specificity (22.7–39.5) for IBP according to the ASAS criteria are described.[6] These studies show that approximately every second patient seen by a rheumatologist fulfils the IBP criteria but does not have axSpA. The diagnostic utility of IBP criteria in a rheumatology setting in several European countries is lower than previously assumed with a (very) low specificity and LR+. This suggests that the distinctive impact of IBP is almost fully expressed when physicians refer their patient to the rheumatologist.

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REFERENCES

1 Braun A, Saracbasi E, Grifka J, *et al*. Identifying patients with axial spondyloarthritis in primary care: how useful are items indicative of inflammatory back pain? *Ann Rheum Dis* 2011;70:1782–7.
2 Abawi O, van den Berg R, van der Heijde D, *et al*. Evaluation of multiple referral strategies for axial spondyloarthritis in the spondyloarthritis caught early (space) cohort. *RMD Open* 2017;3:e000389.
3 Braun A, Gnann H, Saracbasi E, *et al*. Optimizing the identification of patients with axial spondyloarthritis in primary care--the case for a two-step strategy combining the most relevant clinical items with HLA B27. *Rheumatology* 2013;52:1418–24.
4 Sieper J, van der Heijde D, Landewé R, *et al*. New criteria for inflammatory back pain in patients with chronic back pain: a real patient exercise by experts from the Assessment of SpondyloArthritis International Society (ASAS). *Ann Rheum Dis* 2009;68:784–8.
5 Rudwaleit M, Landewé R, van der Heijde D, *et al*. The development of Assessment of SpondyloArthritis International Society classification criteria for axial spondyloarthritis (Part I): classification of paper patients by expert opinion including uncertainty appraisal. *Ann Rheum Dis* 2009;68:770–6.
6 Poddubnyy D, Callhoff J, Spiller I, *et al*. Diagnostic accuracy of inflammatory back pain for axial spondyloarthritis in rheumatological care. *RMD Open* 2018;4:e000825.
7 Rudwaleit M, Khan MA, Sieper J. The challenge of diagnosis and classification in early ankylosing spondylitis: do we need new criteria? *Arthritis Rheum* 2005;52:1000–8.

Refractory sarcoidosis-like systemic granulomatosis responding to ruxolitinib

In 2012, a 51-year-old woman presented with a 2-year history of painful hepatomegaly, chronic asthenia and recurrent fevers. Her medical history comprised augmentation mammoplasty 6 years prior to the onset of her symptoms and she received fenofibrate as a treatment for dyslipidaemia. Physical examination found an increased liver span and splenomegaly, cervical lymphadenopathy, and peritoneal and pleural effusion outbreaks. There was neither rash nor joint pain. Plasma inflammatory markers were constantly raised with C reactive protein (CRP) levels of 80–100 mg/L, increasing when fever and effusions occurred. Subsequently, in the course of the disease, biochemical analysis found hypercalcaemia peaking at 3.81 mmol/L with hypercalciuria. The blood cells count, serum angiotensin conversion enzyme, parathormone (PTH), PTH-rp and plasma protein electrophoresis were constantly unremarkable. Fludeoxyglucose F18 positron-emission tomography (FDG-PET) showed heterogeneous hypermetabolism of the liver, spleen, entire bone frame and several mediastinal, thoracic and abdominal lymph nodes (figure 1). Repetitive pleural and ascites punctures found an exudative, lymphoneutrophilic and aseptic fluid without atypical cells. Over a 5-year period, multiple sites were biopsied including the liver (×3), lymph nodes (×2), bone (×3), minor salivary glands (×1), skin (×1), bronchi (×1), and gastric and colic epitheliomas (×3 each). Liver, lymph nodes, bone and skin specimens were characterised by numerous gigantocellular epithelioid granulomas without caseous necrosis, surrounded by a rich neutrophilic infiltrate, without vasculitis. Other tissues remained healthy. Staining and immunohistochemistry were uninformative. There was neither evidence of infection, immunodeficiency, vasculitis nor other autoimmune diseases, solid cancer, hemopathy and/or drug-induced processes.

Sarcoidosis was suspected in 2012 and the patient was started on a prednisone course of 1 mg/kg/day that led to a regression of clinical features. A relapse of the granulomatous disease occurred 2 months later under a prednisone regimen of 0.5 mg/kg/day. The subsequent relapses prompted a reversal of the diagnosis of sarcoidosis.

The patient was then treated with an empirical antimicrobial therapy (doxycyclin and 1 year of antituberculosis therapy) and

subsequently immunosuppressant drugs, such as hydroxychloroquine, azathioprine, mycophenolate mofetil, cyclophosphamide, metothrexate, tumor necrosis factor (TNF)-α inhibitors or interleukin 1β antagonist with occasional pulse steroid therapy (figure 1), for a sarcoidosis-like systemic granulomatosis.

After 5 years of refractory granulomatous disease, the patient was started on 2 ruxolitinib at 20 mg per day, rapidly increased to 30 mg. At the 3-month mark, clinical and biological remission was obtained. Steroids were then permanently discontinued. Inflammatory markers dropped significantly with CRP values of 17 mg/L, and calcaemia normalised. Clinical and biological remission was maintained at the 12-month and 18-month marks despite weaning of steroid therapy. FDG-PET evaluation showed significantly lower hypermetabolism in previously hypermetabolic sites (figure 1). Overall tolerance of ruxolitinib was good with dosing reduced to and maintained at 25 mg q.d.

To the best of our knowledge, this is the first reported case of refractory systemic granulomatosis treated with—and responding to—ruxolitinib, a Janus kinase (JAK) 1 and 2 inhibitor (JAKi-1 and JAKi-2). Other JAKi, such as tofacitinib, a JAKi-1 and JAKi-3, or baricitinib, a JAKi-1 and JAKi-2, have been shown to be effective in a number of inflammatory and autoimmune diseases (ie, rheumatoid arthritis).[1 2] Filgotinib, a selective JAKi-1, has shown promising results in the granulomatous Crohn's disease.[3] Such effectiveness implies that selective inhibition of JAK1 could be a therapeutic key in most granulomatous diseases. Ruxolitinib is being used in myeloproliferative diseases as well as in graft versus host disease with a good overall efficacy and safety profile.[4] However, its use in inflammatory disease is poorly reported. By inhibiting the JAK/STAT (signal transducers and activators of transcription) pathway, innate and adaptive immunity is attenuated as the effector molecules from the T helper 17 cell pathway, such as interleukin (IL)-17, IL-22 and interferon gamma, are suppressed.[5] This is the very same pathway that is implicated in T-cell granuloma formation.[6]

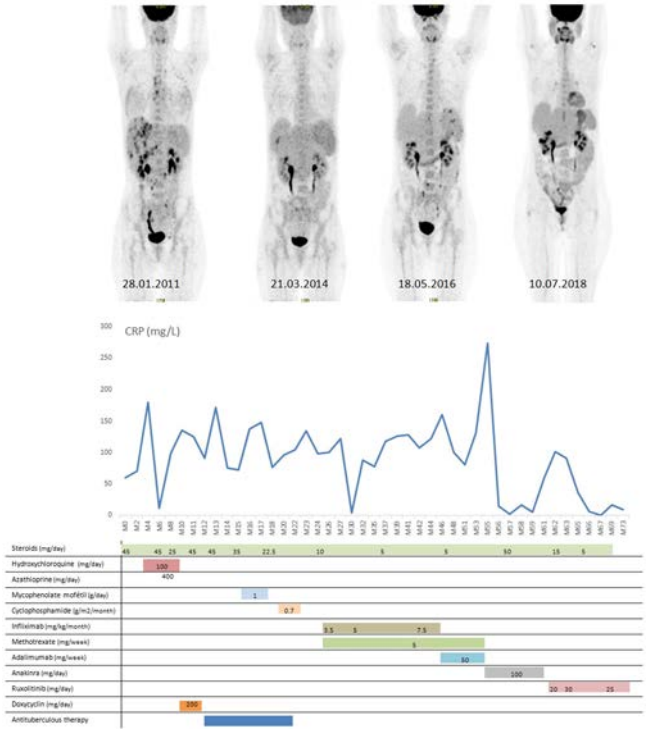

Figure 1 [18F]-FDG-positron emission tomography imaging at the initiation of treatment and during follow-up, and C reactive protein dosing during the course of the disease. CRP, C reactive protein.

Our report illustrates a 'bench to bedside'" approach in a case of refractory sarcoidosis-like systemic granulomatosis. Ruxolitinib appears to be a relatively safe and effective therapeutic option for the management of relapsing granulomatous diseases.

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REFERENCES

1 van Vollenhoven RF, Fleischmann R, Cohen S, *et al*. Tofacitinib or adalimumab versus placebo in rheumatoid arthritis. *N Engl J Med* 2012;367:508–19.
2 Combe B, Balsa A, Sarzi-Puttini P, *et al*. Efficacy and safety data based on historical or pre-existing conditions at baseline for patients with active rheumatoid arthritis who were treated with baricitinib. *Ann Rheum Dis* 2019;78:1135–8.
3 Vermeire S, Schreiber S, Petryka R, *et al*. Clinical remission in patients with moderate-to-severe Crohn's disease treated with filgotinib (the Fitzroy study): results from a phase 2, double-blind, randomised, placebo-controlled trial. *Lancet* 2017;389:266–75.
4 Al-Ali HK, Griesshammer M, le Coutre P, *et al*. Safety and efficacy of ruxolitinib in an open-label, multicenter, single-arm phase 3B expanded-access study in patients with myelofibrosis: a snapshot of 1144 patients in the jump trial. *Haematologica* 2016;101:1065–73.
5 Seif F, Khoshmirsafa M, Aazami H, *et al*. The role of JAK-STAT signaling pathway and its regulators in the fate of T helper cells. *Cell Commun Signal* 2017;15.
6 Sakthivel P, Bruder D. Mechanism of granuloma formation in sarcoidosis. *Curr Opin Hematol* 2017;24:59–65.

Patient and public involvement in biomedical research: training is not a substitute for relationship building

Public and patient involvement (PPI) is increasingly an expectation of funders and policy-makers. Not all areas of health research are obvious or accessible to patients. Involving the public in preclinical and laboratory-based research in a mutually beneficial way can be challenging. Fundamentally, there is confusion among preclinical researchers as to what PPI is and how it is applied.[1] PPI is not about unleashing the public into your laboratory; rather it is about increasing research relevance and

cooperating with people living with rheumatic disease to enable careful and deliberate study design.

In practice, how can you involve patients meaningfully and in line with European League Against Rheumatism recommendations[2] if you have not already established mutual respect, a supportive environment and a relationship built on equal status?

Too often, the literature aimed at guiding researchers refers to 'training' as the solution to involving patients in research. Without some grasp of the relevant science, the public cannot be expected to make informed decisions about research. In the absence of a pre-existing relationship, how can researchers understand a patient's information needs and appropriately plan PPI to ensure their research is accessible?[3]

We used a community-based approach to build our PPI community in advance of engaging in active research involvement. Consequently, we could lay the foundations of building relationships and setting expectations. This allowed us to understand the individual information needs of our public partners and be guided by them. Mutual learning via diversity of experience is key to PPI; hence, a reactive approach rather than a prescriptive training approach has been hugely beneficial in the bespoke environment of PPI. Through this approach, and local community engagement, we built and maintain an active, engaged community of potential research partners.

Our research group carries out predominantly preclinical and biomedical rheumatology research. Science communication is a critical skill for PPI and is a recognised barrier to PPI.[1 4 5] To facilitate meaningful PPI in preclinical research, more groundwork may need to be done then in public-facing research. One of the mechanisms for laying this groundwork is increasing public awareness of ongoing research, not just of the headline results.

In direct response to our PPI community, we held an open research conference conducted entirely in 'plain English' (programme: http://www.ucd.ie/car/t4media/Programme.pdf). On a fundamental level, it allowed researchers and people living with rheumatic disease to meet and discuss their shared interests and initiate cooperative relationships. The focus of the conference was to promote collaborative research, in terms of increasing both public involvement and multidisciplinary research. All presenters at the conference were offered a review of their presentation in advance by patient mentors. Four presenters availed of this, three of whom worked in preclinical research (one postgraduate, one postdoctoral Fellow and one professor, all from different disciplines). They worked with two patient mentors to deconstruct and reassemble their presentations in a more stimulating and clear manner. The result was presentations that were more comprehensible by the public and by other researchers, directly leading to more collaboration in the field.

Increasing evidence demonstrates that public involvement is key to achieving research impact and closing the gap between research production and research use.[6] Dedicating resources and time to building the foundational relationships and communication skills necessary for cooperative research involvement will alleviate some of the challenges to PPI in preclinical research. Yes, the system in which biomedical research operates is stretched and PPI can be viewed as yet another drain on resources and time, but, considering the increasing focus on research impact rather than just output, perhaps the time has come when we can no longer afford not to involve the public.

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REFERENCES

1 Maccarthy J, Guerin S, Wilson AG, *et al*. Facilitating public and patient involvement in basic and preclinical health research. *PLoS One* 2019;14:e0216600.
2 de Wit MPT, Berlo SE, Aanerud GJ, *et al*. European League against rheumatism recommendations for the inclusion of patient representatives in scientific projects. *Ann Rheum Dis* 2011;70:722–6.
3 National Academies of Sciences E, Medicine. *Science literacy: concepts, contexts, and consequences*. National academies press, 2016.
4 Burgess HK, DeBey LB, Froehlich HE, *et al*. The science of citizen science: exploring barriers to use as a primary research tool. *Biol Conserv* 2017;208:113–20.
5 Poliakoff E, Webb TL. What factors predict scientists' intentions to participate in public engagement of science activities? *Sci Commun* 2007;29:242–63.
6 Crocker JC, Ricci-Cabello I, Parker A, *et al*. Impact of patient and public involvement on enrolment and retention in clinical trials: systematic review and meta-analysis. *BMJ* 2018;31.

Clinical evidence guidelines in systemic lupus erythematosus: revaluation

The paper on the First Latin American clinical practice guidelines for the treatment of systemic lupus erythematosus (SLE) points to some conclusions that need to be further clarified since the bulk of evidence points to a different direction.[1] Belimumab safety and efficacy has been well documented since its approval during the last 5 years, and in particular the skin and joint domains appear to have a preferential site of major clinical improvement associated with reduction of flares and steroid tapering.[2] However, the authors when placing the best available evidence of therapy for those domains group belimumab with methotrexate and leflunomide for joint manifestations and indicates superiority favouring methotrexate. For the mucocutaneous manifestations, belimumab is grouped with methotrexate, azathioprine, mycophenolate, cyclosporine and cyclophosphamide, and due to cost and availability, recommendation favours methotrexate and azathioprine.

We feel that this degree of recommendation fails to overview the best medical literature published in the past few years pointing to the superiority of belimumab in improving joint and skin disease when compared with other domains, allowing maintenance of steroid sparing and decrease of adverse events by continuous use of daily steroids also reducing the use of immunosuppressive drugs.[3 4] The authors should explain how that conclusion could be reached even when cost issues are raised since there is well-published literature pointing to benefits of belimumab in the long run for several social services when compared with cheaper immunosuppressive drugs that do not reduce steroid intake and lead to increased adverse events and opportunistic infections.[5] The upcoming opportunity to use the subcutaneous route of administration will certainly create additional opportunities for patients with SLE with active disease that could benefit from add-on existing therapies[6] The recent questioning of expert consensus on expert consensus addresses this approach properly and indicates that, although nice, it is not necessarily relevant to accuracy.[7]

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REFERENCES

1 Pons-Estel BA, Bonfa E, Soriano ER. First Latin American clinical practice guidelines for the treatment of systemic lupus erythematosus: latin American group for the study of lup us (GLADEL, *Grupo Latino Americano de Estudio del Lupus*)–Pan-American League of Associations of Rheumatology (PANLAR). *Ann Rheum Dis* 2018:213512.
2 Marcondes F, Scheinberg M. Belimumab in the treatment of systemic lupus erythematous: an evidence based review of its place in therapy. *Autoimmun Rev* 2018;17:103–7.
3 Fanouriakis A, Adamichou C, Koutsoviti S, *et al*. Low disease activity-irrespective of serologic status at baseline-associated with reduction of corticosteroid dose and number of flares in patients with systemic lupus erythematosus treated with belimumab: a real-life observational study. *Semin Arthritis Rheum* 2018;0172:30803–X.
4 Furie RA, Wallace DJ, Aranow C, *et al*. Long-term safety and efficacy of belimumab in patients with systemic lupus erythematosus: a continuation of a seventy-six-week phase III parent study in the United States. *Arthritis Rheumatol* 2018;70:868–77.
5 Díaz-Cerezo S, García-Aparicio ÁM, Parrondo J, *et al*. [Cost-effectiveness analysis of belimumab in patients with systemic lupus erythematosus in Spain]. *Farm Hosp* 2015;39:161–70.
6 Doria A, Stohl W, Schwarting A, *et al*. Efficacy and safety of subcutaneous belimumab in anti-double-stranded DNA-positive, hypocomplementemic patients with systemic lupus erythematosus. *Arthritis Rheumatol* 2018;70:1256–64.
7 Barosi G, Gale RP. Is there expert consensus on expert consensus? *Bone Marrow Transplant* 2018;53:1055–60.

Response to: 'Clinical evidence guidelines in systemic lupus erythematosus: revaluation' by Scheinberg

We want to thank Dr Morton Scheinberg for his interest[1] in our recent lupus guidelines communication.[2] In his letter, he points out several concerns regarding belimumab which we had recommended at the same therapeutic level as other immunosuppressants for joint and skin manifestations. Few clarifications are needed.

The development of our guidelines followed a rigorous methodology in which the evidence on effect estimates should come, when available, from randomised controlled trials (RCTs). We did not identify any clinical trial comparing belimumab against other immunosuppressants. Therefore, in considering this comparison, the development group had to rely on indirect evidence (the difference in belimumab effect against placebo and other immunosuppressants against placebo) or high risk of bias evidence (from observational studies). In this context, the panel agreed that the certainty that belimumab was better (or worse) than other immunosuppressants was low/very low and therefore decided not to recommend one over the others.

Dr Scheinberg emphasised that in constructing the recommendation we missed relevant information; nevertheless, he did not provide any additional data that could justify this affirmation (RCT about the effects of belimumab in comparison with other immunosuppressants), instead he referenced a non-comparative observational study,[3] an RCT that was included in our review and considered in constructing the recommendations[4] (the reference provided was from a longer follow-up timeframe that was published after the systematic search of our guideline was finished whose results confirmed earlier findings), and a cost-effectiveness analysis that did not model belimumab against other immunosuppressants[5] (only modelled belimumab as add-on therapy) and was based on the information of the BLISS trials,[4 6] also included in our review. We acknowledge that not providing a recommendation (between different immunosuppressants) could not be the best of the guidance as, in practice, guideline users need to decide which one to prescribe; nevertheless, we agreed that in the absence of a head-to-head RCT, a conservative approach (providing the evidence and panel judgements without a recommendation) was the best way to proceed.

We are glad that Dr Scheinberg is concerned about the side effects of glucocorticoids as we do. For that reason, we have emphasised this as an overarching principle.

Finally, the letter mentions difficulties on expert consensus methodology.[7] We agree and have intensively lived that experience in the past. That is why, for these guidelines, we had decided to incorporate a transparent guideline development methodology in which the recommendations were intended to be based on the best available evidence such as the Grading of Recommendations Assessment, Development and Evaluation system, just as described in the editorial.[7]

We very much appreciate Dr Scheinberg's encouraging and insightful comments. We eagerly await new strong clinical evidence of current and new therapeutic options for our patients with lupus.

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REFERENCES

1 Scheinberg M. Clinical evidence guidelines in systemic lupus erythematosus: revaluation. *Ann Rheum Dis* 2018. doi: 10.1136/annrheumdis-2018-214357. [Epub ahead of print: 25 Sep 2018].
2 Pons-Estel BA, Bonfa E, Soriano ER, *et al*. First Latin American clinical practice guidelines for the treatment of systemic lupus erythematosus: Latin American Group for the Study of Lupus (GLADEL, *Grupo Latino Americano de Estudio del Lupus*)–Pan-American League of Associations of Rheumatology (PANLAR). *Ann Rheum Dis* 2018. doi: 10.1136/annrheumdis-2018-213512. [Epub ahead of print 25 Jul 2018].
3 Fanouriakis A, Adamichou C, Koutsoviti S, *et al*. Low disease activity—irrespective of serologic status at baseline—associated with reduction of corticosteroid dose and number of flares in patients with systemic lupus erythematosus treated with belimumab: a real-life observational study. *Semin Arthritis Rheum* 2018. doi: 10.1016/j.semarthrit.2018.02. [Epub ahead of print 23 Feb 2018].
4 Furie RA, Wallace DJ, Aranow C, *et al*. Long-term safety and efficacy of belimumab in patients with systemic lupus erythematosus: a continuation of a seventy-six-week phase iii parent study in the United States. *Arthritis Rheumatol* 2018;70:868–77.
5 Díaz-Cerezo S, García-Aparicio ÁM, Parrondo J, *et al*. [Cost-effectiveness analysis of Belimumab in patients with systemic lupus erythematosus in Spain]. *Farm Hosp* 2015;39:161–7.
6 Navarra SV, Guzman RM, Gallacher AE, *et al*. Belimumab, a BLyS-specific inhibitor, reduced disease activity, flares and prednisone use in patients with active SLE: efficacy and safety results from the phase 3 BLISS-52 study. *Lancet* 2011;377:721–31.
7 Barosi G, Gale RP. Is there expert consensus on expert consensus? *Bone Marrow Transplant* 2018;53:1055–60.

Comment on: 'Lupus Low Disease Activity State (LLDAS) attainment discriminates responders in a systemic lupus erythematosus trial: post-hoc analysis of the Phase IIb MUSE trial of anifrolumab' by Eric Morand *et al*

I read with great interest the paper by Eric Morand and colleagues[1] reporting on the utility of the Lupus Low Disease Activity State attainment using a useful form of discrimination in the phase IIb MUSE Anifrolumab trial published recently in the journal. The manuscript makes a useful contribution to this important debate but does contain one important error which should be corrected.

In the discussion, the authors state 'The assumption that gastrointestinal activity, which is not measured in the SLEDAI-2K or BILAG…' is simply incorrect. British Isles Lupus Assessment Group (BILAG)-2004[2] has a whole section dedicated to capturing gastrointestinal disease. It is one of several facets of lupus activity captured in BILAG but not captured in SLEDAI-2K (others include haemolytic anaemia and ophthalmic disease).

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REFERENCES

1 Morand EF, Trasieva T, Berglind A, *et al*. Lupus Low Disease Activity State (LLDAS) attainment discriminates responders in a systemic lupus erythematosus trial: *post-hoc* analysis of the Phase IIb MUSE trial of anifrolumab. *Ann Rheum Dis* 2018;77:706–13.
2 Yee CS, Farewell V, Isenberg DA, *et al*. Revised British Isles Lupus Assessment Group 2004 index: a reliable tool for assessment of systemic lupus erythematosus activity. *Arthritis Rheum* 2006;54:3300–5.
3 Morand EF, Trasieva T, Berglind A, *et al*. Regarding gastrointestinal activity in SLE, SLEDAI, BILAG, and PGA. *Ann Rheum Dis* 2019;78:e122–e122.

Response to: 'Comment on: 'Lupus Low Disease Activity State(LLDAS) attainment discriminates responders in a systemic lupus erythematosus trial: post-hocanalysis of the Phase IIb MUSE trial of anifrolumab' by Eric Morand et al' by Isenberg

The authors thank Professor Isenberg[1] for pointing out a drafting error on the manuscript reporting the use of the Lupus Low Disease Activity State (LLDAS) in the anifrolumab phase II trial data set.[2] The British Isles Lupus Assessment Group(BILAG) instrument does indeed document gastrointestinal disease activity, while Systemic Lupus Erythematosis (SLE) Disease Activity Index 2000 (SLEDAI-2K) does not. This error does not impact on the findings of the published paper, or the intended meaning of the sentence in which this error occurred, which was a discussion of the need for evidence that the physician global assessment adequately captures gastrointestinal activity in SLE.

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REFERENCES

1 Isenberg DA. Response to: 'Lupus Low Disease Activity State (LLDAS) attainment discriminates responders in a systemic lupus erythematosus trial: post-hoc analysis of the Phase IIb MUSE trial of anifrolumab' by Eric Morand *et al*. *Ann Rheum Dis* 2019;78:e122–e122.
2 Morand EF, Trasieva T, Berglind A, *et al*. Lupus Low Disease Activity State (LLDAS) attainment discriminates responders in a systemic lupus erythematosus trial: *post-hoc* analysis of the Phase IIb MUSE trial of anifrolumab. *Ann Rheum Dis* 2018;77:706–13.

Effectiveness and safety of tocilizumab for the treatment of refractory systemic sclerosis associated interstitial lung disease: a case series

We read with great interest the results of the double-blind phase 2 faSScinate clinical trial,[1] in which there was encouraging (although not statistically significant) numerical improvement in skin thickening and evidence of less decline in lung function in patients with systemic sclerosis (SSc) treated with tocilizumab (TCZ) compared with those receiving placebo. Initial investigations with TCZ in patients with SSc demonstrated improvements in skin sclerosis and polyarthritis.[2 3]

Scleroderma-associated interstitial lung disease (SSc-ILD) is a severely debilitating complication with high mortality in extensive disease. There is no approved disease-modifying treatment, and few effective treatment options are available. One of the most urgent needs is to determine which drugs can be useful as a rescue treatment in patients who do not respond to conventional immunosuppressants (cyclophosphamide (CYC) or mycophenolate mofetil (MMF)). In this sense, TCZ appears as one of the most promising candidates and an ongoing 2-year randomised phase 3 trial of TCZ-SC 162 mg in SSc is under way (ClinicalTrials.gov identifier NCT02453256).

Based on this preliminary evidence,[1–3] we have evaluated TCZ (off-label use) as a rescue therapy in nine selected patients with progressive SSc-ILD (evidence of clinical and functional decline) despite previous treatment with low–medium prednisone doses, immunosuppressants and rituximab (RTX). They were treated with a compassionate use of TCZ for at least 6 months. In all cases, written informed consent was obtained from the patients, and the off-label use of TCZ (and previously of RTX) was approved by our local health authorities.[4]

The median durations of SSc and ILD were 8 years (range: 2–15 years) and 7 years (range: 2–12 years), respectively. All cases corresponded to fibrosing non-specific interstitial pneumonia. Progressive interstitial lung disease (ILD) was defined when there was a worsening of ≥10% in per cent predicted forced vital capacity (%pFVC) or ≥15% in per cent predicted diffusing capacity for carbon monoxide corrected for haemoglobin (%pDLCO) during the follow-up (over 1 year).

Previous or ongoing therapies for SSc-ILD included MMF (100%), CYC (67%), azathioprine (11%) and RTX (100%). In all cases, the time elapsed since the last dose of CYC was greater than 2 years and 6 months in the case of RTX. The mean number of RTX cycles previously administered was 3±1.7 (range: 1–6): in four patients (44%) RTX was discontinued due to adverse events (mainly respiratory or urinary infections and/or transient neutropenia) and in the remaining (56%) due to inefficacy.

TCZ was administered intravenously in two patients (at a dose of 8 mg/kg monthly) and subcutaneously in the remaining 7 (162 mg weekly). In all cases, it was administered with MMF (eight patients received 2 g/day and one patient received 1 g/day). Seven (78%) patients received concomitant treatment with prednisone (≤5 mg/day). Ongoing therapy with MMF and oral prednisone remained initially unchanged in all cases.

The baseline clinical features and outcome of these patients are summarised in table 1. At the end of the follow-up period (median 12 months; IQR 25th–75th: 6–33 months), only four patients (44%) were still in treatment. In the other five patients (56%) TCZ was discontinued, due to serious adverse events in one case and due to inefficacy in the other four cases. One of these,

Table 1 Baseline clinical features and outcome of our nine patients with refractory systemic sclerosis-associated interstitial lung disease treated with tocilizumab

Patient number	1	2	3	4	5	6	7	8	9
Age (years)/sex	59/F	40/F	64/F	63/F	57/F	53/F	52/F	60/F	62/F
SSc duration (years)	15	9	12	6	4	6	2	6	10
SSc cutaneous subset	Diffuse	Limited	Diffuse	Limited	Diffuse	Limited	Limited	Diffuse	Diffuse
Chest HRCT pattern of ILD	Fibrosing NSIP	Fibrosing NSIP	Fibrosing NSIP	Fibrosing NSIP	Fibrosing NSIP	Fibrosing NSIP	Fibrosing NSIP	Fibrosing NSIP	Fibrosing NSIP
ILD duration (years)	12	4	12	6	4	6	2	6	9
Autoantibodies	Scl-70 (+) Ro52 (–)	ACA (+) Ro52: ND	Scl-70 (+) Ro52: ND	ACA (+) Ro52 (–)	Scl-70 (+) Ro52 (–)	ACA (+) Ro52: ND	Scl-70 (+) Ro52 (–)	Scl-70 (+) Ro52 (+)	Scl-70 (+) Ro52 (–)
Previous or ongoing therapies for SSc-ILD	CYC IV, MMF, RTX and PDN.	MMF and RTX.	CYC IV, MMF, RTX and PDN.	AZA, CYC IV, MMF, RTXand PDN.	MMF and RTX.	CYC IV, MMF, RTX and PDN.	MMF, RTX and PDN.	CYC IV, MMF, RTX and PDN.	CYC IV, MMF, RTX and PDN.
No of RTX cycles	4	1	4	3	2	5	1	1	6
Follow-up after first dose of TCZ (months)	23	8	34	33	7	6	8	12	18
Lung responses to TCZ therapy*	*Pre-TCZ/Post-TCZ* %pCVF: 61.7/56.3 (STB). %pTLC: 89.1 /62.6. %pDLCO: 30.5/34.6 (STB). 6MWT: 420 m/393 m. HRCT: STB.	*Pre-TCZ/Post-TCZ* %pCVF: 77.3/80.4 (STB). %pTLC: 88.3/89.1. %pDLCO: 41.4/48.1 (IMPR). 6MWT: 330 m /360 m. HRCT: STB.	*Pre-TCZ/Post-TCZ* %pCVF: 78.8/73.7 (STB). %pTLC: 75.2/79.8 %pDLCO: 47.1/50 (STB). 6MWT: 390 m/399 m. HRCT: STB.	*Pre-TCZ/Post-TCZ* %pCVF: 103/101 (STB). %pTLC: 93/88. %pDLCO: 27/35 (IMPR). 6MWT: 388 m/396 m. HRCT: STB.	*Pre-TCZ/Post-TCZ* %pCVF: 70.2/60.1 (W). %pTLC: 80.7/66. %pDLCO: 42.1/35.5 (W). 6MWT: 388 m/396 m. HRCT: W.	*Pre-TCZ/Post-TCZ* %pCVF: 108/112 (STB). %pTLC: ND/ND. %pDLCO: 69/52 (W). 6MWT: 418 m/335 m. *HRCT: W.*	*Pre-TCZ/Post-TCZ* %pCVF: 70/56 (W). %pTLC: 83/66. %pDLCO: 61/31 (W). 6MWT: ND. *HRCT: W.*	*Pre-TCZ/Post-TCZ* %pCVF: 59/49 (W). %pTLC: 63/57. %pDLCO: 35/30 (W). 6MWT: ND. *HRCT: W.*	*Pre-TCZ/Post-TCZ* %pCVF: 80/91 (IMPR). %pTLC: 91/92. %pDLCO: 65/70 (STB). 6MWT: ND. *HRCT: ND.*
Adverse events	Yes herpeszoster: 1; bacterial infections: 3, needing hospitalisation in one of them (osteomyelitis).	No	No	No	No	No	No	No	No
Discontinuation of the treatment at the endpoint of patient follow-up and reason	Yes, due to adverse events. In *waiting list for lung transplantation.*	No	No	No	Yes, due to inefficacy. Autologous stem cell transplantation	Yes, due to inefficacy.	Yes, due to inefficacy.	Yes; died due to progression of the ILD	No

HRCT was assesed by one chest radiologist blinded to clinical state or change in lung function. Categorisation of radiological response: (1) improvement (no lung fibrotic changes and improvement >20% of the extent of ground glass opacities), (2) stabilisation or (3) worsening (if the extent of lung fibrotic changes and/or ground glass opacities increased >20%).
*The evolution of pulmonary function tests was classified according to definitions from the American Thoracic Society into worsening (a decrease of pre-TCZ %pFVC >10% or %pDLCO >15%), stabilisation (if changes in pre-TCZ %pFVC are less than 10% or 15% in %pDLCO), or improvement (increase of pre-TCZ %pFVC >10% or %pDLCO >15%).
%pDLCO, per cent predicted diffusing capacity for carbon monoxide corrected for haemoglobin; %pFVC, per cent predicted forced vital capacity; %pTLC, per cent predicted total lung capacity; 6MWT, 6 min walk test; ACA, anticentromere antibodies; AZA, azathioprine; CYC, cyclophosphamide; F, female; HRCT, high-resolution CT; ILD, interstitial lung disease; IMPR, improvement; IV, intravenous; m, metres; MMF, mycophenolate; ND, not done; NSIP, non-specific interstitial pneumonia; PDN, prednisone; RTX, rituximab; Scl70, antitopoisomerase I antibodies; SSc, systemic sclerosis; STB, stabilisation; W, worsening.

four patients died due to progression of ILD. The frequency of adverse events was low, occurring in only one patient (11%) who developed repeat infections including an osteomyelitis complicating a digital ulcer requiring hospitalisation.

Although it is difficult to draw any firm conclusions from these data, according with our experience in this small cohort, TCZ appears to be safe. Its effectiveness as a rescue treatment in patients with refractory SSc-ILD seems modest but not negligible, achieving an improvement or stabilisation of pulmonary function[5] in 44% of patients.

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Ethics approval The present report has been approved by of our institutional ethics committee (Clinical Research Ethics Committee of Bellvitge University Hospital-IDIBELL). Written informed consent was obtained from the patients. Their clinical records and information were anonymised prior to analysis. This study was conducted in accordance with the principles of the Declaration of Helsinki and the International Conference for Harmonization.

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REFERENCES

1 Khanna D, Denton CP, Lin CJF, *et al*. Safety and efficacy of subcutaneous tocilizumab in systemic sclerosis: results from the open-label period of a phase II randomised controlled trial (faSScinate). *Ann Rheum Dis* 2018;77:212–20.
2 Shima Y, Kuwahara Y, Murota H, *et al*. The skin of patients with systemic sclerosis softened during the treatment with anti-IL-6 receptor antibody tocilizumab. *Rheumatology* 2010;49:2408–12.
3 Elhai M, Meunier M, Matucci-Cerinic M, *et al*. Outcomes of patients with systemic sclerosis-associated polyarthritis and myopathy treated with tocilizumab or abatacept: a EUSTAR observational study. *Ann Rheum Dis* 2013;72:1217–20.
4 Departament de Salut Generalitat de Catalunya, 2018. Dictamen de la Comissió Farmacoterapèutica de la Medicació Hospital ria deDispensació Ambulatòria sobre l'ús de rituximab, tocilizumab i abatacept per altractament fora d'indicació de l'esclerosi sistèmica refract ria al tractamenthabitual. http://catsalut.gencat.cat/web/.content/minisite/catsalut/proveidors_professionals/medicaments_farmacia/phf_mhda/informes_camse/esclerosi_sistemica/Dictamen-CAMS_-ES_-web.pdf
5 Raghu G, Rochwerg B, Zhang Y, *et al*. An official ATS/ERS/JRS/ALAT clinical practice guideline: treatment of idiopathic pulmonary fibrosis. an update of the 2011 clinical practice guideline. *Am J Respir Crit Care Med* 2015;192:e3–e19.
6 Khanna D, Jahreis A, Lin CJF. Tocilizumab in systemic sclerosis-associated interstitial lung disease. *Ann Rheum Dis* 2019;78:e124.

Response to: 'Effectiveness and safety of tocilizumab for the treatment of refractory systemic sclerosis associated interstitial lung disease: a case series' by Narváez

We read the intriguing findings by Narváez[1] about the effect of tocilizumab (TCZ) in patients with established systemic sclerosis-associated interstitial lung disease (SSc-ILD) with great interest. In the double-blind, randomised, placebo-controlled faSScinate study[2] and open-label extension,[3] we assessed the efficacy and safety of TCZ in patients with systemic sclerosis. There were important differences between our trial and the data presented by Narváez[1]. In the faSScinate study, the patient population shown to benefit from TCZ had shorter duration of disease (mean disease duration of 1.6 years in faSScinate vs 6.9 years in Narváez), increased serum acute phase reactants, progressive skin disease and low normal mean forced vital capacity (FVC) levels (mean FVC% predicted of 81%) at study baseline. Compared with patients receiving placebo, patients receiving TCZ appeared to stabilise their FVC, an exploratory endpoint, and thus preserved their lung function: the mean change from baseline for FVC% predicted for placebo was −6.3% (95% CI −8.9 to 3.8) and for TCZ was −2.6% (−5.2 to −0.1) at week 48, with a delta of 3.7% (0.1 to 7.3); the delta in absolute millilitres for FVC at week 48 was 120 mL (−23 mL, 262 mL). These data are consistent with the findings from De Lauretis *et al*,[4] in which serum interleukin-6 levels appeared to be predictive of disease progression and/or death in patients with mild ILD (defined as FVC% >70%). The effect of TCZ on the lung has been speculated to be related to modulating the activity of M2 macrophages.[2] In contrast, Narváez[1] is studying TCZ as a rescue treatment in patients with SSc with established ILD who have failed rituximab (RTX) in all cases and cyclophosphamide in 67% of cases. All patients were on ongoing mycophenolate mofetil while receiving TCZ, with follow-up after first TCZ dose ranging from 6 to 34 months. TCZ may have contributed to the stabilisation of lung function in a more severe and resistant ILD, which is notable given patients' previous immunotherapy failures. It is interesting to note that all four patients who appeared to have responded to TCZ had pretreatment FVC% predicted of greater than 77% (mean FVC% was 84.8%). For patient 6, whose pretreatment FVC% was 108%, it is unclear if the decline in diffusing capacity for carbon monoxide (DLCO)% predicted represents worsening ILD or concomitant pulmonary vascular disease, especially with normal FVC% predicted (with no change over time) and an FVC to DLCO ratio of 1.56, a risk factor for pulmonary vascular disease in SSc.[5] Given these captivating case reports, controlled studies should be conducted to assess if indeed TCZ is having an effect in patients with severe established SSc lung disease.

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REFERENCES

1 Narváez J, LLuch J, Alegre Sancho JJ, *et al*. Effectiveness and safety of tocilizumab for the treatment of refractory systemic sclerosis associated interstitial lung disease: a case series. *Ann Rheum Dis* 2019;78:e123.
2 Khanna D, Denton CP, Jahreis A, *et al*. Safety and efficacy of subcutaneous tocilizumab in adults with systemic sclerosis (faSScinate): a phase 2, randomised, controlled trial. *The Lancet* 2016;387:2630–40.
3 Khanna D, Denton CP, Lin CJF, *et al*. Safety and efficacy of subcutaneous tocilizumab in systemic sclerosis: results from the open-label period of a phase II randomised controlled trial (faSScinate). *Ann Rheum Dis* 2018;77:212–20.
4 De Lauretis A, Sestini P, Pantelidis P, *et al*. Serum interleukin 6 is predictive of early functional decline and mortality in interstitial lung disease associated with systemic sclerosis. *J Rheumatol* 2013;40:435–46.
5 Coghlan JG, Denton CP, Grünig E, *et al*. Evidence-based detection of pulmonary arterial hypertension in systemic sclerosis: the DETECT study. *Ann Rheum Dis* 2014;73:1340–9.

Will SPAR be useful in the usual patients with scleroderma?

We read with great interest the simplified predictive score 'SPAR model' by Wu *et al*.[1] A simplified predictive score which can predict 'fast progressors' can be used to target and selectively recruit such patients for drug trials and finally lead to improvement in outcomes in the future. However, certain aspects require clarification.

First, the external validity of the patients recruited is unclear. Although early interstitial lung disease (ILD) as defined in the study (<20% CT involvement) would be common, approximately half of the patients of this study could be having forced vital capacity (FVC) >100% (assuming normality). The latter would be a subgroup which would be uncommon. More interestingly, these patients have never been recruited for interventional studies looking at drugs on ILD—both the scleroderma lung studies (refs [2] and [3]) recruited patients with FVC ≤85%; thus we have no idea whether they (FVC>100%) respond to therapy. In the latter study (SLS2), only 30 out of 198 screened systemic sclerosis were excluded due to pulmonary function test, which could be higher or lower FVC and diffusing capacity of the lung for carbon monoxide-. The authors may like to provide their cohort numbers and how many of them fulfilled the inclusion criteria.

Second, the authors found 'arthritis ever' to be significant after multivariate analysis, though a previous study did not show any association of arthritis and ILD progression.[4] However, there is no comment on whether arthritis was persistent and erosive, and did it require treatment in their cohort? Was baseline presence of arthritis also significant? In a patient who comes for the first time, history of arthritis would not be available.

Third, the best multivariate predictive model in this study (model 3 (SpO2≤94%+arthritis ever)) has a sensitivity of only 44%, thus more than half of the progressors would not be detected. Even in a 0–2 SPAR score, the most common score is 1, and that would only identify one-third of progressors![1]

Finally, the authors may like to provide any data on other variables expected to predict progression—baseline extent of ILD on CT (varying from 0% to 20%), oesophageal diameter on high-resolution CT (as shown by other studies[2 5]) and nail fold capillaroscopy. The latter becomes important as low oxygen saturation after the 6 min walk test, when severe ILD and pulmonary artery hypertension (PAH) are excluded (as in this study), may reflect early microvasculature changes in pulmonary bed, which as expected to be generalised and would be reflected in the nail bed capillaries also.

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REFERENCES

1 Wu W, Jordan S, Becker MO, *et al*. Prediction of progression of interstitial lung disease in patients with systemic sclerosis: the SPAR model. *Ann Rheum Dis*. 2018;77:1326–32.
2 Tashkin DP, Elashoff R, Clements PJ, *et al*. Cyclophosphamide versus placebo in scleroderma lung disease. *N Engl J Med Overseas Ed* 2006;354:2655–66.
3 Tashkin DP, Roth MD, *et al*. Sclerodema Lung Study II Investigators. Mycophenolate mofetil versus oral cyclophosphamide in scleroderma-related interstitial lung disease (SLS II): a randomised controlled, double-blind, parallel group trial. *Lancet Respir Med* 2016;4:708–19.
4 Avouac J, Walker UA, Hachulla E, *et al*. Joint and tendon involvement predict disease progression in systemic sclerosis: a EUSTAR prospective study. *Ann Rheum Dis* 2016;75:103–9.
5 Richardson C, Agrawal R, LeeJ AO. Dilated esophagus is an independent risk factor for interstitial lung disease in SSc. Abstract number: 729 Meeting: 2014 ACR/ARHP Annual Meeting.

Response to: 'Will SPAR be useful in the usual patients with scleroderma?' by Chattopadhyay *et al*

Thank you very much for your interest in our article 'Prediction of progression of interstitial lung disease in patients with systemic sclerosis: the SPAR model'[1] and your precious questions 'Will SPAR be useful in the usual scleroderma patients?'.[2] We are glad to respond as below.

EXTERNAL VALIDITY OF THE STUDY COHORT

The external validity of the patients recruited is unclear. The authors may like to provide their cohort numbers and how many of them fulfilled the inclusion criteria.

Regarding the question on the external validity of the study cohort, we would like to stress that our study cohort focused on patients with mild interstitial lung disease (ILD) on high resolution computer tompgraph (HRCT), without defining a limit for forced vital capacity (FVC). That is the reason why our patients had an average normal FVC. We disagree that patients with milder ILD and normal FVC are uncommon in clinical practice—please see our recent study.[3] In fact, many patients with (milder) ILD might be missed if only lung function testing is used for screening.

However, we agree that these patients have not been included in recent interventional clinical trials such as scleroderma lung study (SLS) 1 and 2 which have concentrated on a more severe subpopulation with decreased FVC. Thus, whether the progression of patients with mild systemic sclerosis (SSc)-ILD can be successfully prevented with specific treatments needs to be shown. Notably, the ongoing large randomised placebo controlled SENSCIS trial, which is testing nintedanib versus placebo in patients with SSc-ILD, recruits patients with HRCT involvement >10% and no upper limit of FVC.[4] Thus, post-hoc analysis of this trial in the group of patient with HRCT involvement of 10%–20% and 'normal' FVC might partially address this question.

In the derivation cohort (Zurich cohort) to the present study, we have included 397 patients with SSc with complete data, among which 158 patients had ILD. A total of 98 patients (62%) fulfilled the inclusion criteria of mild ILD. Base on this result, we assume that the current study cohort represents a large subpopulation of patients with SSc-ILD.

THE STATUS OF ARTHRITIS

The authors found 'arthritis ever' to be significant after multivariate analysis, though a previous study did not show any association of arthritis and ILD progression. Was baseline presence of arthritis also significant?

Regarding the question on the status of arthritis, the cited study looked at a different study population: this was a mixture of patients with mild and advanced SSc-ILD, follow-up time was longer and a different definition of SSc-ILD progression was used.[5] Prediction factors for the disease subgroup of more advanced SSc-ILD might be very different from patients with mild SSc-ILD.

The type of arthritis was as follows: only 6.1% patients ever had erosive arthritis in the derivation cohort, and the presence of anti-cyclic citrullinated peptide (CCP) was low in our cohorts (3.5%~6.5%). Methotrexate was the most frequently used immunosuppressant (26.5%) in our study, but we are unaware whether this was given for the indication of arthritis or for other reasons, for example, skin fibrosis. Current arthritis only showed no significant association with ILD progression in the multivariate analysis, indicating that previous inflammatory disease including arthritis is an important parameter for disease characterisation.

PREDICTIVE PERFORMANCE OF THE SPAR MODEL

The best multivariate predictive model in this study (model 3: SpO_2 ≤94%+ arthritis ever) has a sensitivity of only 44%- thus more than half of the progressors would not be detected.

Regarding the question on the predictive performance of the SPAR model, as shown in table 4 of our paper,[1] for example, in the derivation cohort, 91.7% of patients with SPAR score=2 actually had ILD progression, 92.6% of patients with SPAR score=0 actually did not have ILD progression. Moreover, 84.0% of ILD progressors had a SPAR score of 1 or 2, 98.6% of non-progressors had a SPAR score of 0 or 1. All these results indicate that most ILD progressors (>80%) could be identified

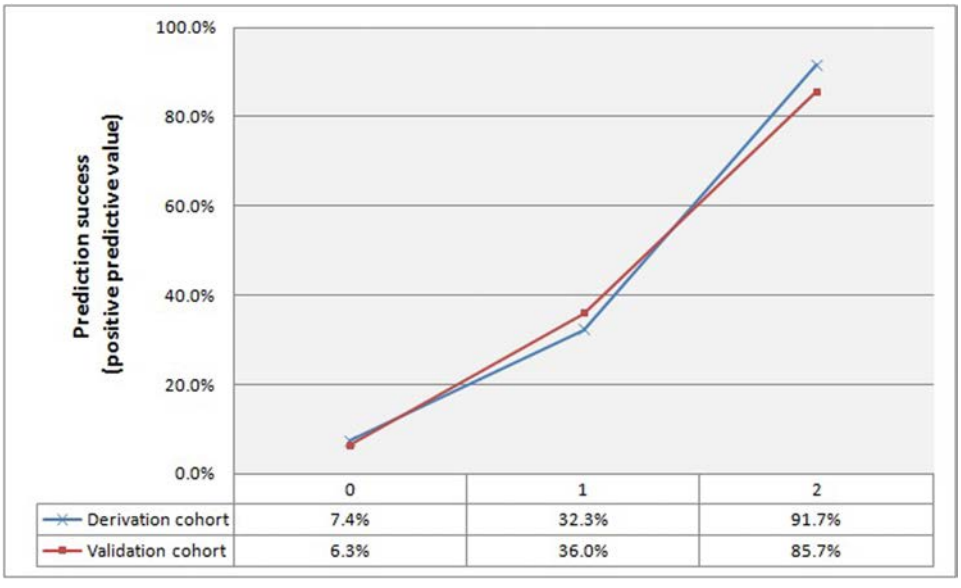

Figure 1 Prediction success rate with SPAR score for progression of mild SSc interstitial lung disease. PPV, positive predictive value.

when the patients fulfilled either of these two characteristics (SpO_2 after 6MWT ≤94%, arthritis ever).

Meanwhile, patients with neither of these two characteristics had a really low chance (<10%) to have a deterioration of ILD in the next 1 year. This is further highlighted in figure 1. Thus, although further external validation and testing in clinical practice is still required, we believe that the SPAR model provides a promising risk-stratification tool in patients with mild SSc-ILD.

OTHER POTENTIAL PREDICTORS

The authors may like to provide any data on other variables expected to predict progression—baseline extent of ILD on CT (varying from 0% to 20%), oesophageal diameter on HRCT and nailfold capillaroscopy.

Regarding the question on other potential predictors, we fully agree that other clinical variables could potentially predict ILD progression in patients with SSc. We collected data from nailfold capillaroscopy (NFC) in the derivation cohort. A total of 9/98 (9.2%) patients showed normal-like patterns, 20/98 (20.4%) patients showed early scleroderma patterns, 33/98 (33.7%) patients showed active scleroderma patterns and 36/98 (36.7%) patients showed late scleroderma patterns, respectively. The percentage of active/late scleroderma pattern did not differ significantly among ILD progressors and non-progressors (72.0% vs 69.9%, p=0.840). After applying multivariate regression, active/late scleroderma pattern in NFC was also not predictive for ILD progression (p=0.138). Additionally, 'SpO_2 after 6 MWT' and 'arthritis ever' were still the only two significant predictors after NFC data were forced in the multivariate regression model.

Unfortunately, we did not have detailed data for exact extent of ILD or oesophageal diameter on HRCT in our cohort. We motivate to include these parameters in further studies.

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REFERENCES

1 Wu W, Jordan S, Becker MO, *et al*. Prediction of progression of interstitial lung disease in patients with systemic sclerosis: the SPAR model. *Ann Rheum Dis* 2018;77:1326–32.
2 Chattopadhyay A, Dhir V, Sharma S, *et al*. Will SPAR be useful in the usual patients with scleroderma? *Ann Rheum Dis* 2019;78:e125.
3 Suliman YA, Dobrota R, Huscher D, *et al*. Brief report: pulmonary function tests: high rate of false-negative results in the early detection and screening of scleroderma-related interstitial lung disease. *Arthritis Rheumatol* 2015;67:3256–61.
4 Distler O, Brown KK, Distler JHW, *et al*. Design of a randomised, placebo-controlled clinical trial of nintedanib in patients with systemic sclerosis-associated interstitial lung disease (SENSCIS™). *Clin Exp Rheumatol* 2017;35:75–81.
5 Avouac J, Walker UA, Hachulla E, *et al*. Joint and tendon involvement predict disease progression in systemic sclerosis: a EUSTAR prospective study. *Ann Rheum Dis* 2016;75:103–9.

M1/M2 polarisation state of M-CSF blood-derived macrophages in systemic sclerosis

We read with interest the work conducted by Soldano and colleagues,[1] exploring M1/M2 macrophage surface markers in circulating blood cells in systemic sclerosis (SSc). Using a wide phenotypic characterisation of circulating cells by flow cytometry, the authors highlight that the percentage of CD14+ cells coexpressing M2 markers (CD206, CD163 and CD204) and M1 markers (TLR4) is higher in patients with SSc than in healthy subjects (HSs). Similarly, CD204+ circulating cells coexpressing M2 markers (CD163, CD206) and M1 markers (TLR4, CD80, CD86) were also over-represented in patients with SSc. These results strengthen the concept of a specific macrophage signature in SSc which goes beyond the dichotomous M1/M2 paradigm.[2–4] However, this study was only conducted on undifferentiated circulating blood cells and the validation of their results on differentiated macrophages is still to be determined.

In support of Soldano's results,[1] we present here, a phenotypic analysis conducted on macrophage-colony stimulating factor (M-CSF) resting blood monocyte-derived macrophages (MDM) from glucocorticoid (GC)-free patients fulfilling ACR/EULAR 2013 classification criteria for SSc in comparison with HSs, evaluating the mean of fluorescence intensity (MFI) ratio of six polarisation markers (CD80, CD206, CD204, CD163, CD169 and CD200R1) and supporting the existence of a mixed M1/M2 signature in SSc MDM. In parallel, to assess the value of these markers as 'polarisation markers' in this model of MDM, MDM from HSs were also polarised in vitro into M1 (IFNɣ+LPS), M2a (IL-4 and IL-13) and M2c (IL-10+ dexamethasone).[5 6]

Our results confirmed, in HS MDM, the higher expression of CD80 and CD169 in polarised M1 in comparison with M2 and unpolarised/resting M0 subtypes (figure 1A). On the contrary, CD200R1 and CD206 were only overexpressed in HS M2a. The expressions of CD163, usually considered as an M2 marker and of CD204, were significantly higher in HS M2a when compared with HS M1 but were not different from those of HS M0 (figure 1A).[6] In SSc M0, CD200R1 and CD204 were significantly decreased in comparison with M0 from HS (figure 1B). The expression of these markers was comparable in SSc M0 and HS M1 (respectively in SSc M0 and HS M1, MFI ratio for CD200R1: 1.83±0.11 vs 1.64±0.096; p>0.05 and

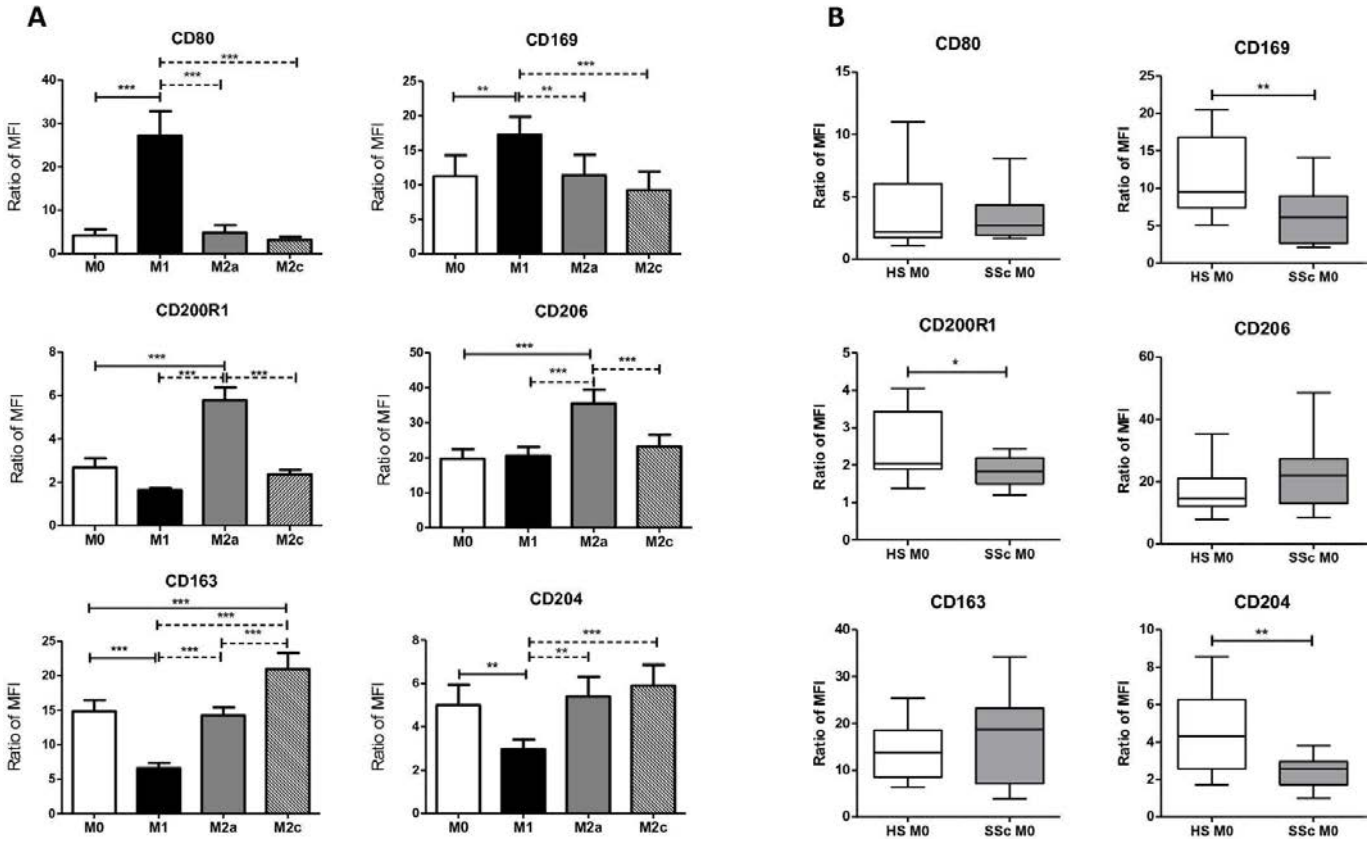

Figure 1 Polarisation markers of M-CSF MDMs from patients with SSc and HSs. (A) Primary human blood monocytes from HSs were differentiated into MDM in vitro in the presence of 50 ng/mL of M-CSF for 6 days. At day 6, media were replaced by a fresh media (RPMI with 5% of fetal bovine serum with 10 ng/mL of M-CSF) and MDM were polarised for additional 24 hours by the addition of 20 ng/mL IFNɣ and 20 ng/mL LPS (M1), by 20 ng/mL IL-4 and 20 ng/mL IL-13 (M2a) and by 20 ng/mL IL-10 with 10 nM of dexamethasone (M2c) or unpolarised/resting (M0). Cells were then harvested, stained and the expression of cell surface molecules was analysed on a LSRII Fortessa flow cytometer. Data are expressed as MFI relative to isotype control (ratio)±SEM for at least six independent experiments. ANOVA followed by Newman-Keuls' multiple comparison test, **p<0.01 and ***p<0.001. (B) Primary human blood monocytes from HSs and patients with SSc were differentiated into MDM in vitro in the presence of 50 ng/mL of M-CSF for 6 days. At day 6, media were replaced by a fresh media (RPMI with 5% of fetal bovine serum with 10 ng/mL of M-CSF) for additional 24 hours. These M0 unpolarised/resting cells were then harvested, stained and the expression of cell surface molecules was analysed on a LSRII Fortessa flow cytometer. Data are expressed as MFI relative to isotype control (ratio)±SEM for at least 13 HSs and for 11–16 patients with SSc. Student unpaired t-test, *p<0.05 and **p<0.01. ANOVA, analysis of variance; HSs, healthy subjects; MDM, monocyte-derived macrophages; MFI, mean fluorescence intensity; SSc, systemic sclerosis.

MFI ratio for CD204: 2.45±0.24 vs 2.97±0.44; p>0.05). By contrast, the expression of CD169 was significantly decreased in SSc M0 when compared with both HS M0 (figure 1B) and HS M1 (SSc M0 vs HS M1, MFI ratio for CD169: 6.20±1.23 vs 17.31±2.56; p<0.001). The expression of this marker was comparable in SSc M0 and HS M2c (SSc M0 vs HS M2c: MFI ratio for CD169: 6.20±1.23 vs 9.21±2.71; p>0.05).

Therefore, in this phenotypic analysis based on MFI, M0 MDM from patients with SSc showed a specific phenotype expressing some markers in the same way as M1 and other markers similarly to M2 or unpolarised M0 macrophages. Within a different approach, this result supports the existence of a mixed M1/M2 signature in SSc MDM, as suggested by Soldano *et al* concerning subpopulations of circulating cells.[1] Our results on marker expressions among polarised MDM in vitro also alert on the value of so-called M1 or M2 markers, as CD163 may be considered as an M2a marker in comparison with M1 but is similar to M0 resting macrophages (figure 1A). CD163 appears to be more specific to dexamethasone-induced M2c in comparison with M2a (figure 1A), a result that could be especially important when considering patients with GCs. CD163 could even be induced in M1 when dexamethasone is added to the medium.[5 7] These results also bring light to the results from Soldano's figure 2B, pointing the increased percentage of CD163+/CD206+ among CD204+cells in patients with SSc with GCs in comparison with both patients with SSc without GCs and HSs.[1]

Acknowledging that the small sample size of our data represents a limitation for our conclusions, we agree with Soldano's suggestion to think outside the box: beyond the dichotomous M1/M2 paradigm, using new phenotyping approaches may offer a more encompassing vision of macrophages in SSc.[4–6 8–10]

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REFERENCES

1 Soldano S, Trombetta AC, Contini P, *et al*. Increase in circulating cells coexpressing M1 and M2 macrophage surface markers in patients with systemic sclerosis. *Ann Rheum Dis* 2019;77:1842–5.
2 Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. *Nat Rev Immunol* 2008;8:958–69.
3 Moreno-Moral A, Bagnati M, Koturan S, *et al*. Changes in macrophage transcriptome associate with systemic sclerosis and mediate *GSDMA* contribution to disease risk. *Ann Rheum Dis* 2018;77:596–601.
4 Behmoaras J, Petretto E. Cell function in disease: there are more than two parties at play. *Ann Rheum Dis* 2019;78:e20.
5 Murray PJ, Allen JE, Biswas SK, *et al*. Macrophage activation and polarization: nomenclature and experimental guidelines. *Immunity* 2014;41:14–20.
6 Chávez-Galán L, Olleros ML, Vesin D, *et al*. Much More than M1 and M2 macrophages, there are also CD169(+) and TCR(+) Macrophages. *Front Immunol* 2015;6:263.
7 Tedesco S, Bolego C, Toniolo A, *et al*. Phenotypic activation and pharmacological outcomes of spontaneously differentiated human monocyte-derived macrophages. *Immunobiology* 2015;220:545–54.
8 Christmann RB, Sampaio-Barros P, Stifano G, *et al*. Association of Interferon- and transforming growth factor β-regulated genes and macrophage activation with systemic sclerosis-related progressive lung fibrosis. *Arthritis Rheumatol* 2014;66:714–25.
9 Taroni JN, Greene CS, Martyanov V, *et al*. A novel multi-network approach reveals tissue-specific cellular modulators of fibrosis in systemic sclerosis. *Genome Med* 2017;9:27.
10 Martinez FO, Gordon S. The M1 and M2 paradigm of macrophage activation: time for reassessment. *F1000Prime Rep* 2014;6:13.

Monocyte and macrophage phenotypes: a look beyond systemic sclerosis. Response to: 'M1/M2 polarisation state of M-CSF blood-derived macrophages in systemic sclerosis' by Lescoat *et al*

We read with interest the new data from Lescoat *et al* and their interesting comments concerning our research letter on the identification of circulating cells coexpressing M1 and M2 phenotype markers in patients affected by systemic sclerosis (SSc) compared with healthy subjects (HSs).[1 2]

In their study, Lescoat *et al* evaluated the mean of fluorescence intensity of specific markers of M1 and M2 phenotypes (CD80, CD206, CD204, CD163, CD169 and CD200R1) in macrophage colony-stimulating factor (M-CSF) resting blood monocyte-derived-macrophages (MDMs) from HSs exposed to in vitro stimuli aiming to polarise them towards the M1, M2a and M2c phenotypes. The markers were also evaluated on the cell surface of the MDMs obtained from 11 to 16 SSc patients not treated with glucocorticoids and compared with those obtained from 13 HSs.[1] The important conclusion highlighted is cultured circulating MDMs from patients with SSc showcells with a mixed M1/M2 signature, supporting our results.[2]

We will soon be able to complement these data with the clinical implications seen in a new study that is going to demonstrate, in patients with SSc, how the higher percentages of circulating cells showing a mixed M1/M2 phenotype correlate significantly with important SSc clinical complications, such as functional and structural lung damage (data under publication).

Lescoat *et al* appropriately asserted that the results showing a mixed phenotype of circulating monocyte/macrophage cells in patients with SSc might arise both methodological and conceptual questions with respect to the M1/M2 definition.

Lescoat's results further highlighted how macrophages can evolve to exhibit characteristics that are shared by more than one macrophage population, similarly to secondary colours in a colour wheel, as postulated by Mosser and Edwards.[3]

Although it is true that our study was conducted on circulating and theoretically less differentiated cells, it is also possible to hypothesise that the in vitro differentiation of monocytes through the stimulation with M-CSF as well as GM-CSF probably does not fully reproduce the process induced by the in vivo microenvironmental signals.[4 5] Also, the comparison with cultured macrophages differentiated from human cell lines, such as primarily PMA-treated THP-1 cell line, would be even less accurate.[6]

Furthermore, the higher percentages of differentiated and activated myeloid-derived circulating cells in patients with SSc compared with HSs make part of the most interesting results of our research. Our data and those from Lescoat *et al* could in any case coexist, without being in contrast with the different findings observed at tissue level, that is, in internal organs affected by SSc, since the different tissues are able to direct the inflammatory response in a proinflammatory direction or towards resolution and repair, determining the expression of a more polarised and stable cell phenotype in the periphery (ie, M1 and/or M2).[7]

Nevertheless, the more differentiated phenotype observed at the tissue level could not mirror the one observed in circulating monocyte/macrophage precursors or that derived from in vitro MDMs. Additionally, it was very recently demonstrated that different cell phenotypes could be observed in different tissues especially in SSc and, particularly for innate immune cells, given their plasticity.[4 5 8]

Finally, the acquisitions on monocyte/macrophage polarisation have shown at least that the approach based on the evaluation of single or few markers for the determination of a cell signature is no longer conceivable. In fact, the strong interest currently found in the contribution of the innate immune cells to pathogenic processes should probably be directed towards the clarification of the relationships between the phenotype of circulating cells and that of more differentiated ones observed in peripheral tissues and to a wider phenotype study and definition.

The results described in our study and that presented by Lescoat *et al* contributed to identify possible new cell players involved in the pathophysiology of SSc.

In accordance with Behmoaras and Petretto, the question regarding whether and how the circulating mixed M1/M2 cells described in our study and/or the MDMs investigated in vitro by Lescoat *et al* could reflect the context-specific activation of macrophages in the different SSc tissues (ie, lung and skin) remains open and matter of our further research.[4]

Starting from these results, the functional role of mixed M1/M2 cells in the pathogenesis of SSc and in other fibrotic diseases, as well as the possible effects of modulators and the related clinical complications, should be better addressed.[1 5 9] These considerations on the innate immune cell plasticity could apply to the contribution to SSc pathogenesis and to other fibrotic diseases and pathological or even physiological conditions.[7]

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REFERENCES

1 Lescoat A, Ballerie A, Jouneau S. M1/M2 polarization state of M-CSF blood-derived macrophages in systemic sclerosis. *Ann Rheum Dis* 2019;78:e127–e127.
2 Soldano S, Trombetta AC, Contini P, *et al*. Increase in circulating cells coexpressing M1 and M2 macrophage surface markers in patients with systemic sclerosis. *Ann Rheum Dis* 2018;77:1842–5.
3 Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. *Nat Rev Immunol* 2008;8:958–69.
4 Behmoaras J, Petretto E. Cell function in disease: there are more than two parties at play. *Ann Rheum Dis* 2019;78:e20–e20. 10.1136/annrheumdis-2018-213121.
5 Moreno-Moral A, Bagnati M, Koturan S, *et al*. Changes in macrophage transcriptome associate with systemic sclerosis and mediate *GSDMA* contribution to disease risk. *Ann Rheum Dis* 2018;77:596–601.
6 Forrester MA, Wassall HJ, Hall LS, *et al*. Similarities and differences in surface receptor expression by THP-1 monocytes and differentiated macrophages polarized using seven different conditioning regimens. *Cell Immunol* 2018;332:58–76.
7 Matzinger P, Kamala T. Tissue-based class control: the other side of tolerance. *Nat Rev Immunol* 2011;11:221–30.
8 Taroni JN, Greene CS, Martyanov V, *et al*. A novel multi-network approach reveals tissue-specific cellular modulators of fibrosis in systemic sclerosis. *Genome Med* 2017;9:27.
9 Soldano S, Pizzorni C, Paolino S, *et al*. Correction: alternatively activated (M2) macrophage phenotype Is inducible by endothelin-1 in cultured human macrophages. *PLoS One* 2017;12:e0175238.

Immune checkpoint inhibitor rechallenge in patients with immune-related myositis

Therapeutic management of many cancers has been revolutionised by the development of immune checkpoint inhibitors (ICI) targeting antiprogrammed death 1 (PD-1)/ligand 1 (PDL1) and anticytotoxic T-lymphocyte antigen 4 leading to durable responses.[1] ICIs however can induce several immune-related adverse events (irAE) including musculoskeletal irAEs.[2] Among them, ICI-related myositis can be severe and sometimes life threatening.[3 4] The current management includes permanent discontinuation of ICIs and steroid treatment. To date, very little is known about the risk of irAE recurrence in case of ICI rechallenge,[5 6] especially in myositis for which no case of rechallenge has yet been reported. Through two cases, we report the safety of resuming anti-PD-1/PDL1 in patients who experienced severe ICI-related myositis.

An 87-year-old patient with metastatic Merkel cell carcinoma (MCC) received avelumab as first-line treatment. After three infusions, he developed slight head dropped syndrome with increased creatine kinase (CK) level up to 3.5 times the upper limit normal (ULN) range. Electromyography showed myogenic syndrome (left trapezius and right sternocleidomastoid muscles) and ^{18}F-fluorodeoxyglucose-positron emission tomography revealed significant hypermetabolism of axial muscles (table 1). Myositis-specific autoantibodies were negative. Myocarditis was ruled out. At that time the radiologic evaluation showed a partial tumour response. Avelumab was stopped and the patient received prednisone (tapering from 0.5 mg/kg) during 6 weeks, which allowed myositis remission, but MCC recurred 7 months later. Avelumab was resumed and prednisone was preventively given during 3 months, starting at 20 mg/day. With a 9-month follow-up, no irAE, including myositis, occurred and MCC returned in partial response.

A 61-year-old patient with metastatic melanoma developed ptosis, diplopia, dysphagia and muscle weakness 3 weeks after first infusion of ipilimumab combined with nivolumab as first-line treatment. CK levels raised up to 40 ULN. Electromyography showed myogenic pattern of the trapezius, without decrement. Muscular biopsy with focal necrosis/regeneration lesions, HLA-1 and C5b9 positive sarcoplasmic staining of the suffering myofibres and T cell infiltrates confirmed the myositis.

Table 1 Patient characteristics

		Patient 1	Patient 2
Cancer history	Stage IV cancer	Merkel cell carcinoma	Melanoma
	ICI	Avelumab 10 mg/kg/2 weeks, 3 infusions	Ipilimumab 3 mg/kg+nivolumab 1 mg/kg, 1 infusion
ICI-related myositis	Onset of symptoms	Week 6	Week 3
	Clinical symptoms	Dropped head syndrome	Myalgia, muscle weakness
		Neck pain	Ptosis, diplopia, dysphagia
		Fatigue	Fatigue
	Maximum	×3.5 ULN	×40 ULN
	Creatine kinase		
	Electromyography	Myogenic syndrome: Left trapezius and right sternocleidomastoid muscle. No decrement.	Myogenic sundrome: Right trapezius. No decrement.
	FDG-PET	Significant hypermetabolism of trapezius, erector muscles of the spine, pilar muscle of the diaphragm	Slight diffuse muscular hypermetabolism
	Muscular biopsy	Contraindication (anticoagulant treatment for atrial fibrillation)	Positive with focal necrosis/regeneration lesions, HLA-I and C5b9 positive staining, presence of T cell and macrophage inflammatory cells
	Cardiac examination ECG	Permanent atrial fibrillation (>5 years)	Normal
	High-sensitive troponin-T (<14 ng/L)	475	298
	Echocardiography	Normal	Normal
	Cardiac MRI	Contraindication (pacemaker)	–
	Cardiac FDG PET	No myocarditis	No myocarditis
	Treatment of irAE	Oral prednisone 0.5 mg/kg/day, tapered and withdrawn within 6 weeks	Methylprednisolone 1 g/day×3 days, followed by oral prednisone 1 mg/kg, tapered and withdrawn within 9 weeks
Rechallenge	Response to initial treatment with ICI	Partial response	Progressive disease
	Time from first ICI treatment to ICI rechallenge	7 months	8 months
	ICI	Avelumab 10 mg/kg/2 weeks, 20 infusions	Pembrolizumab 2 mg/kg/3 weeks, 2 infusions
	Associated treatment	Prednisone 20 mg/day, tapered and withdrawn within 3 months	–
	irAE	None	None
	ICI-myositis	No recurrence of myositis with no symptoms, normal CK level, normal electromyography and absence of muscle hypermetabolism on TEP	No recurrence of myositis without any symptoms, normal CK level
	Tumour response	Ongoing partial response after 9 months	Death due to progressive disease at week 6

CK, creatine kinase; FDG-PET, fluorodeoxyglucose-positron emission tomography; HLA, human leucocyte antigen; ICI, immune checkpoint inhibitor; irAE, immune-related adverse event; ULN, upper limit normal.

Neither myositis-specific nor myasthenia gravis autoantibodies were detected. ICIs were stopped and three pulses of methylprednisolone followed by tapering doses of prednisone were given leading to complete remission within 8 weeks. Because of the lack of efficacy of the single infusion of ICI combination followed by three infusions of dacarbazine, pembrolizumab was introduced 8 months after the myositis episode. The patient presented no irAEs or myositis (table 1) but died due to melanoma progression.

Despite the risk of recurrent irAEs, rechallenging ICIs after discontinuation due to previous irAEs remains critical, when considering their potential benefits in terms of survival.[5] These two cases suggest that resuming ICIs can be safe in patients displaying persistent remission of ICI-related myositis.

Julie Delyon,[1,2,3] Florence Brunet-Possenti,[3,4] Sarah Leonard-Louis,[5,6] Dimitri Arangalage,[3,7,8] Mathilde Baudet,[9] Barouyr Baroudjian,[1] Celeste Lebbe,[1,2,3] Baptiste Hervier,[6,10,11] on behalf of the PATIO Group

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REFERENCES

1 Wolchok JD, Chiarion-Sileni V, Gonzalez R, *et al*. Overall survival with combined nivolumab and ipilimumab in advanced melanoma. *N Engl J Med* 2017;377:1345–56.
2 Kostine M, Rouxel L, Barnetche T, *et al*. Rheumatic disorders associated with immune checkpoint inhibitors in patients with cancer—clinical aspects and relationship with tumour response: a single-centre prospective cohort study. *Ann Rheum Dis* 2018;77:393–8.
3 Touat M, Maisonobe T, Knauss S, *et al*. Immune checkpoint inhibitor-related myositis and myocarditis in patients with cancer. *Neurology* 2018;91:e985–94.
4 Eggermont AMM, Blank CU, Mandala M, *et al*. Adjuvant pembrolizumab versus placebo in resected stage III melanoma. *N Engl J Med* 2018;378:1789–801.
5 Pollack MH, Betof A, Dearden H, *et al*. Safety of resuming anti-PD-1 in patients with immune-related adverse events (irAEs) during combined anti-CTLA-4 and anti-PD1 in metastatic melanoma. *Ann Oncol* 2018;29:250–5.
6 Menzies AM, Johnson DB, Ramanujam S, *et al*. Anti-PD-1 therapy in patients with advanced melanoma and preexisting autoimmune disorders or major toxicity with ipilimumab. *Ann Oncol* 2017;28:368–76.

Coffee consumption and gout: a Mendelian randomisation study

I have read with great interest the article by Larsson and Carlström[1] regarding coffee consumption and gout. This Mendelian randomisation (MR) analysis demonstrates that coffee consumption may lower the risk of gout. When a randomised controlled trial is lacking because of an unethical or infeasible issue, MR studies may help address the causal relationship. However, it has some methodological issues. The primary concern relates to whether the MR study has adequate statistical power to detect an association. An increase in the variance in the trait of interest explained by the genetic instrument leads to improvement in the power of the MR analysis. However, most genetic variants for an exposure may only explain a small proportion of variance in that exposure. As genetic variants typically explain a small proportion of the variance in an exposure, the statistical power to detect an association between the variant and the outcome in an MR analysis can be limited or low.[2] I wonder how much amount of the variance in coffee consumption is explained by the five single nucleotide polymorphisms (SNP) in the sample. A second concern relates to whether the causal effect in the MR study remained significant in the sensitivity tests. As all the variants used in MR may not be the valid instruments, methods for sensitivity analysis including MR-Egger regression and the weighted median approach have been developed.[3 4] The use of both methods is recommended when multiple genetic variants must be assessed for robustness of any causal finding to different sets of assumptions.[5] MR-Egger regression has been proposed to test for directional pleiotropy and provides an estimate of the causal effect adjusted for its presence, and has been shown to be robust against invalid instruments.[3] MR-Egger regression provides a useful additional sensitivity analysis to the standard inverse variance weighted approach that assumes that all variants are valid instruments. Although MR-Egger regression revealed that directional pleiotropy was unlikely to have biased the result, this study did not show the data on the causal effect adjusted for pleiotropy by MR-Egger analysis. A 'leave-one-out' analysis may also be needed to evaluate if the causal association by MR estimate is driven or biased by a unique SNP that might have a particularly large horizontal pleiotropic effect. Thus, I believe that the findings of this MR study should be interpreted by taking the aforementioned methodological concerns into consideration.

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REFERENCES

1 Larsson SC, Carlström M. Coffee consumption and gout: a mendelian randomisation study. *Ann Rheum Dis* 2019;77:1842–5.
2 Swerdlow DI, Kuchenbaecker KB, Shah S, *et al*. Selecting instruments for mendelian randomization in the wake of genome-wide association studies. *Int J Epidemiol* 2016;45:1600–16.
3 Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int J Epidemiol* 2015;44:512–25.
4 Bowden J, Davey Smith G, Haycock PC, *et al*. Consistent estimation in mendelian randomization with some invalid instruments using a weighted median estimator. *Genet Epidemiol* 2016;40:304–14.
5 Burgess S, Bowden J, Fall T, *et al*. Sensitivity analyses for robust causal inference from mendelian randomization analyses with multiple genetic variants. *Epidemiology* 2017;28:30–42.