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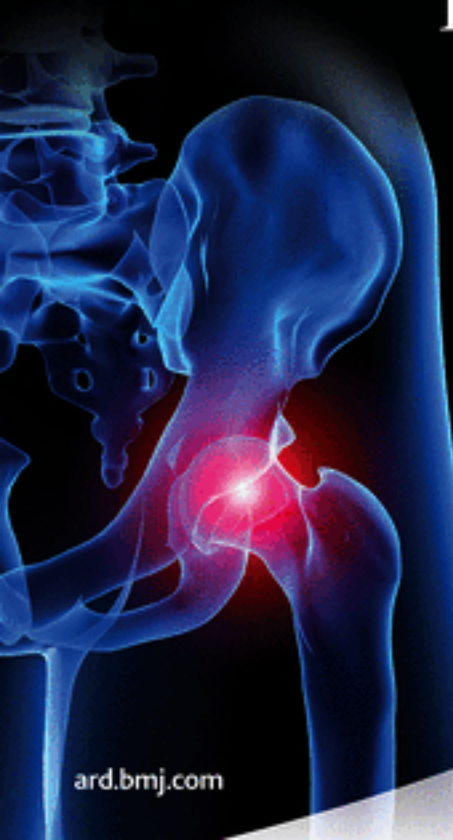

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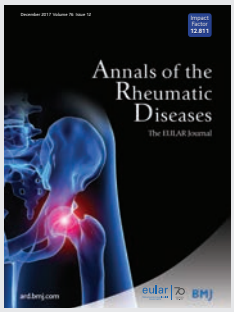

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Classification and diagnostic criteria in Sjögren's syndrome: a long-standing and still open controversy

Claudio Vitali,[1,2] Nicoletta Del Papa[2,3]

Most rheumatic diseases are multisystem disorders that are heterogeneous in their presentation, course and outcome. These conditions still lack a single clinical, laboratory, pathological or radiological feature that could serve as a 'gold standard' in support of diagnosis and/or classification. Thus, the development of criteria for use in clinical care and research studies has been an important challenge in these disorders.[1]

From the theoretical and methodological point of view, classification and diagnostic criteria are quite different. Classification criteria are standardised tools that are aimed at selecting well-defined and homogenous groups of patients for research and at guaranteeing comparability across studies. They are not designed to be used for the clinical diagnosis in individual patients and may be defective in capturing some cases with a less common clinical presentation or course.[2]

Diagnostic criteria are generally less stringent and usually include a wider variety of disease features. Their aim is to accurately identify as many people with that condition as possible.[1] Given the complexity of systemic rheumatic disorders, the development of diagnostic criteria in these diseases is certainly difficult. Therefore, optimal diagnostic criteria have not been defined for most of the rheumatic diseases and the diagnosis, given the suspicion of one of these disorders, is commonly based on a decision-making process by physicians who have to evaluate a complex combination of symptoms, signs, diagnostic tests and rule out other confounding or similar diseases.

As a consequence of these theoretical assumptions, the diagnostic criteria are commonly characterised by high sensitivity and negative predictive value, whereas the classification criteria classically possess high specificity and positive predictive value to minimise the risk of classifying false positive patients as having the disease. Sensitivity and specificity show an inverse relationship where to any increase of the former corresponds a decrease of the latter and vice versa.[3] The receiver operating characteristic (ROC) curve is the statistical and graphical description of this process, showing the equilibrium between sensitivity and specificity.[4]

Because of the lack of diagnostic criteria for many rheumatic disorders, no studies on direct comparison between classification and diagnostic criteria for the same disease are traceable in the medical literature.[5] Conversely, the performance of classification criteria as a diagnostic tool has been explored in a number of studies where the expert clinician's judgement was considered the gold standard for the diagnosis. As expected, specific classification criteria did not demonstrate to be a reliable instrument in making a correct diagnosis in the different disorders.[5] In spite of their deceptive diagnostic performance in individual patients, the use of classification criteria as a diagnostic tool is commonplace in daily rheumatological practice. Classification criteria are regarded as a useful guide for diagnosis, and, in addition, they may have a role in education and training in medicine.[5]

A large number of studies comparing different classification criteria for the same disorder have been carried out, often aimed at measuring the performance of newly proposed criteria to that of the older ones. In this regard, Tsuboi *et al*[6] report a study performed in a large cohort of Japanese (JPN) patients where the sensitivity and specificity of the new 2016 American College of Rheumatology (ACR)-European League Against Rheumatism (EULAR) classification criteria for primary Sjögren's syndrome (pSS)[7] were compared with those of the 1999 revised JPN Ministry of Health diagnostic criteria,[8] the 2002 American-European Consensus Group (AECG)[9] and the 2012 ACR[10] classification criteria for this disease. On the whole, the results of this comparison indicate that the 2016 ACR-EULAR criteria have higher sensitivity and lower specificity in the classification of patients with pSS than the other three sets of criteria. Furthermore, the degree of agreement of the ACR-EULAR classification criteria with all the other three sets of criteria was low.

Looking in details at the results of this study,[6] and namely at the subanalysis of 383 cases—that is certainly more reliable for the higher similarity of the considered diagnostic items across the different criteria sets—it is rather surprising to see that the JPN criteria are the ones with the highest specificity and the lower sensitivity. This result is rather unexpected since the JPN criteria are the only ones defined as diagnostic criteria among the criteria compared in the study.

Taking in mind the theoretical considerations discussed above on the critical differences between classification and diagnostic criteria, to compare JPN criteria, which were defined as diagnostic, to other classification criteria for pSS could be 'per se' an invalidating procedural defect. However, considering the general policy and procedures adopted in the development of the revised JPN diagnostic criteria, in which it was outlined that one of the goals should be to make only definite diagnoses and to exclude probable cases, in other words, to have a high specificity,[8] one can conclude that the JPN criteria should have been more correctly defined as classification rather than diagnostic criteria.

Other factors may have conditioned the results of this study. It is well known that both the classification and diagnostic criteria performance may vary in different clinical and geographical settings.[11 12] This may greatly depend on the prevalence and clinical pattern of presentation that a disease may have in different geographical regions and in different clinical backgrounds.[12 13] Thus, it is likely that the best performance of any criteria set can be reached in the clinical setting and geographical area where the criteria set has been developed. This performance variability is expected to be wider for diagnostic criteria that include more disease descriptors, but may also be observed, to a lesser extent, in applying classification criteria.

The low level of agreement between the ACR/EULAR and AECG criteria observed in the study by Tsouboi *et al* is in contrast with what was reported before.[7] This discrepancy can be largely reduced, and the agreement between the ACR/EULAR

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and AECG criteria consistently improved, reconsidering the 19 patients of this cohort who were classified as having pSS by only the ACR/EULAR criteria.[6] They have positive lip biopsy (11 patients) or positive anti-SSA/Ro antibodies (8 patients), plus reduced salivary (18 patients) or lachrymal flow (1 patient). Most of these patients could also have met the AECG criteria if the presence of dry eye and dry mouth symptoms had been investigated by the AECG-validated questionnaires for sicca symptoms. The authors did not specify the way they explored sicca complaints in their retrospective study.

The fact that, in the study of Tsouboi *et al*, the ACR/EULAR classification criteria for pSS have demonstrated higher sensitivity and, consequently, lower specificity than all of the other criteria sets is not completely unexpected. The appearance of new therapeutic agents with a favourable risk–benefit profile and the potential to change the long-term prognosis of rheumatic disorders has outlined the need to define new classification criteria with a higher sensitivity and therefore able to recognise patients with early disease. With the support of and following the methodological procedures approved by both the ACR and EULAR 'ad hoc' committees,[4 14] newer classification criteria for different disorders have been proposed and validated in multicentre multinational frameworks.[7 15–18] Of course, a loss of specificity may be the counterpart to the increased sensitivity of the new criteria. Consequently, more 'liberal' criteria should be used with caution when a therapeutic agent with an unclear safety profile is under investigation in a trial. By moving along the ROC curve designed for the new classification criteria, and then applying the criteria in a flexible way, one can find a different sensitivity/specificity ratio capable of greatly reducing the risk of selecting and treating false positive cases. A cut-off point of 5 instead of 4, for instance, raises the specificity of the ACR-EULAR classification criteria for pSS from 89% to 98%.[7]

The new ACR-EULAR classification criteria for pSS are the final result of an international cross-cultural collaboration and are derived by a well-established and validated methodology. At the best of present knowledge, these criteria describe the key shared features defining this condition and may represent the common language to be used in the next future to make the scientific communication easier, favour the exchange of information and stimulate the development of collaborative studies.

Contributors CV is the main author. NDP contributed to writing the paper and revising the cited literature.

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2017 European League Against Rheumatism/ American College of Rheumatology classification criteria for adult and juvenile idiopathic inflammatory myopathies and their major subgroups

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ABSTRACT

Objective To develop and validate new classification criteria for adult and juvenile idiopathic inflammatory myopathies (IIM) and their major subgroups.

Methods Candidate variables were assembled from published criteria and expert opinion using consensus methodology. Data were collected from 47 rheumatology, dermatology, neurology and paediatric clinics worldwide. Several statistical methods were used to derive the classification criteria.

Results Based on data from 976 IIM patients (74% adults; 26% children) and 624 non-IIM patients with mimicking conditions (82% adults; 18% children), new criteria were derived. Each item is assigned a weighted score. The total score corresponds to a probability of having IIM. Subclassification is performed using a classification tree. A probability cut-off of 55%, corresponding to a score of 5.5 (6.7 with muscle biopsy) 'probable IIM', had best sensitivity/specificity (87%/82% without biopsies, 93%/88% with biopsies) and is recommended as a minimum to classify a patient as having IIM. A probability of ≥90%, corresponding to a score of ≥7.5 (≥8.7 with muscle biopsy), corresponds to 'definite IIM'. A probability of <50%, corresponding to a score of <5.3 (<6.5 with muscle biopsy), rules out IIM, leaving a probability of ≥50 to <55% as 'possible IIM'.

Conclusions The European League Against Rheumatism/American College of Rheumatology (EULAR/ACR) classification criteria for IIM have been endorsed by international rheumatology, dermatology, neurology and paediatric groups. They employ easily accessible and operationally defined elements, and have been partially validated. They allow classification of 'definite', 'probable' and 'possible' IIM, in addition to the major subgroups of IIM, including juvenile IIM. They generally perform better than existing criteria.

INTRODUCTION

Idiopathic inflammatory myopathies (IIM), collectively known as myositis, are heterogeneous disorders characterised by muscle weakness and muscle inflammation.[1] The most common subgroups in adults are dermatomyositis (DM), polymyositis (PM) and inclusion body myositis (IBM),[2] and in children, juvenile DM (JDM).

The International Myositis Assessment and Clinical Studies (IMACS) Group has developed consensus on outcome measures and definitions of improvement to be used in clinical trials for myositis.[3 4] A prerequisite for clinical trials and other clinical studies is the inclusion of well-defined patient groups. A wide variety of diagnostic or classification criteria for myositis are used,[2 5–16] but are generally derived empirically and not validated. The criteria of Bohan and Peter[7 8] are most widely used, but have limitations. Because they do

This criteria set has been approved by the European League Against Rheumatism (EULAR) Executive Committee and the American College of Rheumatology (ACR) Board of Directors as Provisional. This signifies that the criteria set has been quantitatively validated using patient data, but it has not undergone full validation based on an independent dataset, using both cases and controls. This validation step is still needed before the criteria are fully validated.

not clearly specify how to exclude other forms of myopathy, they may misclassify IBM patients as PM,[13 17–19] and muscular dystrophies with inflammation as myositis, and each criterion is not defined explicitly. New discoveries in the last decade, such as myositis-specific autoantibodies that are associated with distinct clinical phenotypes,[2 20–22] may provide opportunities to improve the precision of classification, but have not been tested adequately.[11 23]

The aim of this project was to develop classification criteria for adult and juvenile IIM. The specific goal was to define the minimum essential, easily available clinical and laboratory features to (1) distinguish IIM from mimicking conditions with high sensitivity and specificity, and (2) distinguish the major subgroups of IIM.

METHODS

Study design

The International Myositis Classification Criteria Project (IMCCP), an international collaboration with experts from adult and paediatric rheumatology, neurology, dermatology, epidemiology and biostatistics, was established in 2004 and followed at our best the European League Against Rheumatism (EULAR) and American College of Rheumatology (ACR) recommendations for development of classification criteria from that time or published soon thereafter.[24 25] A steering committee (online supplementary 1) and a larger working committee with experts in IIM were formed (see online supplementary appendix).

Using the nominal group technique, experts in IIM from the steering committee and the working committee[26–29] designed the study and validation experiments, assembled and defined candidate criteria from published myositis criteria[2 5–16] and other characteristics of myositis, and determined and assembled the IIM subgroup diagnoses and comparator conditions that were studied. A pilot study to assess the practicality of capturing the items showed a fair agreement of data availability from IIM and non-IIM cases (online supplementary 2). Input was obtained from myositis experts, by email to the IMACS network and requesting comments on the items, to maximise face and content validity.[24 25] The steering committee revised the list of variables based on the comments and further suggestions from the IMACS network and 93 variables (online supplementary 3) were selected by the steering committee for study in cases and comparators. A glossary and definitions were developed according to an ACR glossary[30 31] (online supplementary 4). Data were abstracted from patients' records and entered into a web-based database.

Inclusion criteria for cases and comparators were (1) diagnosis for at least 6 months prior to study inclusion; (2) physician certainty of diagnosis—either known IIM or, as comparators, known non-IIM cases where myositis was considered in the initial differential diagnosis; and (3) patients with the most recent and complete data were prioritised to acquire the most complete data in a consistent manner. A maximum of 40 cases and an equal number of comparators were collected from each centre.

The study was approved by the ethics committees at each site.

Data analysis and candidate criteria selection

The association of each variable with the diagnosis (IIM, non-IIM) was assessed by ORs and tested with Fisher's exact test. The treating physician diagnosis was considered the gold standard for analysis. Three classification techniques were explored: (1) a sum-of-items model in which a patient was classified as a case if the patient had a specified number of items from a set of items, (2) a probability-score model and (3) a classification tree. The ensuing candidate criteria were examined with respect to statistical performance and clinical relevance. Due to the observed superior discriminating performance of the probability-score model, the other models were set aside.

Criteria development

The probability-score model summed score points associated with the signs and symptoms present. The score points were obtained as coefficients of a logistic regression model used to combine multiple variables for predicting IIM. The statistical significance of the resulting increase in the goodness-of-fit of the model was assessed using the Wald test. The improvement in predictive ability was measured by the increment in specificity and sensitivity and summarised by the area under the receiver operating characteristic curve (AUC).

Paediatric experts are using fewer muscle biopsies for classification of JDM in clinical practice than adult rheumatologists. Thus, a second model not including biopsy variables was developed. Assessment of statistical performance for each score/probability cut-off value provided the basis for a recommendation of a cut-off value for IIM classification by the steering committee. The proposed cut-offs were then defined as possible, probable and definite IIM. To facilitate use of the new criteria, a web-based calculator for the probability-score model was developed.

The new classification criteria were compared with previous IIM criteria. Their statistical performance, and number of patients per IIM subdiagnosis classified as IIM by the different criteria sets, were calculated.

To distinguish subgroups of patients classified with IIM according to the new criteria, a classification tree was developed. The tree was based on the variables in the new classification criteria, statistical analyses, as described in a separate methodology paper and on expert opinion.

Validation

The new criteria were internally cross-validated. Samples of equal size to the original sample were drawn from the entire population at random with replacement, so-called 'bootstrap' samples.[32] The bootstrap sample represented the training sample, and the remaining subjects not contained in the bootstrap sample constituted the validation sample. The probability score was applied to each bootstrap training sample separately and then used to predict IIM in the validation sample. The procedure was repeated in over 200 bootstrap samples, and the average AUC was calculated.

The performance of the new criteria for IIM including the subgroups was tested for sensitivity in two independent cohorts, the Euromyositis Register (https://euromyositis.eu/) and the Juvenile Dermatomyositis Cohort Biomarker Study and Repository (JDRG) (UK and Ireland) (https://www.juvenile dermatomyositis.org.uk/).

The program Stata V.13 (StataCorp) was used for data management and statistical analyses. The statistical program R (R Core Team (2014). R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/) was used for some analyses.

A report detailing the methodology will be submitted as a separate publication (manuscript submitted).

Table 1 Demographic data of the International Myositis Classification Criteria Project cohort

	IIM (n=976)	Comparators (n=624)
Sex, n (%)		
Female	652 (66.8)	369 (59.1)
Male	324 (33.2)	255 (40.9)
Adult onset disease*, n (%)	727 (74.5)	509 (81.6)
Childhood onset disease*, n (%)	249 (25.5)	115 (18.4)
Age at onset of symptoms, median (IQR), years	44.0 (14.7–57.0)	41.0 (20.0–56.0)
Age at diagnosis, median (IQR), years	45.5 (16.2–59.3)	45.0 (25.8–58.0)
Disease duration from time of first symptom†, median (IQR), years	4.0 (2.0–8.0)	4.0 (1.0–9.0)
Disease duration from time of diagnosis‡, median (IQR), years	3.0 (1.0–6.0)	1.8 (0.0–4.5)
Ethnicity, n (%)		
Caucasian	611 (62.6)	360 (57.7)
Asian	177 (18.1)	156 (25.0)
Hispanic	51 (5.2)	25 (4.0)
African	40 (4.1)	28 (4.5)
Native American	18 (1.8)	4 (0.6)
Pacific Islander	3 (0.3)	1 (0.2)
Mixed	37 (3.8)	22 (3.5)
Unknown	54 (5.5)	32 (5.1)
Disease onset§, n (%)		
Acute (days to 2 weeks)	45 (4.6)	64 (10.3)
Subacute (>2 weeks to ≤2 months)	237 (24.3)	88 (14.1)
Insidious (>2 months to years)	648 (66.4)	444 (71.2)
NA	46 (4.7)	28 (4.5)

*Onset of first symptoms assumed to be related to the disease.
†Time from first symptom to last clinical evaluation.
‡Time from diagnosis to last clinical evaluation.
§Onset and progression of the first symptoms of the syndrome to the full disease presentation.
IIM, idiopathic inflammatory myopathies; NA, information not available.

RESULTS

Study population

Data from 976 IIM patients (74.5% adults; 25.5% children) (table 1) were collected between 2008 and 2011 from 23 European, 17 North American, 1 South American and 6 Asian sites, representing IIM subgroups of JDM (n=248), PM (n=245), DM (n=239), IBM (n=176), amyopathic DM (ADM) (n=44), hypomyopathic DM (n=12), immune-mediated necrotising myopathy (IMNM) (n=11) and juvenile PM (n=1). A total of 624 comparators (81.6% adults; 18.4% children) (table 1) representing a broad spectrum of conditions that can mimic IIM were included, comprising systemic inflammatory diseases (36.5%), muscle dystrophies (16.0%), drug-associated or toxin-associated myopathies (7.9%), motor neuron diseases/neuropathies (7.7%), metabolic myopathies (6.9%), myalgias (4.5%), dermatological diseases (3.7%), endocrine myopathies (3.7%), infectious myopathies (4.5%), mitochondrial myopathies (2.4%), neuromuscular diseases (2.6%), other myopathies (1.9%), immune-mediated skin conditions (0.5%) as well as other diagnoses (1.3%) (online supplementary 5 and 6).

CANDIDATE CRITERIA SELECTION AND CRITERIA DEVELOPMENT

Based on statistical models, 16 variables from six categories best distinguished IIM cases from comparators (table 2), and each variable was assigned a weight (score) based on its influence to discriminate IIM from non-IIM. A total score was computed by adding score points corresponding to each criterion being present. The score can be converted into a probability of IIM (figure 1A,B) by:

Probability of IIM including muscle biopsy=1/[1+exponential (5.33–score)]

or,

Probability of IIM without muscle biopsy=1/[1+exponential (6.49–score)]

or by using the online web calculator (www.imm.ki.se/biostatistics/calculators/iim).

Sensitivity and specificity for varying probability cut-offs are shown in figure 1C,D.

Cut-points for classification

The best balance between sensitivity and specificity was found for a probability of 55%–60% for the criteria not including muscle biopsy data, and 55%–75% when including muscle biopsies, or a total aggregated score of score of ≥5.5 and ≤5.7 (≥6.7 and ≤7.6 if biopsy is available). The IMCCP proposes that a patient may be classified as IIM if the probability exceeds a predetermined cut-off of at least 55% (corresponding to a score of ≥5.5, or ≥6.7 if biopsies are included) based on maximisation of statistical performance and best balance between sensitivity and specificity. The level of probability ≥55% and <90% was defined as 'probable IIM'. The steering committee recommends, based on expert opinion, that 'definite IIM' should equal a probability of ≥90%, corresponding to having total aggregate score of ≥7.5 without muscle biopsy and ≥8.7 with muscle biopsy.

Patients falling in the probability range ≥50% and <55% will be classified as 'possible IIM'. For a patient to be classified as a non-IIM patient, the probability would have to be <50% (score of < 5.3 without biopsies; < 6.5 with biopsies).

As suggested by paediatric experts and dermatologists, for patients with pathognomonic skin rashes of DM or JDM, classification criteria were developed, which did not include muscle biopsy data (table 2). However, where no skin rash is present, a muscle biopsy is required for classification, as determined by a consensus of expert opinion within the IMCCP steering and working committees. Both sets apply equally well to adult IIM patients and to juvenile patients with DM and should be used when IIM is suspected and no better explanation for the symptoms exists, as agreed on by expert opinion. Definitions for the criteria items are presented in table 2.

IDENTIFICATION OF SUBGROUPS

A patient classified with IIM by the EULAR/ACR classification criteria (probability of IIM ≥55%) can be further subclassified with a classification tree (figure 2). Age at onset of first symptom (≥18 years of age) distinguishes adult from juvenile IIM. Thereafter, clinical findings and muscle biopsy features subclassify adult IIM patients into PM, IBM, ADM or DM. Based on our dataset, juvenile patients with skin rash can be classified into JDM. Three subgroups cannot be further separated using our criteria because of small sample sizes: juvenile PM, IMNM and hypomyopathic DM.

Among patients with IIM by the EULAR/ACR classification criteria (probability of IIM ≥55%), and with sufficient data to allow subclassification (n=703), the number of cases in the subgroups as defined according to the classification tree was

Table 2 The European League Against Rheumatism/American College of Rheumatology (EULAR/ACR) classification criteria for adult and juvenile idiopathic inflammatory myopathies (IIMs)

When no better explanation for the symptoms and signs exists, these classification criteria can be used

Variable	Score points		
	Without muscle biopsy	With muscle biopsy	Definition
Age of onset			
Age of onset of first symptom assumed to be related to the disease ≥18 years and <40 years	1.3	1.5	18≤Age (years) at onset of first symptom assumed to be related to the disease <40
Age of onset of first symptom assumed to be related to the disease ≥40 years	2.1	2.2	Age (years) at onset of first symptom assumed to be related to the disease ≥40
Muscle weakness			
Objective symmetric weakness, usually progressive, of the proximal upper extremities	0.7	0.7	Weakness of proximal upper extremities as defined by manual muscle testing or other objective strength testing, which is present on both sides and is usually progressive over time
Objective symmetric weakness, usually progressive, of the proximal lower extremities	0.8	0.5	Weakness of proximal lower extremities as defined by manual muscle testing or other objective strength testing, which is present on both sides and is usually progressive over time
Neck flexors are relatively weaker than neck extensors	1.9	1.6	Muscle grades for neck flexors are relatively lower than neck extensors as defined by manual muscle testing or other objective strength testing
In the legs, proximal muscles are relatively weaker than distal muscles	0.9	1.2	Muscle grades for proximal muscles in the legs are relatively lower than distal muscles in the legs as defined by manual muscle testing or other objective strength testing
Skin manifestations			
Heliotrope rash	3.1	3.2	Purple, lilac-coloured or erythematous patches over the eyelids or in a periorbital distribution, often associated with periorbital oedema
Gottron's papules	2.1	2.7	Erythematous to violaceous papules over the extensor surfaces of joints, which are sometimes scaly. May occur over the finger joints, elbows, knees, malleoli and toes
Gottron's sign	3.3	3.7	Erythematous to violaceous macules over the extensor surfaces of joints, which are not palpable
Other clinical manifestations			
Dysphagia or oesophageal dysmotility	0.7	0.6	Difficulty in swallowing or objective evidence of abnormal motility of the oesophagus
Laboratory measurements			
Anti-Jo-1 (anti-histidyl-tRNA synthetase) autoantibody present	3.9	3.8	Autoantibody testing in serum performed with standardised and validated test, showing positive result
Elevated serum levels of creatine kinase (CK)* *or* lactate dehydrogenase (LD)* *or* aspartate aminotransferase (ASAT/AST/SGOT)* *or* alanine aminotransferase (ALAT/ALT/SGPT)*	1.3	1.4	The most abnormal test values during the disease course (highest absolute level of enzyme) above the relevant upper limit of normal
Muscle biopsy features—presence of:			
Endomysial infiltration of mononuclear cells surrounding, but not invading, myofibres		1.7	Muscle biopsy reveals endomysial mononuclear cells abutting the sarcolemma of otherwise healthy, non-necrotic muscle fibres, but there is no clear invasion of the muscle fibres
Perimysial and/or perivascular infiltration of mononuclear cells		1.2	Mononuclear cells are located in the perimysium and/or located around blood vessels (in either perimysial or endomysial vessels)
Perifascicular atrophy		1.9	Muscle biopsy reveals several rows of muscle fibres, which are smaller in the perifascicular region than fibres more centrally located
Rimmed vacuoles		3.1	Rimmed vacuoles are bluish by H&E staining and reddish by modified Gomori trichrome stains

*Serum levels above the upper limit of normal.

enumerated (table 3). The agreement between the classification tree subgroups and the physician-diagnosed subgroups in the dataset was high (92.6% agreement, kappa=0.90, p<0.00001). The agreement proportions, with a probability of 55%, were 1.00 for JDM, 0.89 for DM, 0.94 for ADM, 0.92 for IBM and 0.93 for PM. Raising the probability cut-off of IIM to 90% yielded 94.9% agreement, kappa=0.93, p<0.00001. With a probability cut-off of 90%, the agreement proportions were 1.00 for JDM, 0.96 for DM, 0.95 for ADM, 0.93 for IBM and 0.88 for PM.

Performance of EULAR/ACR criteria compared with published criteria

Performance of the EULAR/ACR criteria was compared with published criteria for IIM[7 8 10 11 14 15] using the IMCCP dataset (table 4). The new criteria including muscle biopsy features displayed high sensitivity (93%) and specificity (88%). There was slightly lower performance without biopsy variables (sensitivity and specificity 87% and 82%, respectively). Among the assessed criteria, the Targoff criteria[11] showed the highest sensitivity (93%) and specificity (89%). Other criteria had either high

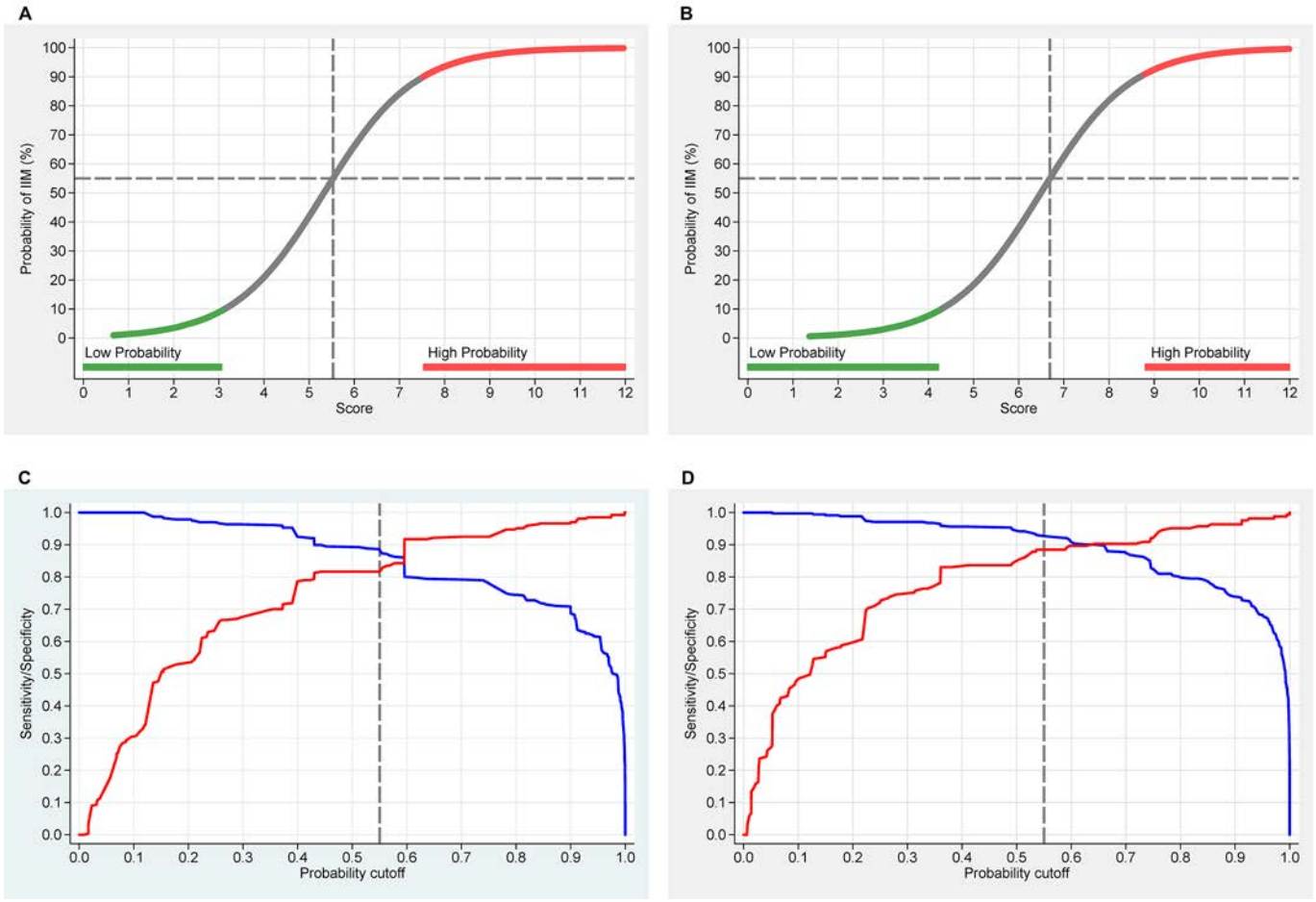

Figure 1 Probability of having idiopathic inflammatory myopathies (IIM) based on the EULAR/ACR classification criteria for IIM. Each score obtained from the classification criteria corresponds to a probability of having the disease, without muscle biopsy data (A) or with muscle biopsy data (B). Each score and probability of disease display a unique set of sensitivity (blue line) and specificity (red line) measurements for the classification criteria not including muscle biopsy data (C) or including muscle biopsy data (D). The most optimal point of accuracy should be stated in publications and be appropriate to the intended purpose, with the recommendation of using a minimum of 55% probability (score of 5.5 without biopsies; 6.7 with biopsies) for classifying a case as IIM ('probable IIM') (dotted line). 'Definite IIM' corresponds to a probability of at least 90% (score of ≥ 7.5 without biopsies; ≥ 8.7 with biopsies). ACR, American College of Rheumatology; EULAR, European League Against Rheumatism.

sensitivity and low specificity (Bohan and Peter[7 8] and Tanimoto criteria[10]), or low sensitivity and high specificity (Dalakas and Hohlfeld[14] and ENMC criteria[15]).

We studied how different criteria could classify patients with diverse IIM subdiagnoses in the IMCCP dataset (table 4). The EULAR/ACR classification criteria correctly classified most patients with all IIM subdiagnoses. When biopsy data were used, the performance improved for IBM (94% with biopsy data vs 58% without biopsy data) and PM (86% with biopsy data vs 79% without biopsy data). The Bohan and Peter,[7 8] Tanimoto[10] and Targoff[11] criteria correctly classified all IIM subdiagnoses except ADM, a diagnosis not included in those criteria. The Dalakas and Hohlfeld criteria[14] could not classify any subdiagnoses. The ENMC criteria[15] correctly classified DM and JDM cases but no other subdiagnoses.

A comparison between the EULAR/ACR classification criteria (55% probability cut-off) and the Bohan and Peter criteria[7 8] showed 89% agreement (kappa=0.71, p<0.00001) without including muscle biopsy data, and 93% agreement (kappa=0.73, p<0.00001) using muscle biopsy findings. Comparison between the newly developed criteria and the Targoff criteria[11] demonstrated that the agreement was 89% (kappa=0.74, p<0.00001) and 93% (kappa=0.82, p<0.00001) without or with inclusion of muscle biopsy data, respectively.

VALIDATION

Internal validation

Using the criteria without muscle biopsy data, 733 observations were used, resulting in AUC=0.942 and cross-validated area=0.933. Using the criteria with muscle biopsy data, 507 observations were included, resulting in AUC=0.962 and cross-validated area=0.942.

External validation for sensitivity

Data from 592 cases (PM=281, DM=256, IBM=33, JDM=18 and ADM=4) in the Euromyositis register were used where clinical, laboratory and muscle biopsy data were available (Karolinska University Hospital, Stockholm, Sweden; Prague Hospital, Prague, Czech Republic; Oslo University Hospital, Oslo, Norway) (online supplementary 7). When there was sufficient information available, the EULAR/ACR classification criteria confirmed IIM diagnosis using a 55% probability cut-off for classification of IIM with no misclassification, yielding 100% sensitivity. Using the criteria without muscle biopsies, 489 (83%) patients were classified as IIM, and 103 (17%) patients could not be classified due to missing data. For the criteria with biopsies, 204 (34%) were classified as IIM and 388 (66%) could not be classified due to missing muscle biopsy data in the register.

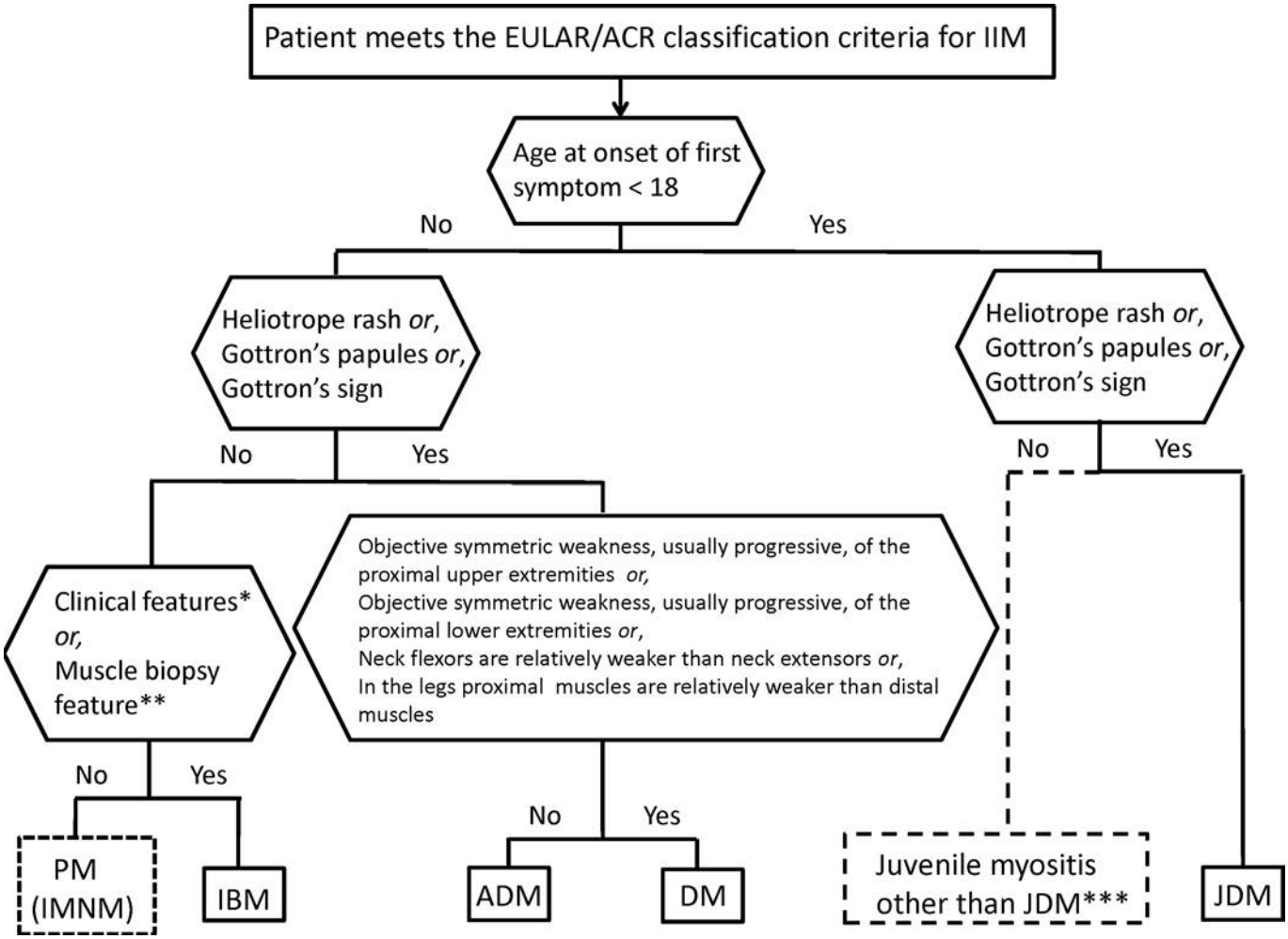

Figure 2 Classification tree for subgroups of IIM. A patient must first meet the EULAR/ACR classification criteria for IIM (probability of IIM ≥55%). The patient can then be subclassified using the classification tree. The subgroup of PM patients includes patients with IMNM. For IBM classification, one of the following, *finger flexor weakness and response to treatment: not improved, or **muscle biopsy: rimmed vacuoles, is required for classification. ***Juvenile myositis other than JDM was developed based on expert opinion. IMNM and hypomyopathic DM were too few to allow subclassification. ACR, American College of Rheumatology; ADM, amyopathic dermatomyositis; DM, dermatomyositis; EULAR, European League Against Rheumatism; IBM, inclusion body myositis; IIM, idiopathic inflammatory myopathies; IMNM, immune-mediated necrotising myopathy; JDM, juvenile dermatomyositis; PM, polymyositis.

Results for the IBM and PM subgroups improved when biopsy data were included: 97% of IBM cases could be classified compared with 73% when biopsy data were not included. For PM, 80% and 76%, respectively, could be classified. Raising the IIM classification cut-off from 55% to 90% decreased the total number of cases that could be classified to only 63% (not including muscle biopsies) or 28% (including muscle biopsies) due to absence of some muscle biopsy variables in the Euromyositis registry database.

The Juvenile Dermatomyositis Biomarker Study and Repository (UK and Ireland)

The JDRG register included 332 juvenile IIM cases in the study (definite JDM=292, probable JDM=20, definite juvenile PM=4, probable juvenile PM=2, focal myositis=6 and other IIM=8) (online supplementary 8). Muscle biopsy data were not available for all, thus the EULAR/ACR classification criteria without muscle biopsy data were used to test sensitivity in this dataset. Three hundred and seven (92%) cases could be classified using the 55% cut-off and no case was misclassified, yielding 100% sensitivity. The remaining 25 cases (8%) could not be classified due to missing data. Raising the cut-off stepwise to 60%, 70%, 80% or 90% yielded classification of 92%, 88%, 87% or 64% cases, respectively, where classification was possible.

Web calculator

A web calculator was developed (www.imm.ki.se/biostatistics/calculators/iim) as an aid to use the EULAR/ACR classification criteria. A probability range of classification can be obtained, providing the minimum and maximum probability. In addition to the probabilities acquired, the aggregated scores will be displayed. Whenever sufficient data are entered, the subclassification will be displayed.

DISCUSSION

Classification criteria are essential for inclusion of comparable patients in studies. No validated classification criteria for IIM currently exist. The EULAR/ACR classification criteria for IIM offer advantages that previous criteria lack. They are data driven, exhibit high sensitivity and specificity, and use a limited number of accessible, defined clinical and laboratory variables. Internal

Table 3 Comparison of physician-diagnosed IIM subgroups with IIM subgroups defined according to the classification tree among patients meeting the EULAR/ACR classification criteria for IIM

Physician-diagnosed subgroups	Classification tree subgroups*					
	JDM	DM	ADM	IBM	PM	Total
JDM	235	0	0	0	0	235
DM	0	191	6	2	15	214
ADM	1	1	30	0	0	32
IBM	0	0	0	66	5	71
PM	0	7	0	3	131	141
IMNM	0	0	0	0	10	10
Total	236	199	36	71	161	703
% of all IIM	33.6	28.3	5.1	10.1	22.9	
% of adult IIM	–	42.6	7.7	15.2	34.5	

*Classification of IIM by the EULAR/ACR classification criteria for IIM, using a 55% probability cut-off for classification, followed by the classification tree for subclassification.
ACR, American College of Rheumatology; ADM, amyopathic dermatomyositis; DM, dermatomyositis; EULAR, European League Against Rheumatism; IBM, inclusion body myositis; IIM, idiopathic inflammatory myopathies; IMNM, immune-mediated necrotising myopathy; JDM, juvenile dermatomyositis; PM, polymyositis.

validation and testing in external cohorts confirmed excellent performance. Importantly, the new criteria capture the most frequent IIM subgroups and can be used for both adults and children for research studies and clinical trials.

The new EULAR/ACR classification criteria provide a score with a corresponding probability of having IIM. This provides investigators flexibility in inclusion criteria for different types of studies, for example, clinical trials requiring high specificity would warrant a high probability of IIM in the inclusion criteria, whereas epidemiological studies requiring high sensitivity would need inclusion criteria with lower probability of IIM.

The new criteria are based on data from children and adults with different ethnicities from centres in Europe, America and Asia, and use symptoms, signs and other measures that are routinely assessed. A limitation is still that a majority of the patients were Caucasian, and even though we included data from 298 patients from Asia, we cannot exclude that there can be differences in manifestations between different ethnic groups, hence we still need to validate the criteria in Asian and African populations. Importantly, in patients with a typical DM skin rash, the criteria can be used without muscle biopsy data. For JDM, 97% of patients were correctly classified using the new criteria without muscle biopsy data. The new criteria also offer practical advantages in the number of variables needed to be tested. If a sufficient probability is reached, there is no requirement to test all items. Each criterion is well defined, lessening the opportunities for ad hoc interpretation. The skin rash typical of DM contributed with high weights in the probability score. Skin biopsy is recommended in the absence of muscle symptoms.[33 34] The EULAR/ACR classification criteria are the first myositis criteria to be validated and tested for sensitivity in other cohorts and revealed no misclassification.

Compared with most previous criteria, the new criteria are superior in sensitivity, specificity and classification accuracy. Classification criteria should have high sensitivity and specificity. The EULAR/ACR criteria demonstrated sensitivity and specificity of 87% and 82%, respectively, with even higher accuracy when muscle biopsies were included, 93% and 88%, respectively. Correctly classified patients were 86% and 91%, respectively, with and without inclusion of biopsies, and the criteria performed equally well for adult and juvenile cases. The Targoff criteria[11] also showed good statistical properties, but were not able to capture all subgroups of IIM as ADM patients were not included. Furthermore, the variables were not clearly defined in the Targoff criteria, and testing of more variables is required,

Table 4 Performance of the EULAR/ACR classification criteria for IIM and existing classification and diagnostic criteria for IIM

Performance (%)	EULAR/ACR classification criteria for IIM*		Bohan and Peter†[7 8]	Tanimoto *et al*[10]	Targoff *et al*†[11]	Dalakas and Hohlfeldt†[14]	ENMC Hoogendijk *et al*†[15]
	Without muscle biopsy	With muscle biopsy					
Mean (95% CI)							
Sensitivity	87 (84 to 90)	93 (89 to 95)	98 (96 to 99)	96 (94 to 97)	93 (90 to 95)	6 (5 to 8)	52 (48 to 55)
Specificity	82 (77 to 87)	88 (83 to 93)	55 (50 to 61)	31 (25 to 37)	89 (84 to 92)	99 (98 to 100)	97 (95 to 98)
Mean							
Positive predictive value	90	94	85	80	95	92	96
Negative predictive value	79	85	90	73	85	43	57
Correctly classified	86	91	86	79	91	45	70
Correct classification of IIM per subgroup‡ (%)							
Amyopathic dermatomyositis	94	60	25	14	0	0	0
Dermatomyositis	96	98	100	96	99	7	83
Hypomyopathic dermatomyositis	83	100	80	40	67	0	20
Immune-mediated necrotising myopathy	100	100	100	100	100	0	10
Inclusion body myositis	58	94	97	97	91	1	1
Juvenile dermatomyositis	97	96	100	96	98	5	86
Polymyositis	79	86	95	100	85	11	9

*Cut-off for probability: 55%.
†Definite and probable polymyositis and dermatomyositis.
‡Classification as idiopathic inflammatory myopathy per subgroup out of total number of cases per subgroup, expressed as mean.
ACR, American College of Rheumatology; ENMC, European Neuromuscular Centre; EULAR, European League Against Rheumatism; IIM, idiopathic inflammatory myopathies.

including electromyography, which is not always easily accessible and may be painful for patients. Importantly, the EULAR/ACR criteria can be applied to patients with myositis with overlap diagnoses, such as mixed connective tissue disease or systemic lupus erythematosus with myositis, since these patients were included among IIM cases.

There are limitations of the study; no controls or comparators were included in the external validation cohort since the IMCCP study was designed before those recommendations from ACR/EULAR were in place, requiring future validation. A validation study using comparators is underway, but we encourage additional validation studies in different populations. Another limitation largely unavoidable in observational data is the high frequency of missing data in the derivation dataset and validation samples, reflecting differences in practice patterns in evaluating patients. Nevertheless, 80% of cases and comparators had muscle biopsy data available, whereas MRI data and electromyography were only available for 38% and 29% of cases, respectively, reflecting their limited usage in clinical diagnosis. However, MRI data and electromyography examination are still important for diagnostic purposes of IIM. Patients studied had to have their disease for at least 6 months, which did not allow us to study new-onset patients. Importantly, these criteria are proposed as classification criteria in research and in clinical trials, not as diagnostic criteria.[35] There is also some possibility that the cut-points established for probable and definite myositis will need adjustment when tested with new populations of patients.

It took almost 10 years to assemble sufficient numbers of patients with these rare diseases, and three subgroups did not have enough subjects to study adequately. During this period, a new IIM subgroup became recognised, IMNM,[36] of which only a few cases were included into the study. IMNM cases could thus not be distinguished from PM in the subclassification tree. Another subgroup with few cases was juvenile PM, making a data-derived distinction from JDM impossible. However, paediatric rheumatology experts in the IMCCP recommended that the adult subclassification of IIM could be used for juvenile PM by extrapolation (figure 2). IBM cases were identified in the subclassification tree by the clinical features of finger flexor weakness and no response to treatment, or by the presence of rimmed vacuoles in muscle biopsies.[37]

Another limitation was the low frequency of myositis-specific autoantibodies documented. Five myositis-specific autoantibodies were included: anti-Jo-1, anti-Mi-2, anti-SRP, anti-PL7 and anti-PL12 antibodies, and all were strongly associated with IIM. However, only anti-Jo-1 autoantibody had a significant number of observations (n=1062) to permit analyses and inclusion in the classification criteria. A future update of the EULAR/ACR classification criteria should include the more recently identified myositis-specific autoantibodies,[21 22] in addition to more patients with IMNM, ADM, hypomyopathic DM and juvenile cases other than JDM.

RECOMMENDATIONS

- Patients with pathognomonic skin rashes (heliotrope rash, Gottron's papules and/or Gottron's sign) of JDM or DM are accurately classified with the EULAR/ACR classification criteria without including muscle biopsy data. For patients without these skin manifestations, muscle biopsy is recommended. For DM patients without muscle involvement, a skin biopsy is recommended.
- The EULAR/ACR classification criteria provide a score and a corresponding probability of having IIM. Each probability displays a unique sensitivity and specificity. The best balance between sensitivity and specificity can be found for a probability of 55%–60% (total aggregated score of ≥5.5 and ≤5.7) for the criteria not including muscle biopsy data, and 55%–75% (total aggregated score ≥6.7 and ≤7.6) when including muscle biopsies. These cases are designated 'probable IIM'. The recommended cut-off needed for classifying a patient as IIM is ≥55%.
- 'Definite IIM' corresponds to a probability of ≥90% or a total aggregate score of 7.5 or more without muscle biopsy and 8.7 with muscle biopsy, and is recommended in studies where a high specificity is required.
- A patient is termed 'possible IIM' if the probability is ≥50% and <55% (a minimum score of 5.3 without biopsies and 6.5 with biopsies).
- For clarity and transparency, both the descriptive term ('possible', 'probable' or 'definite') and the probability and the aggregated score should be reported in studies.

CONCLUSIONS

New classification criteria for IIM and the major IIM subgroups have been developed. These data-driven criteria have a good feasibility, high sensitivity and specificity, have been partly validated in external cohorts and are superior to previous criteria in capturing different subgroups of IIM. Revision of the criteria in the future will be important when additional validated myositis autoantibody tests, imaging and other tests are available in more IIM cases and comparator cases without IIM.

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Collaborators The collaborators are included in the appendix.

Contributors All authors were involved in drafting the article or revising it critically for important intellectual content and approved the final version to be published. All authors had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study conception and design: IEL, AT, MB, VPW, CP, MdV, LA, AAA, RJB, MHL, JAS, KD, BMF, HK, PAL, BAL, FWM, LGR. Acquisition of data: IEL, AT, MB, VPW, CP, MdV, LA, AAA, RJB, MHL, JAS, RA, SA, HC, RGC, KD, MMD, BMF, IG-DLT, PG, TH, JDK, HK, PAL, BAL, YL, CVO, MO, AMR, LR-S, HS, AS-O, YWS, JV, SRY, FWM, LGR, The International Myositis Classification Criteria Consortium, working committee members. Analysis and interpretation of data: IEL, AT, MB, VPW, CP, MdV, LA, AAA, RJB, MHL, JAS, RA, BMF, IG-DLT, PG, HK, PAL, BAL, YL, FWM, LGR.

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European evidence-based recommendations for the diagnosis and treatment of childhood-onset lupus nephritis: the SHARE initiative

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ABSTRACT

Lupus nephritis (LN) occurs in 50%–60% of patients with childhood-onset systemic lupus erythematosus (cSLE), leading to significant morbidity. Timely recognition of renal involvement and appropriate treatment are essential to prevent renal damage. The Single Hub and Access point for paediatric Rheumatology in Europe (SHARE) initiative aimed to generate diagnostic and management regimens for children and adolescents with rheumatic diseases including cSLE. Here, we provide evidence-based recommendations for diagnosis and treatment of childhood LN. Recommendations were developed using the European League Against Rheumatism standard operating procedures. A European-wide expert committee including paediatric nephrology representation formulated recommendations using a nominal group technique. Six recommendations regarding diagnosis and 20 recommendations covering treatment choices and goals were accepted, including each class of LN, described in the International Society of Nephrology/Renal Pathology Society 2003 classification system. Treatment goal should be complete renal response. Treatment of class I LN should mainly be guided by other symptoms. Class II LN should be treated initially with low-dose prednisone, only adding a disease-modifying antirheumatic drug after 3 months of persistent proteinuria or prednisone dependency. Induction treatment of class III/IV LN should be mycophenolate mofetil (MMF) or intravenous cyclophosphamide combined with corticosteroids; maintenance treatment should be MMF or azathioprine for at least 3 years. In pure class V LN, MMF with low-dose prednisone can be used as induction and MMF as maintenance treatment. The SHARE recommendations for diagnosis and treatment of LN have been generated to support uniform and high-quality care for all children with SLE.

INTRODUCTION

In 2012, the Single Hub and Access point for paediatric Rheumatology in Europe (SHARE) initiative was launched with the aim to optimise and disseminate diagnostic and management regimens for children and adolescents with rheumatic diseases, including childhood-onset systemic lupus erythematosus (cSLE).[1] cSLE is rare, with a prevalence of 1.9–25.7 per 100 000 children and incidence of 0.3–0.9 per 100 000 children-years worldwide.[2–4] cSLE in general has a more severe phenotype than adult-onset disease.[5–8] Fifty to sixty per cent of patients with cSLE will develop lupus nephritis (LN).[5–8] Timely and accurate recognition of renal involvement combined with appropriate treatment choices will optimise clinical outcome and decrease renal-associated morbidity and mortality.[1]

Consensus treatment recommendations for proliferative LN in children are available,[9 10] but do not include a paediatric-specific systematic literature review, nor do they focus on recommendations regarding diagnosis of LN or treatment in non-proliferative LN.

SHARE recommendations for paediatric antiphospholipid syndrome, juvenile dermatomyositis, familial Mediterranean fever and auto-inflammatory diseases have been published.[11–14] SHARE recommendations for diagnosis and treatment of cSLE (excluding LN) have also been published.[15] Here, the SHARE recommendations for LN are presented. These recommendations will support clinicians caring for children with or without suspected LN in carrying out a stepwise diagnostic process and guide them in treatment decision-making.

METHODS

SHARE is a European Union (EU)-funded project; therefore, representative paediatric rheumatologists from across Europe formed a panel of 16 members, with representation of paediatric nephrology. Disease experts from outside the EU also contributed to the project. The European League Against Rheumatism (EULAR) standardised operating procedures for developing best practice recommendations were followed.[16]

Systematic literature search and study selection

A systematic literature search, based on specific research questions was performed in the electronic databases PubMed/MEDLINE, EMBASE and Cochrane in July 2013 (see online supplementary table S1), using a validated filter to search articles pertaining to children and adolescents only.[17] All titles and abstracts were screened independently by two reviewers (NG, NdG). Articles fulfilling the inclusion criteria were sent to the experts for validity assessment and data extraction (see online

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supplementary table S2). While the literature search included terms regarding cSLE generally and paediatric antiphospholipid syndrome (APS), these topics are discussed separately.[14 18] Here, we report the LN-specific studies identified.

Validity assessment

All articles were analysed by the expert panel (two reviewers per article), using standardised data extraction and scoring forms. Any discrepancies were resolved by a third expert (SK or MWB) to reach consensus. Adapted classification tables for diagnostic[19] and therapeutic[20] studies were used to determine the level of evidence and strength of each recommendation[16] (see online supplementary tables S3 and S4).

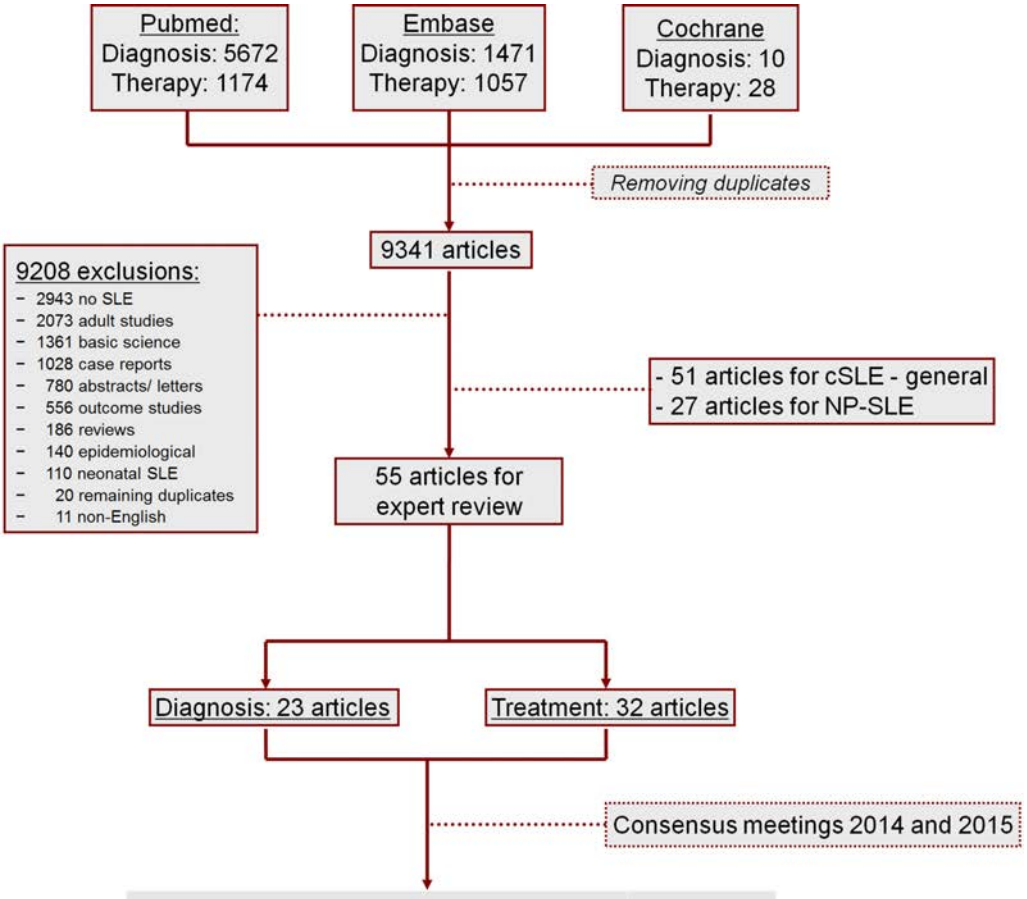
Establishment of recommendations

Based on this evidence base, provisional statements regarding diagnosis and treatment of LN were formulated (NG, NdG, SK, MWB). Adult-derived literature was consulted if no evidence in children was found. Provisional statements were presented to the expert committee (n=15) in an online survey (100% response rate). Recommendations were revised according to responses and discussed at two sequential face-to-face consensus expert meetings in March 2014 (Genova, n=16) and March 2015 (Barcelona, n=14). Nominal group technique was used to reach consensus,[21] where final recommendations were formulated. Recommendations were accepted when a predefined ≥80% of the experts agreed.

RESULTS

Literature review

Figure 1 summarises the literature review. The initial search yielded 9341 articles regarding diagnosis, treatment and management of cSLE. After screening title and abstract, and assessing full texts for relevance, 55 articles were used (see online supplementary table S5).



Recommendations regarding:	Number
Diagnosis	6
Treatment	
General	5
LN Class I	2
LN Class II	2
LN Class III/IV	4
LN Class V	2
Flares and refractory disease	5

Figure 1 Summary results from the systematic literature review. cSLE, childhood-onset systemic lupus erythematosus; LN, lupus nephritis; NP, neuropsychiatric.

Table 1 Recommendations for LN—diagnosis

	L	S	Agreement (%)
1. In case of isolated mild proteinuria* in a patient with (suspected) cSLE, exclude orthostatic proteinuria by collecting first morning urine sample (collected directly after waking up). For female patients, the urine sample needs to be obtained when patient is not menstruating.	4	D	100
2. Suspicion of renal involvement—in particular when finding reproducible proteinuria† should be an indication for renal biopsy, after excluding orthostatic proteinuria‡.	3	C	100
3. Proteinuria† and/or an impaired GFR§ should prompt the consultation of a paediatric nephrologist to discuss the need for a biopsy.	4	D	100
4. LN should be classified by the ISN/RPS 2003 classification system.	3	C	100
5. The expertise of an experienced renal pathologist to evaluate the renal biopsies should be sought even when one is not available in your own centre.	4	D	100
6. In class I or II LN, persistent proteinuria after 3 months is very unusual. Diagnosis and renal pathology needs to be reassessed in such cases.	3/4	C/D	100

1B, randomised controlled study; 2A, controlled study without randomisation; 2B, quasi-experimental study; and for treatment studies: 1A, meta-analysis of randomised controlled trial; B, based on level 2 or extrapolated from level 1; C, based on level 3 or extrapolated from level 1 or 2; D, based on level 4 or extrapolated from level 3 or 4 expert opinion.[16] Agreement indicates % of experts agreeing on the recommendation during the final voting round of the consensus meeting; for diagnostic and observational studies: 1A, meta-analysis of cohort studies; L, level of evidence; S, strength of recommendation: A, based on level 1 evidence; 3, descriptive study; 4, expert opinion.[19 20]
*Mild proteinuria: UP:CR 50–100 mg/mmol.
†Proteinuria: ≥0.5 g/24 hours or UP:CR ≥50 mg/mmol in a urine sample.
‡This statement is based on the EULAR recommendations for adults with SLE.[9]
§Impaired eGFR: <80 mL/min/1.73 m^2, calculated using the modified Schwartz formula.
cSLE, childhood-onset systemic lupus erythematosus; eGFR, estimated glomerular filtration rate; ISN/RPS, International Society of Nephrology/Renal Pathology Society; LN, lupus nephritis; UP:CR, urinary protein:creatinine ratio.

RECOMMENDATIONS FOR LN—DIAGNOSIS

Renal symptoms that could be indicative of LN include: renal dysfunction (acute kidney injury, acute-on-chronic kidney disease), hypertension, macroscopic or microscopic haematuria and/or proteinuria. Proteinuria is not always related to LN. Orthostatic proteinuria or postural proteinuria is the most common cause of proteinuria in teenagers, and should therefore be excluded as a cause of mild proteinuria in patients with (suspected) cSLE.[22 23] Confirmation and classification of renal involvement with consultation with paediatric nephrologist is recommended, proceeding to a percutaneous renal biopsy (table 1).

The International Society of Nephrology/Renal Pathology Society (ISN/RPS) 2003 classification system is commonly used to classify LN[9 24] (see online supplementary table S6). Studies using the ISN/RPS classification system showed that class of nephritis is associated with severity of renal disease and long-term renal outcome. Therefore, treatment strategies were based on the ISN/RPS 2003 classification system.[25 26]

Assessment of renal biopsies can be challenging. A renal pathologist experienced in LN should be consulted for biopsy evaluation.[27] Even so, misclassification of a renal biopsy is possible. For example, patients diagnosed with class I or II LN should not generally have proteinuria after 3 months of treatment. If proteinuria persists after 3 months, the possibility of misclassification of the biopsy or progression to class III or IV LN must be considered.[28] To avoid unnecessary repeat biopsy, the expert group recommends re-evaluating the initial biopsy as a first step.

RECOMMENDATIONS FOR LN—TREATMENT

As clinical symptoms are not reliable enough to reflect severity of renal disease, a renal biopsy is needed to guide treatment strategy. Treatment strategies for the different classes of LN are discussed in table 2 and summarised in figure 2. Renal biopsy is not always possible (eg, critical clinical condition; lack of resources to safely perform the procedure). As nephrotic syndrome, hypertension and impaired renal function are all correlated with class III/IV LN,[29–31] these symptoms should be considered as reflecting class III/IV LN and treated likewise if renal biopsy cannot be performed.

The long-term aim for treatment of LN should be complete renal response, with early morning urine protein:creatinine ratio (UP:CR) of <50 mg/mmol (or urine albumin:creatinine ratio of <35 mg/mmol) and normal renal function (estimated glomerular filtration rate >90 mL/min/1.73 m^2). Within 6–12 months after initiation of treatment, partial renal response, defined as ≥50% reduction in proteinuria to at least subnephrotic levels and normal or near-normal renal function should be achieved.[9] Degree of proteinuria at baseline was not a statistical significant predictor of renal function deterioration among patients with (membranous) LN, and herewith is not a decisive factor for specific treatment strategies.[32–36]

Several studies have reported on the antiproteinuric effect of ACE-inhibitors (ACE-I) or angiotensin-II receptor blockers (ARB) in renal disease. Evidence in patients with adult-onset SLE shows that these inhibitors of the renin-angiotensin system have a protective effect on the kidneys in case of proteinuria.[37 38] Additional treatment with ACE-I and/or ARB in children with LN and proteinuria should be advocated, guided by consultation with a paediatric nephrologist. Notably, the use of hydroxychloroquine is recommended in all patients with cSLE.[15]

ISN/RPS class I and II LN

Although class I LN is more common in cSLE compared with adult-onset SLE, no specific articles on treatment of class I LN were identified. Based on adult literature and consensus, class I LN could be treated with low-dose oral corticosteroid therapy.[39] If other organ systems are involved and class I LN has been found, treatment choice should be guided by these other clinical features. If class I LN is the only clinically active feature, adding other disease-modifying antirheumatic drugs (DMARDs) is generally not necessary (table 2, figure 2).

Class II LN generally responds well to low-dose oral corticosteroid therapy, tapered over a 3–6 months period (starting dose 0.25–0.5 mg/kg/day, maximum of 30 mg/day; often 0.25 mg/kg/day is sufficient). If proteinuria is persistent after 3 months or corticosteroid dose cannot be effectively weaned, renal biopsy should be re-evaluated by an experienced renal pathologist to exclude misclassification. Adding a DMARD to the treatment or switching to another DMARD effective for LN (eg, MTX to AZA)

Table 2 Recommendations for LN—treatment

	L	S	Agreement (%)
Treatment—general			
1. Immunosuppressive treatment should be guided by a diagnostic renal biopsy*.	3	C	100
2. Partial renal response† should be achieved preferably by 6 months but no later than 12 months following initiation of treatment*.	3	C	100
3. Treatment should aim for complete renal response with UP:CR<50 mg/mmol and normal or near-normal renal function (within 10% of normal GFR)*.	3	C	100
4. In case of LN with proteinuria, ACE-inhibitors or ARBs should be considered as additional treatment. Combined use of ACE-inhibitors and ARBs should be guided by paediatric nephrologists.	3	C	100
5. Where biopsy is not possible, patients with nephrotic syndrome, hypertension and impaired renal function should be treated as if it were class IV LN.	3	C	100
Treatment—class I LN			
6. Low-dose prednisone (<0.5 mg/kg/day) could be considered in class I LN, although treatment choice should be guided mainly by other clinical features.	3	C	100
7. For the treatment of class I LN alone, adding a DMARD is not necessary.	3	C	100
Treatment—class II LN			
8. First-line treatment of class II LN should be prednisone (with a starting dose of 0.25–0.5 mg/kg/day, with a maximum of 30 mg/day) tapering over a total duration of 3–6 months.	3	C	100
9. For the treatment of active class II LN, a DMARD is necessary in persistent proteinuria‡ and/or when failing to taper corticosteroids after 3 months of low-dose prednisone§.	3	C	100
Treatment—class III/IV LN with or without class V LN			
10. First choice of induction treatment of class III or IV LN should be MMF or intravenous CYC, in combination with corticosteroids.	3	C	93
11. First choice of maintenance treatment of class III or IV LN should be MMF or AZA.	3	C	100
12. Although specific paediatric data are lacking, maintenance treatment for class III and IV LN should be administered for at least 3 years.	4	D	100
13. When poor compliance is suspected while treating class III and IV LN, treatment with intravenous CYC should be considered.	4	D	100
Treatment—class V LN			
14. In pure class V LN, MMF in combination with oral prednisone (0.5 mg/kg/day) may be used as initial treatment based on better efficacy/toxicity ratio. CYC, CNI (ciclosporin or tacrolimus) or rituximab are recommended as alternative options or for non-responders*.	3	C	100
15. In class V LN the first choice of maintenance treatment should be MMF or AZA*.	3	C	100
Treatment—flares and refractory disease			
16. For a mild flare of class III/IV or V LN, the dose of prednisone should be increased, and a switch of DMARD should be considered.	4	D	100
17. In case of severe disease+, intravenous methylprednisolone pulses and high-dose prednisone (initially 1–2 mg/kg/day, gradually weaned) should be added to the treatment of LN.	3	C	100
18. In refractory class III/IV with or without class V LN, either because of lack of effect or in case of a partial response†, treatment should be changed to another therapeutic agent, for example, MMF, intravenous CYC or rituximab. However, treatment adherence must be assessed and current treatment must be optimised before this switch.	3	C	100
19. In refractory cases of class III and IV with or without class V LN, rituximab should be considered as induction/maintenance treatment in combination with another DMARD.	3	C	100
20. CNI (ciclosporin or tacrolimus) can be considered as a treatment option of LN in selected cases, with the consideration of potential nephrotoxicity*.	3	C	100

1B, randomised controlled study; 2A, controlled study without randomisation; 2B, quasi-experimental study; and for treatment studies: 1A, meta-analysis of randomised controlled trial; B, based on level 2 or extrapolated from level 1; C, based on level 3 or extrapolated from level 1 or 2; D, based on level 4 or extrapolated from level 3 or 4 expert opinion.[14] Agreement indicates % of experts agreeing on the recommendation during the final voting round of the consensus meeting; for diagnostic and observational studies: 1A, meta-analysis of cohort studies; L, level of evidence; S, strength of recommendation: A, based on level 1 evidence; 3, descriptive study; 4, expert opinion.[16 17]
*This statement is based on the EULAR recommendations for adults with SLE.[9]
†Partial response is defined as ≥50% reduction in proteinuria to subnephrotic levels (UP:CR <250–300 mg/mmol) and normal or near-normal renal function.
‡Persistent proteinuria: presence of proteinuria for >3 months.
§See also table 1, recommendation 6.
¶Severe disease: impaired GFR (<80 mL/min/1.73 m^2), nephrotic range proteinuria (>3.5 g/24 hours), biopsy-proven crescentic glomerulonephritis.
ARB, angiotensin receptor blockers; AZA, azathioprine; CNI, calcineurin inhibitors; CYC, cyclophosphamide; DMARD, disease-modifying antirheumatic drug; EULAR, European League Against Rheumatism; GFR, glomerular filtration rate; ISN/RPS: International Society of Nephrology/Renal Pathology Society; LN, lupus nephritis; MMF, mycophenolate mofetil; UP:CR, urinary protein:creatinine ratio.

is recommended (table 2, figure 2).[40–42] Notably, if treatment of class II LN remains unchanged despite the lack of renal response or prednisone dependency, renal impairment or even renal failure may develop.[43] There is little evidence for a specific DMARD in class I/II LN. Only case series or cohorts with limited number of patients are available and report the use of, mycophenolate mofetil (MMF), tacrolimus and cyclophosphamide (CYC) with variable effects.[44–46]

ISN/RPS class III and IV LN with or without class V LN

Class III and IV LN (proliferative LN) are the most common and severe forms of LN in cSLE.[6 29 30 47–49] Combination of class III or IV LN with class V LN is prevalent. As class III and IV LN generally show a less favourable disease course than class V LN, treatment strategies advised for proliferative LN should be followed in case of combined class III or IV with class V LN.

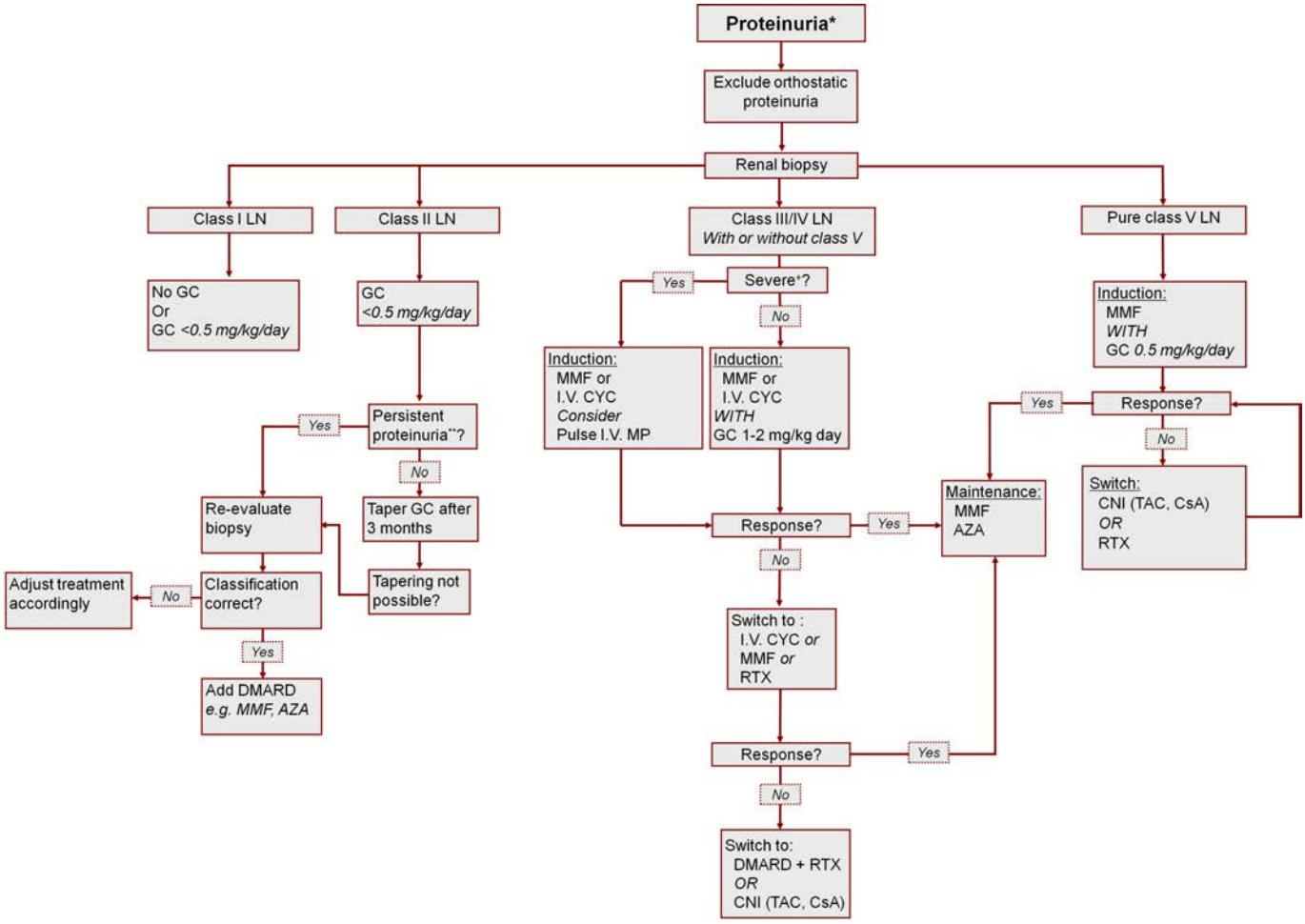


Figure 2 Treatment strategies for the different classes of LN definitions: *proteinuria: 0.5 g/24 hour or UP:CR >50 mg/mmol in a urine sample; **persistent proteinuria: presence of proteinuria for >3 months; DMARD: MMF, AZA, CNI, intravenous CYC; +severe disease, eg, impaired eGFR, estimated glomerular filtration rate (<80 mL/min/1.73 m^2), nephrotic range proteinuria (>1 g/m^2/day), biopsy-proven crescentic glomerulonephritis. AZA, azathioprine; CNI, calcineurin inhibitors; CsA, ciclosporin; CYC, cyclophosphamide; DMARD, disease-modifying antirheumatic drug; GC, corticosteroids; LN, lupus nephritis as classified by the ISN/RPS 2003 classification system; MMF, mycophenolate mofetil; MP, methylprednisolone; RTX, rituximab; TAC, tacrolimus.

Induction treatment of ISN/RPS class III and IV LN with or without ISN/RPS class V LN

In adults, evidence for induction treatment of class III and IV LN is based on several randomised controlled trials (RCT).[50 51] Equal efficacy and toxicity ratios are present for low-dose intravenous CYC (in adults: fixed dose 500 mg/pulse, six pulses given every 2 weeks), and high-dose CYC (500–750 mg/m^2/pulse, if tolerated increase to 750 mg/m^2/pulse, maximum dose 1000–1200 mg/pulse, 6 monthly pulses), adjusting appropriately in cases of renal dysfunction.[50] When comparing high-dose intravenous CYC with MMF (in adults: starting 1000 mg/day, increase to maximum dose 2000–3000 mg/day), renal outcomes were similar.[51] Recently, a network meta-analysis including only RCTs investigated comparative efficacy and toxicity of multiple treatment regimens for induction and/or maintenance treatment of proliferative adult-onset LN. This concluded that induction treatment with MMF, calcineurin inhibitors (CNIs) or a combination thereof, when added to corticosteroids, were the most effective treatments compared with intravenous CYC.[52]

In cSLE, there are no RCTs on this topic but several observational cohort studies and case series describe treatment of class III/IV LN. Intravenous CYC is generally used as induction treatment, with good results in most patients.[53–61] Three studies compared intravenous CYC induction therapy with azathioprine (AZA) in proliferative LN, one including patients with acute renal failure at diagnosis, showing similar efficacy.[55 57 59] Notably, patients with acute renal failure at diagnosis had excellent renal outcome.[57]

When comparing MMF with intravenous CYC in 13 patients with class III LN, complete or partial remission was achieved by more patients in the MMF group than in the intravenous CYC group.[60] MMF is well tolerated as induction treatment.[62] Initial MMF monotherapy combined with ciclosporin after 4 weeks has been shown to be safe and effective therapy after 12 months follow-up for 16 patients.[63]

When considering these adult and cSLE-derived data, the consensus group concluded that MMF (standard dose 1200 mg/m^2/day, maximum 2000 mg/day; when poor response option to increase to maximum of 1800 mg/m^2/day, maximum dose 3000 mg/day, but toxicity increases with higher dose) or intravenous CYC combined with high-dose prednisone (1–2 mg/kg/day, maximum 60 mg/day) should be considered for induction treatment of proliferative LN in cSLE.[10 50–68] The dosing of intravenous CYC (high or lower-dose, see above) is left to the discretion of the treating physician. The toxicity profile of MMF is more favourable when compared with intravenous CYC and may be preferred for this reason. In case of suspected non-compliance to oral medication, intravenous CYC should be considered (table 2, figure 2).[51 66] Notably, in contrast to high-dose, low-dose intravenous CYC does

not seem to impact ovarian reserve as measured by anti-Mullerian hormone.[69]

Maintenance treatment of ISN/RPS class III and IV LN with or without ISN/RPS class V LN

RCT in adults demonstrate that both MMF and AZA are good options for maintenance treatment in class III and IV LN,[64 65 70 71] although a higher relapse rate is seen in patients treated with AZA.[64 65 71] Additionally, a recent network meta-analysis showed that MMF was the most effective strategy to maintain remission for proliferative LN.[52]

Studies of proliferative LN in cSLE show similar results for MMF and AZA. Some studies indicate better outcomes for MMF, others for AZA.[55–57 59 60 62 63 72 73] The expert group therefore advises to use MMF (dosing: see above) or AZA (2–3 mg/kg/day, maximum 150 mg/day) as maintenance treatment for LN. Of note, AZA is associated with a higher flare risk in a meta-analysis of adult LN RCT.[70] Intravenous CYC can be effective as maintenance treatment,[53–55 58 59 61 72 73] but is not advised due to higher toxicity when compared with MMF or AZA (eg, increased risk of a reduced ovarian reserve/premature ovarian failure, inhibition of spermatogenesis, increased risk of bladder carcinoma).[74]

Duration of maintenance treatment in LN in the cSLE from the literature search was variable (1–5 years). Adult proliferative LN RCT studying maintenance therapy treated patients up to 3 years with good results.[65 71] The expert panel agreed that adopting this time frame was the best strategy, while accepting additional supportive evidence is necessary (table 2, figure 2).

Corticosteroid use in ISN/RPS class III/IV LN

Corticosteroids are generally used concomitantly with induction/maintenance regimen for class III/IV LN. Comparative studies regarding corticosteroid dose and oral versus intravenous use are not available. EULAR/European Renal Association–European Dialysis and Transplant Association (ERA-EDTA) and American College of Rheumatology (ACR) guidelines for treatment of proliferative LN in adult-onset SLE, recommend intravenous methylprednisolone pulse therapy in the initial treatment strategy, followed by oral prednisone (0.5–1 mg/kg/day) and tapered to the minimal amount necessary to control disease. This recommendation is based on expert opinion and extrapolation from controlled studies.[9 75] The Childhood Arthritis and Rheumatology Research Alliance (CARRA), a North American-based research collaboration specifically for paediatric rheumatic diseases, have provided consensus treatment plans for induction therapy of proliferative LN in cSLE.[10] These plans include three different dosing regimens combining oral corticosteroids with intravenous methylprednisolone-pulses based on expert opinion and by evidence from gene-expression arrays suggesting that intravenous methylprednisolone pulses but not oral prednisone have the potential to eliminate the interferon-alpha gene expression signature in cSLE.[10] However, no clinical data available reports that eliminating the interferon-alpha gene expression signature is associated with better renal outcomes.

As there is no robust evidence for the ideal dosing strategy of corticosteroids in proliferative LN, the expert group has not specified this in a recommendation. Most studies in cSLE report the use of oral prednisone 1–2 mg/kg/day (maximum 60 mg/day) as initial dosing in proliferative LN where children <30 kg mostly are dosed up to 2 mg/kg/day.[46 55 56 60 61 73] Intravenous methylprednisolone pulse therapy (30 mg/kg/dose intravenous for three consecutive days, maximum 1000 mg/dose) may be added to induction treatment before start of oral prednisone, especially in case of severe disease (eg, impaired GFR (<80 mL/min/1.73 m^2); nephrotic range proteinuria (>3.5 g/24 hours); biopsy-proven crescentic glomerulonephritis). An example for a prednisone-tapering schedule that may be used is tapering by 10%–20% at 1-week or 2-week interval based on clinical improvement.[50 51 66 71]

ISN/RPS class V LN

When comparing the use of corticosteroids with intravenous CYC with corticosteroids alone, combination therapy was superior in the only RCT for adults with pure class V LN available.[32] A pooled analysis of patients with pure class V LN included in two RCTs showed that MMF was equally efficacious when compared with intravenous CYC as induction treatment.[33] Patients with class V LN with or without class III or IV LN were also included in RCT for LN in adults, showing no difference between the use of MMF or high-dose intravenous CYC as induction treatment.[66] Evidence for treatment strategies in the literature search for children with class V LN was very limited. Good renal outcome has been shown in a cohort (n=30, 90% achieved renal remission as defined by the ACR[76]) of cSLE with pure class V LN. Thirty-three per cent of the total cohort were treated with DMARDs (AZA/ciclosporin/MMF).[77]

When combining the evidence of adult-onset SLE and cSLE, the expert group recommends the use of MMF in combination with low-dose oral prednisone (0.5 mg/kg/day) as induction treatment for pure class V LN in cSLE. MMF or AZA are recommended as maintenance treatment. CNI (ciclosporin, tacrolimus), rituximab or intravenous CYC are recommended as alternative options or for non-responders, with consideration of their respective toxicity profiles[32 33 51 77] (figure 2, table 2).

Renal flares and refractory disease

In general, in a patient not responding to the prescribed treatment as expected or developing disease flare, medication non-compliance should first be explored. Lack of adherence to therapy can be as high as 50%, and has been associated with higher persistent disease activity and poorer renal outcomes.[78–81] Measuring medication (trough) levels to unmask non-compliance is advisable.[15] RCTs in adult LN have shown that time is needed to reach complete renal response for at least 3–6 months.[51] However, if a patient shows hardly any response within 3 months of induction treatment, it is generally accepted to change the principle induction agent.

Renal flares can occur in up to 50% of patients with cSLE during maintenance treatment.[49 82 83] After excluding non-compliance, restarting or increasing corticosteroid dose (oral prednisone or intravenous methylprednisolone pulses) and a switch of DMARD should be considered. Defining renal response criteria or other outcomes of renal disease was outside the scope of these recommendations. In persistent active or refractory cases of lupus nephritis class III and IV, with or without class V LN, treatment should be changed to another therapeutic agent. For example, when treating with MMF this should be changed to rituximab or intravenous CYC. Adherence must be re-assessed and dosing of current treatment must be optimised first. Two RCTs in adults testing rituximab for LN did not reach their primary end point, and is not recommended as primary treatment for LN.[84 85] However, in observational studies of LN in adults, rituximab has been successfully used as rescue treatment for refractory LN.[86 87] There is limited evidence for the use of rituximab for LN in cSLE.[45 56] An observational cohort study in cSLE reported the effects of rituximab treatment in 63 children,

LN was the indication to start rituximab treatment in 36% of the patients. Rituximab was well-tolerated and improved disease activity in these children with a significant reduction in oral corticosteroid dose.[88] The expert group recommends that rituximab should be considered in refractory LN, in addition to the DMARD currently used.

CNI (tacrolimus, ciclosporin) can be considered as a treatment option for LN in selected cases, although with the consideration of potential nephrotoxicity especially related to ciclosporin after long-term use.[89]

DISCUSSION

Six recommendations regarding diagnosis and 20 recommendations regarding treatment for LN in children were accepted with ≥93% agreement among a European-wide group of cSLE experts, including paediatric nephrology.

Recommendations for treatment of LN in cSLE are available.[9 10] The CARRA cSLE subcommittee have published consensus treatment plans for newly diagnosed class III and IV LN.[10] These plans correspond well with the SHARE LN recommendations. Differences do exist, specifically regarding the use of concomitant corticosteroid use. The EULAR/ERA-EDTA have also published recommendations for management of adult and paediatric lupus nephritis. These recommendations mainly focus on evidence obtained in adult studies of LN. Notably, these recommendations underline the importance of a well-coordinated transition programme in the care for children with LN.[9] The expert group fully supports this recommendation. As specific EULAR guidelines for transition programmes for young people with rheumatic diseases have been published,[90] we have refrained from this subject in these SHARE guidelines.

The SHARE recommendations are the first to specifically focus on evidence in cSLE for diagnosis and treatment of all classes of LN using a systematic literature search. Evidence in cSLE was limited and the need for new high-quality studies in this field is clear.

In conclusion, the SHARE project has resulted in evidence-based recommendations for diagnosis and treatment of LN, to support uniform and high-quality care for all children with LN.

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The 2017 EULAR standardised procedures for ultrasound imaging in rheumatology

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ABSTRACT

Background In 2001, the European League Against Rheumatism developed and disseminated the first guidelines for musculoskeletal (MS) ultrasound (US) in rheumatology. Fifteen years later, the dramatic expansion of new data on MSUS in the literature coupled with technological developments in US imaging has necessitated an update of these guidelines.

Objectives To update the existing MSUS guidelines in rheumatology as well as to extend their scope to other anatomic structures relevant for rheumatology.

Methods The project consisted of the following steps: (1) a systematic literature review of MSUS evaluable structures; (2) a Delphi survey among rheumatologist and radiologist experts in MSUS to select MS and non-MS anatomic structures evaluable by US that are relevant to rheumatology, to select abnormalities evaluable by US and to prioritise these pathologies for rheumatology and (3) a nominal group technique to achieve consensus on the US scanning procedures and to produce an electronic illustrated manual (ie, App of these procedures).

Results Structures from nine MS and non-MS areas (ie, shoulder, elbow, wrist and hand, hip, knee, ankle and foot, peripheral nerves, salivary glands and vessels) were selected for MSUS in rheumatic and musculoskeletal diseases (RMD) and their detailed scanning procedures (ie, patient position, probe placement, scanning method and bony/other landmarks) were used to produce the App. In addition, US evaluable abnormalities present in RMD for each anatomic structure and their relevance for rheumatology were agreed on by the MSUS experts.

Conclusions This task force has produced a consensus-based comprehensive and practical framework on standardised procedures for MSUS imaging in rheumatology.

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INTRODUCTION

Over the last two decades, increasing numbers of rheumatologists worldwide have incorporated musculoskeletal (MS) ultrasound (US) into their clinical practice as both a valuable diagnostic and monitoring tool[1–6] and a means to guide interventions (injections and biopsies).[7 8] MSUS is a multiplanar and dynamic imaging modality. It has a number of benefits over other imaging techniques; of particular note, it is safe and well tolerated by patients and provides point-of-care scanning allowing immediate and direct correlations between imaging findings and clinical data, which can improve the management of patients with rheumatic and musculoskeletal diseases (RMDs). The increasing miniaturisation of scanning machines and hence portability have improved access to the use of MSUS in different clinical settings. MSUS has been applied to a wide range of RMD including inflammatory and degenerative joint diseases, crystal arthropathy, connective tissue diseases, vasculitis and regional pain syndromes.

In 2001, the European League Against Rheumatism (EULAR) developed and disseminated the first Guidelines for Musculoskeletal Ultrasound in Rheumatology based on both the available literature at the time and the expert opinion of a panel of European rheumatologists highly experienced in MSUS.[9] These guidelines set the technical standards for the use of MSUS in rheumatology and established a standardised MSUS scanning method in RMD. They have been widely used in clinical practice and research by the rheumatology community and have been widely cited in the literature. However, since their inception, there have been significant developments in technology and an increasing literature base with respect to validation and clinical application of MSUS for RMD, including the first incorporation of MSUS findings in rheumatological disease classification criteria.[10–13] Furthermore, scientific rheumatology and radiology societies such as EULAR, the American College of Rheumatology (ACR), the Pan American League of Association for Rheumatology (PANLAR), the European Society of Musculoskeletal Radiology (ESSR), the European Musculoskeletal Ultrasound Study Group (EURO-MUSCULUS) and the Ultrasound Study Group in Physical and Rehabilitation Medicine (USPRM) have produced evidence and expert

opinion-based recommendations on the use of MSUS in the clinical management of RMD.[14–19]

To this end, a new EULAR-endorsed task force was created with the following objectives:

1. To update the standardised scanning procedures (ie, patient position, probe placement and scanning method) for MSUS assessment of the joint areas accessible to US evaluation involved in RMD;
2. To produce standardised imaging procedures (ie, patient position, probe placement and scanning method) for US assessment of other articular and non-articular accessible anatomic structures of importance in rheumatology;
3. To select and prioritise the abnormalities evaluable by MSUS present in RMD;
4. To create an electronic illustrated manual (ie, application (App)) of these images and technique procedures accessible to all with interest in performing MSUS in their practice.

METHODS

The task force was composed of a steering group (ie, the convenors (IM and MB), two rheumatologists with high expertise in MSUS and anatomy (EN and DB), the methodologist (LC) and two fellows (IJ and SO)) and a panel consisting of 28 rheumatologists and radiologists highly experienced in MSUS performance, teaching and research in RMD. These task force members have been involved in education in MSUS within EULAR and in international multicentre research projects under the OMERACT (Outcome Measures in Rheumatology) initiative over the past 10–15 years and have worked and published on standardisation of MSUS scanning methods and definitions and criteria for MSUS abnormalities. In addition, the task force included two health professionals (HP) experienced in MSUS (ie, a podiatrist (HS) and a radiographer (GS)) and one patient representative (DH). The members of the task force represented 22 countries in Europe, the Americas and Asia (Austria, Bulgaria, Canada, Colombia, Denmark, France, Finland, Germany, Hungary, Hong Kong, Ireland, Italy, México, Netherlands, Norway, Poland, Romania, Serbia, Spain, UK, USA and Venezuela).

The project consisted of the following steps: (1) a systematic scoping review (SSR) on how MSUS is performed and what pathologies can be assessed by MSUS in RMD; (2) a Delphi survey aiming at selecting MS and non-MS anatomic structures evaluable by US and relevant to RMD, selecting pathologies evaluable by US and prioritising these abnormalities for rheumatology and (3) a nominal group technique was convened to achieve consensus on the scanning procedures summarised from the literature review for the MS and non-MS anatomic structures selected in the previous Delphi step and to produce the corresponding images for the EULAR US Scanning App.

Scoping review

A scoping literature review was performed by two fellows (IJ and SO) under the supervision of the steering group. Both fellows conducted the literature search independently and disagreement was resolved by discussion with the steering group. The systematic search strategy was based on the following PICO (Population, Intervention, Comparator, Outcome)-adapted components: body parts, ultrasound and scanning procedures. Online supplementary table 1 shows the synonyms used for each component. The search excluded animal studies, prenatal or postpartum US and surgery-related studies. Owing to the great number of synonyms for body parts, we divided the review into two separate searches, one for MS structures, mainly related to joints, and second for non-MS structures, that is, salivary glands, vessels and nerves.

The literature search was performed in Medline and Embase from their inception on the 1 May 2015. Online supplementary table 2 shows the literature search strategy. References identified were imported into a bibliographic manager (EndNote(R)) and duplicates were removed. The remaining articles were assessed by title and abstract to identify eligible studies, that is, those in which a description of scanning procedures of RMD-related body parts were detailed. Only articles in English, German, French, Spanish and Italian were retained.

Data about the examined area, patient position, probe placement, scanning method, landmarks and pathologies were extracted from each article using a predefined data collection form. The results were provided to the full expert panel. The review did not include an evaluation for the risk of bias of the individual studies as the objective was not to evaluate the diagnostic value of the technique but to collect narrative formulae of procedures. An update of the literature search was performed at the end of the project.

Delphi survey

The steering group developed an English-language survey that included six MS anatomic areas, that is, shoulder, elbow, wrist and hand, hip, knee and ankle and foot, and three non-MS organs/systems, that is, peripheral nerves, salivary glands and large vessels. For each anatomic area/organ/system, a variable number of structures and pathologies (1–14 per structure) derived from the literature review were included. These included 39 structures for the shoulder, 36 for the elbow, 15 for the wrist, 17 for the hand, 28 for the hip, 41 for the knee, 64 for the ankle, 12 for the foot, 20 for the peripheral nerves, 3 for the salivary glands and 18 for the large vessels.

The questionnaire consisted of nine tables (ie, one table for each MS anatomic area/non-MS organ/system) with the recipients required to respond to four statements. The first two statements addressed whether the respondent actually assessed the structure ('Examination included in my practice') and his/her satisfaction with that visualisation ('Quality of visualization of the structure'). The second two statements evaluated the respondents' opinion as to whether that visualisation enabled them to detect pathology ('Capability of evaluation of the abnormality') and if it was relevant to their practice ('Relevance for rheumatology clinical practice').

The questionnaire was sent by email to a broad group of rheumatologist and radiologist experts in MSUS in RMD. An explanation of the purpose of the survey was provided along with the questionnaire. The Delphi participants included rheumatologists with more than 5 years of experience in MSUS and EULAR level 2 in MSUS competency, European Federation of Societies for Ultrasound in Medicine or Biology (EFSUMB) level 3 in MSUS competency or faculty members of international MSUS courses organised by other societies and radiologists from a list provided by the ESSR based on their proven expertise in practice, teaching and research in MSUS.

The surveyed experts were asked to rate each statement on a 1–5 Likert scale as follows: 1=never and 5=always for the statement 'Examination included in my practice'; 1=very poor and 5=excellent for the statements 'Quality of visualization of the structure' and 'Capability of evaluation of the abnormality' and 1=minimal and 5=maximal for the statement 'Relevance for rheumatology clinical practice'. Those structures that scored both ≥3 for the statement 'Examination included in my practice'

and ≥4 for the statement 'Quality of visualization of the structure' by ≥70% of the respondents were selected for the subsequent steps. Those pathologies of the selected structures by the first two statements that scored ≥4 by ≥70% of the respondents for both statements 'Capability of evaluation of the abnormality' and 'Relevance for rheumatology clinical practice' were selected.

Nominal group technique

A subgroup of the task force panel composed of 14 rheumatologists (including those from the steering group), 3 radiologists, the methodologist, the patient and 2 HP attended a 2-day meeting in Madrid (Spain). The tables with the selected anatomic structures obtained from the Delphi survey and their US scanning method extracted from the literature review were sent by email to these panellists 3 weeks before the nominal meeting.

During the meeting, participants worked in small groups to define optimal US scanning procedures regarding patient position, probe placement, scanning method and bony landmarks of the selected structures. These experts scanned healthy models using seven top-end US machines (LOGIQ E9 XDclear; GE Medical Systems Ultrasound and Primary Care Diagnostics, Wauwatosa, Wisconsin, USA) equipped with a multifrequency linear matrix array transducer (ML6–15 MHz) used for the shoulder, elbow, wrist, hip, knee, ankle, salivary glands, vessels and peripheral nerves in deep areas and a multifrequency linear hockey-stick transducer (L8–18 MHz) used for the hand, feet, vessels and peripheral nerves in superficial areas. Grey-scale and power/colour Doppler settings were optimised for the different joints assessed. The results of the small work groups were then presented to the group as a whole to achieve consensus regarding the production of the final images.

Production of the US scanning App

The final phase of the nominal group meeting consisted of photographing of the US scanning procedures and the capture of static US images and videos for the online US scanning App.

Patient and HP perspective

The patient representative was asked to participate in the small and large group discussions as well as the scanning and recording sessions and to provide her feedback from the patient's perspective in order to obtain optimal imaging with the least discomfort to the patient. The HP were also instructed to give their opinion on the procedures from their unique perspective.

Statistical analysis

Simple descriptive and summary statistics were calculated from the responses to the survey.

RESULTS

Scoping review

The literature search resulted in 7706 articles, of which 176 articles were selected for detailed review and 47 articles provided the most relevant information.[20–74] Online supplementary figure 1 shows the study flowchart for the article selection. The main reason for the article exclusion after full-text review was the lack of standardised examination description. The resulting tables with the description of the scanning procedures as they were presented to the panel are available on request.

Delphi survey

A total of 227 international MSUS experts who fulfilled the selection criteria were identified and were sent the Delphi survey. One hundred thirteen experts (107 rheumatologists, 6 radiologists; 86 European, 27 non-European) responded to the survey (response rate 49.8%).

General recommended procedures for MSUS assessment in RMD

MSUS is a real-time, highly dynamic imaging technique. The 'dynamic' nature refers to the ability to visualise the structure of interest while it is in motion or being actively stressed and to the necessity of moving the probe and, therefore, the US beam. The ability to produce optimal US images, either as a single image or as a cine clip, is dependent on the examiner's anatomic knowledge, his/her technical proficiency and the quality and correct adjustment of the settings of the equipment. General recommended procedures for MSUS in RMD are presented in box 1 and online supplementary text. HP and a patient perspective are shown in online supplementary text and online supplementary tables 3 and 4.

Standardised procedures for MSUS assessment in RMD

Structures from nine anatomic areas/organs/systems (ie, shoulder, elbow, wrist and hand, hip, knee, ankle and foot, peripheral nerves, salivary glands and large vessels) were selected for MSUS in RMD as well as detailed scanning procedures (ie, patient position, probe placement, scanning method and bony/other landmarks) shown as downloadable text in the EULAR US Scanning App (www.eular.org; http://ultrasound.eular.org/).

Abnormalities evaluable by MSUS in RMD and prioritisation for rheumatology

The US-evaluable and relevant for rheumatology abnormalities present in RMD for each anatomic structure are displayed in online supplementary tables 5–14. Although the detection of features of Sjögren syndrome in salivary glands was considered highly relevant for >80% of the participants in the survey, less than 70% of them considered US highly capable to evaluate this pathology.

US scanning App

The final product of the task force was the elaboration of the EULAR US Scanning App which is a comprehensive electronic illustrated manual of didactic image acquisition in rheumatological MSUS. This tool displays the procedures (ie, images and/or videos on patient position, probe placement, scanning method, sonoanatomy and anatomical landmarks as well as additional downloadable text corresponding to these aspects for each structure ordered by anatomic region, anatomical location and type of structure) for MSUS assessment of the principal joint areas and non-articular anatomic regions of importance in RMD (www.eular.org; http://ultrasound.eular.org/).

DISCUSSION

The increasing utility of MSUS in rheumatology has led to a dramatic increase in the demand for education in the appropriate use of this imaging modality among rheumatologists worldwide. The rheumatologist as an ultrasonographer has the unique advantage of correlating the clinical picture with the imaging in a more advanced way and we have not made enough of the advantages of this heretofore. As all imaging assessments, MSUS is highly dependent on operator expertise mainly owing to the intrinsic real-time nature of image acquisition. Standardisation of the scanning procedures is an important requisite for

Box 1 General recommended procedures for MSUS assessment in RMD

- MSUS includes two principal modes: B-mode (or grey scale) that provides us with morphological information of the anatomic structures and Doppler mode (colour Doppler or power Doppler) that allows us to evaluate blood flow.
- MSUS should be performed with high-resolution linear transducers (ie, probes) with frequencies between 6 and 14 MHz for deep/intermediate areas to ≥15 MHz for superficial areas.
- Tissue harmonic imaging, spatial compound imaging, extended field of view (panoramic) and virtual convex imaging are some of the software capabilities that may be useful in MSUS.
- When scanning a joint, the probe should be oriented as perpendicular or parallel to the bony cortical surface (bony acoustic landmark) so that the cortical margin appears bright, sharp and hyperechoic.
- A dynamic scanning technique by means of slight movements of translation (side-to-side, back-to-front), angulation and rotation of the probe should be carried out in order to allow the best visualisation of the structure(s) of interest.
- MS structures should be evaluated as they move smoothly either actively or passively.
- To avoid anisotropy (ie, hypoechoic/anechoic appearance of a normally hyperechoic structure that mainly affects tendons) and the common pitfalls that accompany it, the probe should be continuously adjusted to maintain the beam perpendicular to the tendon fibres especially in insertional regions.
- When the long axis of the structure of interest corresponds to the cranial-caudal orientation of the anatomic position, the most proximal aspect of the structure is usually placed on the left-hand side of the screen. However, other options are acceptable as long as the movement of the image on the screen is kept parallel to the direction of the probe on the patient. Our preference for short axis is to align the structure of interest on the screen as if the observer is looking at the patient.
- Probe compression can be helpful in distinguishing a compressible liquid collection from a non-compressible solid. Little or no compression is important when performing Doppler examination to avoid cessation of flow in small vessels.
- A generous amount of gel should be used for superficial structures especially when little or no pressure is indicated.
- The machine setting for B-mode and Doppler mode should be properly adjusted to optimise the US image acquisition process.[68 69]
- Note: MSUS, musculoskeletal ultrasound; RMD, rheumatic and musculoskeletal disease.

the skilled and safe use of this technique in clinical practice and research.

Fifteen years after the publication of the Guidelines for Musculoskeletal Ultrasound in Rheumatology,[9] a thorough revision of the procedures for US imaging in rheumatological practise with the inclusion of new anatomic regions relevant to RMD was performed by an international panel of experts in MSUS. The principal aim was to enhance the standardisation and improve the quality of the scanning of anatomic structures evaluable by US and relevant for rheumatology through a consensus process among rheumatologists and radiologists who practice, teach and pursue research in MSUS in RMD. As expected, many of the US scans resulting from our task force were similar to those published by the ESSR 7 years ago.[21] However, our product is broader in terms of anatomic areas and structures and includes static images and videos on patient position, probe placement, scanning method and sonoanatomy. In addition, the task force has created an illustrated online App of these techniques as a useful educational tool accessible to all with interest in incorporating MSUS into their practice. It is the goal of this panel and its sponsor, EULAR, that this application will become a primary teaching and reference resource for rheumatologists, radiologists, non-medical HP[75] and other specialties involved in the management of RMD worldwide, and as a result, enhance the standardisation of the ultrasound assessment.

To achieve this, the pathologies evaluable by MSUS and relevant for rheumatology were elucidated through the Delphi survey process. The objective of our task was to collect expert opinion on the technical capability of US to assess abnormalities in RMD and the degree of priority of US assessment of these abnormalities in their clinical practice and not to establish evidence-based indications for MSUS as some scientific societies have done and published.[14–19] We selected anatomic structures that scored >3 by the majority of the respondents regarding the inclusion in their practice to ensure that there was sufficient experience with the visualisation of that structure which, in turn, enabled us to consistently score the second statement as to the respondents' perception of the quality of that visualisation. The acceptance value for this criterion was purposefully set lower than the other criteria in order to capture new structures that now with advances in the overall knowledge of rheumatic diseases, along with advances in instrumentation and the ultrasound skillset including anatomic knowledge, are now becoming part of MSUS in RMD. Our results indicated an advanced level of US practise among our respondents and a great interest in a wide spectrum of MSUS pathologies detectable in RMD. The use of MSUS for evaluation of the non-MS structures, that is, the peripheral nerves, salivary glands and large vessels, was relatively limited which we felt could be related either to a general lack of experience along the respondents or, possibly, a lack of evidence validating their use. It was the opinion of the panel that standardisation of the scanning procedures for these structures would further facilitate their clinical application in MSUS practice and encourage further research into this group of structures as they relate to RMD.

Some limitations of our project should be mentioned. The number of radiologists who participated in the Delphi survey and consensus meeting was small compared with that of rheumatologists. This can be explained by the dramatic expansion and implementation of MSUS among the rheumatologists, who are highly motivated to collaborate in the enhancement of MSUS use in practice and research. In addition, other MS specialists (eg, physiatrics, pain physicians, sport physicians) who could have enriched the procedures, particularly for certain pathologies, were missing. Furthermore, for logistic reasons, only a subgroup of the experts involved in the Delphi process were able to participate in the nominal group meeting where the detailed scanning method was agreed on and established. However, we believe that this subgroup was sufficiently representative of the entire community of MSUS experts.

Finally, the addition of the patient and the HP to the panel has provided a unique perspective providing technical and practical advice in improving the US experience for the patient, whose

active involvement in US investigations should be essential,[76] and all participants.

In conclusion, we expect this enhanced consensus-based comprehensive and practical framework for MSUS procedures in rheumatology to be a valuable educational tool and provide a standard reference for MSUS practice and research in RMD. EULAR and EFSUMB offer a structured curriculum to be followed to achieve competency in MSUS in rheumatology.

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EXTENDED REPORT

Comparison of performance of the 2016 ACR-EULAR classification criteria for primary Sjögren's syndrome with other sets of criteria in Japanese patients

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ABSTRACT

Objectives To compare the performance of the new 2016 American College of Rheumatology (ACR)-European League Against Rheumatism (EULAR) classification criteria for primary Sjögren's syndrome (SS) with 1999 revised Japanese Ministry of Health criteria for diagnosis of SS (JPN), 2002 American-European Consensus Group classification criteria for SS (AECG) and 2012 ACR classification criteria for SS (ACR) in Japanese patients.

Methods The study subjects were 499 patients with primary SS (pSS) or suspected pSS who were followed up in June 2012 at 10 hospitals in Japan. All patients had been assessed for all four criteria of JPN (pathology, oral, ocular, anti-SS-A/SS-B antibodies). The clinical diagnosis by the physician in charge was set as the 'gold standard'.

Results pSS was diagnosed in 302 patients and ruled out in 197 patients by the physician in charge. The sensitivity of the ACR-EULAR criteria in the diagnosis of pSS (95.4%) was higher than those of the JPN, AECG and ACR (82.1%, 89.4% and 79.1%, respectively), while the specificity of the ACR-EULAR (72.1%) was lower than those of the three sets (90.9%, 84.3% and 84.8%, respectively). The differences of sensitivities and specificities between the ACR-EULAR and other three sets of criteria were statistically significant ($p<0.001$). Eight out of 302 patients with pSS and 11 cases out of 197 non-pSS cases satisfied only the ACR-EULAR criteria, compared with none of the other three sets.

Conclusions The ACR-EULAR criteria had significantly higher sensitivity and lower specificity in diagnosis of pSS, compared with the currently available three sets of criteria.

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INTRODUCTION

Sjögren's syndrome (SS) is an autoimmune disease that affects mainly exocrine glands including the salivary and lacrimal glands, and is often associated with extraglandular manifestations, such as interstitial lung and kidney diseases, and neurological, haematological and musculoskeletal involvements.[1] It is characterised by lymphocytic infiltration into the exocrine glands and other organs, leading to dry mouth, dry eyes and various extraglandular symptoms. SS is subcategorised into primary SS (pSS) which is not associated with other well defined connective tissue diseases (CTDs), and secondary SS which is associated with other well defined CTDs.[2]

In Japan, the revised criteria for the diagnosis of SS proposed by the Japanese Ministry of Health (JPN) (1999),[3] as well as the American-European Consensus Group classification criteria for SS (AECG) (2002)[2] have been used commonly in both daily clinical practice and clinical studies in this decade. In 2012, the American College of Rheumatology (ACR) published the 2012 ACR classification criteria for SS, which were proposed by the Sjögren's International Collaborative Clinical Alliance (SICCA).[4] These three sets of criteria have also been applied for the diagnosis or classification of SS in Japan in the last 3 years. We previously analysed 694 Japanese patients with SS or suspected SS, and showed that the sensitivities of JPN, AECG and ACR in the diagnosis of SS were 79.6%, 78.6% and 77.5%, respectively, with respective specificities of 90.4%, 90.4% and 83.5%, when considering the clinical diagnosis as the 'gold standard'.[5] We concluded in that study the superiority of the JPN criteria in the diagnosis of SS in Japanese patients compared with the ACR and AECG criteria.[5]

Recently the 2016 new ACR-European League Against Rheumatism (EULAR) classification criteria for pSS (ACR-EULAR) were published.[6 7] Investigators from the SICCA team and the EULAR Sjögren's Task Force formed the International SS Criteria Working Group to develop this single set of classification criteria that combined features of the ACR and AECG criteria, based on methodology consistent with the current ACR and EULAR guidelines.[6 7] The working group adopted the methodology based on both data and expert clinical judgement, and finally defined the new classification criteria comprising five objective tests or items, and a total score of ≥4 as the cut-off for the diagnosis of pSS. The total score is derived from the sum of the weights assigned to each positive

test or item as follows: focal lymphocytic sialadenitis in labial salivary gland with Focus Score (FS) of ≥1 (based on number of foci/4 mm^2) and positive anti-SS-A/Ro serology with the highest weights (3 for each positive test), and Ocular Staining Score (OSS) of ≥5 (or Van Bijsterveld Score of ≥4) on at least one eye, Schirmer's test of ≤5 mm/5 min on at least one eye, and unstimulated whole saliva (UWS) flow rate of ≤0.1 mL/min with a weight of 1 for each positive test.[6 7]

Comparison of the above four sets of criteria (table 1) shows certain differences in the adopted items. In addition to the adopted items, the purpose of these criteria sets also differs. Importantly, the JPN criteria were formulated for the diagnosis of SS as the diagnostic criteria, while other three sets of criteria (the ACR-EULAR, AECG and ACR criteria) were formulated for research purposes as the classification criteria. The purpose of the present study was to compare the performance of the new ACR-EULAR criteria with the former sets of criteria, such as the JPN, AECG and ACR criteria in Japanese patients.

PATIENTS AND METHODS

Study population

The study subjects were 499 patients (38 men and 461 women) with the diagnosis of pSS or suspected pSS, who had been checked for all four criteria of the JPN (pathology, oral, ocular, anti SS-A/Ro and SS-B/La antibody), and were followed up in June 2012 at 10 hospitals across Japan (Kanazawa Medical University Hospital, Nagasaki University Hospital, Hyogo Medical University Hospital, Keio University Hospital, Tokyo Women's Medical University Hospital, Tsurumi University Hospital, Kyushu University Hospital, University of Occupational and Environmental Health Hospital, Kyoto University Hospital, and University of Tsukuba Hospital), which form parts of the Research Team for Autoimmune Diseases, The Research Program for Intractable Disease of the Japan Ministry of Health, Labor and Welfare (MHLW).

Table 1 Comparison of items adopted in the 2016 ACR-EULAR, JPN, AECG and ACR criteria

Items	ACR-EULAR	JPN	AECG	ACR
Ocular symptoms	Not adopted	Not adopted	Adopted	Not adopted
Oral symptoms	Not adopted	Not adopted	Adopted	Not adopted
Ocular signs				
Schirmer's test	Adopted (1 point)	Adopted	Adopted	Not adopted
Ocular staining	Adopted (1 point)	Adopted	Adopted	Adopted
Labial salivary gland biopsy	Adopted (3 points)	Adopted	Adopted	Adopted
Salivary gland involvements				
Salivary secretion	Adopted (1 point)	Adopted	Adopted	Not adopted
Sialography	Not adopted	Adopted	Adopted	Not adopted
Scintigraphy	Not adopted	Adopted	Adopted	Not adopted
Autoantibodies				
SS-A/Ro	Adopted (3 points)	Adopted	Adopted	Adopted
SS-B/La	Not adopted	Adopted	Adopted	Adopted
ANA	Not adopted	Not adopted	Not adopted	Adopted
RF	Not adopted	Not adopted	Not adopted	Adopted

ACR, American College of Rheumatology ACR criteria for SS; ACR-EULAR, American College of Rheumatology (ACR)-European League Against Rheumatism (EULAR) classification criteria for pSS; AECG, The American-European Consensus Group classification criteria for SS; ANA, antinuclear antibody; JPN, The revised Japanese Ministry of Health criteria for the diagnosis of SS; pSS, primary SS; RF, rheumatoid factor; SS, Sjögren's syndrome; SS-A/Ro, anti-SS-A/Ro antibody; SS-B/La, anti-SS-B/La antibody.

Data collection and analysis

We collected clinical data through a questionnaire from the above 10 hospitals. We retrospectively examined the clinical diagnosis by the physician in charge, satisfaction of ACR-EULAR, JPN, AECG and ACR criteria. Because the OSS adopted in the ACR-EULAR and ACR criteria is not commonly performed in Japan, we regarded patients with Van Bijsterveld Score ≥4 in the Rose Bengal test, lissamine green test or fluorescein staining test to have satisfied OSS in the ACR-EULAR criteria, and patients who had positive Rose Bengal or lissamine green test (Van Bijsterveld Score ≥3) or fluorescein staining test to have satisfied OSS in the ACR criteria. Similarly, because numerous cases (116/499 cases) lacked results of the UWS, which was not adopted in the JPN criteria,[3] we regarded patients who had UWS ≤0.1 mL/min, gum test ≤10 mL/10 min or Saxon test ≤2 g/2 min to have satisfied low salivary volume in the ACR-EULAR and AECG criteria.

Moreover, we performed the subanalysis using 383 cases who were examined for UWS, excluding 116 cases who lacked results of the UWS mentioned above. We examined satisfaction for each criteria set more strictly in this subanalysis than in whole analysis of 499 cases. For salivary volume, we regarded patients who had gum test ≤10 mL/10 min or Saxon test ≤2 g/2 min to have satisfied decreased salivary volume in JPN criteria, while UWS ≤0.1 mL/min in the ACR-EULAR and AECG criteria. For ocular staining, we regarded patients with Van Bijsterveld Score ≥3 in the Rose Bengal test, lissamine green test or fluorescein staining test, and/or positive fluorescein staining test to have satisfied positive ocular staining in JPN and ACR criteria, while Van Bijsterveld Score ≥4 in AECG and ACR-EULAR criteria.

We considered the clinical diagnosis by the physician in charge as the 'gold standard' for the diagnosis of pSS in this study. In all cases, the diagnosis established by the physician in charge was based on clinical findings, laboratory and serological tests of blood and saliva samples, sialography, scintigraphy, and histopathological examination of biopsy material. We regarded the clinical diagnosis by the physician in charge to be appropriate for the 'gold standard', because the clinical diagnosis was decided by senior and experienced clinicians belonging to 10 hospitals which form parts of the Research Team for Autoimmune Diseases, The Research Program for Intractable Disease of the Japan MHLW described above. We compared the sensitivity and specificity between the ACR-EULAR, JPN, AECG and ACR criteria in the diagnosis of pSS.

Statistical analysis

The differences of sensitivities and specificities between all possible pairs of the four sets of criteria were evaluated using the McNemar's test and the Newcombe's square-and-add method. A p value <0.05 denoted the presence of a statistically significant difference.

RESULTS

Diagnosis of pSS and denial of pSS

None of the 499 patients had other well defined CTDs. pSS was diagnosed in 302 patients, whereas pSS was excluded in 197

patients by the physician in charge, and these judgements were considered as the 'gold standard' in the present study. For subanalysis using 383 cases that were examined for UWS, pSS was diagnosed in 203 patients, while pSS was excluded in 180 patients based on clinical judgements.

Sensitivity and specificity in diagnosis of pSS by different sets of criteria

For all the 499 patients, the sensitivities of the ACR-EULAR, JPN, AECG and ACR criteria in the diagnosis of pSS were 95.4% (95% CI 93.0% to 97.1%), 82.1% (79.6% to 84.1%), 89.4% (86.8% to 91.6%) and 79.1% (76.2% to 81.6%), respectively, considering the diagnosis by the physician in charge as the 'gold standard' (table 2). The respective specificities were 72.1% (68.4% to 74.7%), 90.9% (87.0% to 93.8%), 84.3% (80.2% to 87.6%) and 84.8% (80.3% to 88.5%) (table 2).

For subanalysis using 383 cases that were examined for UWS, the sensitivities of the ACR-EULAR, JPN, AECG and ACR criteria in the diagnosis of pSS were 94.1% (90.8% to 96.4%), 74.9% (71.3% to 77.6%), 85.7% (82.1% to 88.7%) and 79.8% (75.8% to 83.2%), respectively. The respective specificities were 76.7% (73.0% to 79.3%), 90.6% (86.5% to 93.7%), 86.1% (82.0% to 89.4%) and 81.1% (76.6% to 85.0%) (table 2).

The sensitivity of the ACR-EULAR criteria was statistically significantly higher than those of other three sets of criteria in both whole analysis and subanalysis ($p<0.001$) (table 3). The specificity of the ACR-EULAR criteria was statistically significantly lower than those of other three sets of criteria in whole analysis ($p<0.001$) (table 3). Although the specificity of the ACR-EULAR criteria was statistically significantly lower than those of the JPN and AECG criteria in subanalysis ($p<0.001$), the difference between the ACR-EULAR and ACR criteria was not statistically significant ($p=0.117$) (table 3).

These findings indicate that the ACR-EULAR criteria have higher sensitivity and lower specificity in the diagnosis of pSS, compared with the JPN, AECG and ACR in both whole analysis and subanalysis.

Agreement of ACR-EULAR criteria with the other three sets of criteria

Table 4 shows the satisfaction for these four sets of criteria in each case. The data showed that the ACR-EULAR criteria were satisfied by much more cases than the other three sets of criteria for both pSS (288 cases) and non-pSS (55 cases) groups. Many pSS (220/302 cases, 72.8%) satisfied all four sets of criteria, while many non-pSS (135/197 cases, 68.5%) satisfied none of four sets of criteria (table 4). Although 8 non-pSS cases satisfied all four sets of criteria, 11 patients with pSS did not satisfy any set of criteria (table 4). There was no case that satisfied all the other three sets of criteria except for the ACR-EULAR criteria among both pSS and non-pSS groups (table 4).

Importantly, 8 out of the 302 patients with pSS diagnosed by the physician in charge and 11 cases out of the 197 clinically non-pSS cases satisfied only the ACR-EULAR criteria, compared with none of the other three sets of criteria. These 19 cases explained the low agreement between the ACR-EULAR criteria and the other three sets of criteria. Further analysis of positivity for each item adopted in the ACR-EULAR criteria among the 8 patients with pSS who satisfied only the ACR-EULAR criteria indicated that they had positive FS (62.5%, 5/8 cases) or positive SS-A/Ro (37.5%, 3/8 cases), together with decreased salivary (87.5%, 7/8 cases) or lacrimal (12.5%, 1/8 cases) secretion, resulting in a total score of 4 in these 8 patients (figure 1A). The 11 non-pSS cases who satisfied only the ACR-EULAR criteria had positive FS (54.5%, 6/11 cases) or positive SS-A/Ro (45.5%, 5/11 cases), together with decreased salivary secretion (100%, 11/11 cases), resulting in a total score of 4 in these 11 cases (figure 1B).

Considered together, the above analyses suggest that judgement by the ACR-EULAR criteria for both diagnosis and exclusion of pSS was different from those by the JPN, AECG and ACR criteria (table 4).

DISCUSSION

In the present study, we compared the sensitivity and specificity of the 2016 ACR-EULAR criteria for pSS with those of the JPN, AECG and ACR criteria, for the diagnosis of pSS using clinical data of 499 Japanese patients with pSS or suspected pSS. The results showed clearly that the 2016 ACR-EULAR criteria had higher sensitivity and lower specificity in the diagnosis of pSS, compared with the other three sets of criteria in both whole analysis (n=499) and subanalysis (n=383) using cases that were examined for UWS. Moreover, the degree of agreement of the ACR-EULAR criteria with the three sets of criteria for both diagnosis and exclusion of pSS was low. These results are different from those reported in a recent study by Shiboski *et al*,[6 7] which showed high sensitivity (96%, 95% CI 92% to 98%) and specificity (95%, 95% CI 92% to 97%) for the ACR-EULAR criteria and high agreement rate with both AECG

Table 2 Sensitivity and specificity for the diagnosis of pSS by the four sets of criteria

Analysis	Criteria sets	Diagnosis by the physician in charge as the 'gold standard'			
		Sensitivity (%)	95% CI	Specificity (%)	95% CI
Whole n=499	ACR-EULAR	95.4 (288/302)	93.0 to 97.1	72.1 (142/197)	68.4 to 74.7
	JPN	82.1 (248/302)	79.6 to 84.1	90.9 (179/197)	87.0 to 93.8
	AECG	89.4 (270/302)	86.8 to 91.6	84.3 (166/197)	80.2 to 87.6
	ACR	79.1 (239/302)	76.2 to 81.6	84.8 (167/197)	80.3 to 88.5
Subanalysis n=383	ACR-EULAR	94.1 (191/203)	90.8 to 96.4	76.7 (138/180)	73.0 to 79.3
	JPN	74.9 (152/203)	71.3 to 77.6	90.6 (163/180)	86.5 to 93.7
	AECG	85.7 (174/203)	82.1 to 88.7	86.1 (155/180)	82.0 to 89.4
	ACR	79.8 (162/203)	75.8 to 83.2	81.1 (146/180)	76.6 to 85.0

Of the 499 enrolled patients, pSS was diagnosed in 302 patients, whereas pSS was excluded in 197 patients by the physician in charge. For subanalysis using 383 cases that were examined for unstimulated whole saliva (UWS), pSS was diagnosed in 203 patients, while pSS was excluded in 180 patients based on clinical judgements.
ACR-EULAR, American College of Rheumatology (ACR)-European League Against Rheumatism (EULAR) classification criteria for pSS; AECG, American-European Consensus Group classification criteria for SS; JPN, The revised Japanese Ministry of Health criteria for the diagnosis of SS; pSS, primary SS; SS, Sjögren's syndrome.

Table 3 Differences of the sensitivities and specificities (with 95% CI) for the diagnosis of pSS among the four sets of criteria

	Competitor	JPN	AECG	ACR
Whole n=499				
Sensitivities	ACR-EULAR	13.2 (9.2 to 17.7) p<0.001	6.0 (3.0 to 9.4) p<0.001	16.2 (12.2 to 20.7) p<0.001
	JPN	–	−7.3 (−11.8 to −2.9) p=0.001	3.0 (−0.3 to 6.4) p=0.072
	AECG	–	–	10.3 (5.6 to 15.1) p<0.001
Specificities	ACR-EULAR	−18.8 (−24.7 to −13.1) p<0.001	−12.2 (−17.2 to −7.3) p<0.001	−12.7 (−18.6 to −6.8) p<0.001
	JPN	–	6.6 (0.8 to 12.5) p=0.024	6.1 (2.4 to 10.3) p=0.001
	AECG	–	–	0.0 (−6.9 to 5.9) p=0.873
Subanalysis n=383				
Sensitivities	ACR-EULAR	19.2 (13.7 to 25.2) p<0.001	8.4 (4.3 to 13.1) p<0.001	14.3 (9.6 to 19.6) p<0.001
	JPN	–	−10.8 (−17.3 to −4.4) p=0.001	−4.9 (−10.2 to 0.3) p=0.059
	AECG	–	–	5.9 (−0.3 to 12.1) p=0.058
Specificities	ACR-EULAR	−13.9 (−19.6 to −8.6) p<0.001	−9.4 (−14.4 to −4.8) p<0.001	−4.4 (−10.2 to 1.2) p=0.117
	JPN	–	4.4 (−1.5 to 10.5) p=0.131	9.4 (4.9 to 14.5) p<0.001
	AECG	–	–	5.0 (−1.6 to 11.6) p=0.128

Of the 499 enrolled patients, pSS was diagnosed in 302 patients, whereas pSS was excluded in 197 patients by the physician in charge. For subanalysis using 383 cases that were examined for unstimulated whole saliva (UWS), pSS was diagnosed in 203 patients, while pSS was excluded in 180 patients based on clinical judgements.
The p values and confidence limits were computed by the McNemar's test and the Newcombe's square-and-add method.
–, not examined; ACR-EULAR, American College of Rheumatology (ACR)-European League Against Rheumatism (EULAR) classification criteria for pSS; AECG, American-European Consensus Group classification criteria for SS; JPN, The revised Japanese Ministry of Health criteria for the diagnosis of SS; pSS, primary SS; SS, Sjögren's syndrome.

Table 4 Satisfaction of the ACR-EULAR, JPN, AECG and ACR criteria in clinically diagnosed pSS and non-pSS cases

Cases						
pSS	non-pSS		ACR-EULAR	JPN	AECG	ACR
220	8		O	O	O	O
14	0		O	O	O	X
11	8		O	O	X	O
7	3		O	X	O	O
0	0		X	O	O	O
0	1		O	O	X	X
27	19		O	X	O	X
1	5		O	X	X	O
2	0		X	O	O	X
0	1		X	O	X	O
0	0		X	X	O	O
8	11		O	X	X	X
1	0		X	O	X	X
0	1		X	X	O	X
0	5		X	X	X	O
11	135		X	X	X	X
Total 302	197	pSS	288	248	270	239
		non-pSS	55	18	31	30
		Total numbers of cases that satisfied each criteria set				

Of the 499 enrolled patients, pSS was diagnosed in 302 patients, whereas pSS was excluded in 197 patients by the physician in charge.
ACR-EULAR, American College of Rheumatology (ACR)-European League Against Rheumatism (EULAR) classification criteria for pSS; AECG, American-European Consensus Group classification criteria for SS; JPN, The revised Japanese Ministry of Health criteria for the diagnosis of SS; O, satisfaction; pSS, primary SS; SS, Sjögren's syndrome; X, non-satisfaction.

(κ coefficient: 0.91) and ACR criteria (κ coefficient: 0.82). These disagreements seem to be somewhat related to our 8 patients with pSS and 11 non-pSS cases who did not satisfy any of the JPN, AECG and ACR criteria, while they satisfied only the ACR-EULAR criteria. Among these 19 cases, 11 non-pSS cases had positive FS (6/11 cases) or positive anti-SS-A/Ro (5/11 cases), and decreased salivary volume (11/11 cases). These 11 non-pSS cases might cause the low specificity of the ACR-EULAR criteria. However, one has to pay enough attention to the adopted methods for assessment of salivary volume in this study. As mentioned in the Patients and methods section, we regarded patients with UWS ≤0.1 mL/min, gum test ≤10 mL/10 min or Saxon test ≤2 g/2 min to have satisfied decreased salivary volume in the ACR-EULAR and AECG criteria, because numerous cases (116/499 cases) lacked the results of UWS, which was not adopted in the JPN criteria.[3] Importantly, 6 out of 11 non-pSS cases who satisfied only the ACR-EULAR criteria, were considered to have decreased salivary volume based on the gum and/or Saxon test instead of UWS (data not shown). These six cases might explain the low specificity of the ACR-EULAR criteria in the present study. Actually, in the subanalysis of 383 cases that were examined for UWS, the specificity (76.7%) of the ACR-EULAR criteria was higher than in the whole analysis (72.1%). On the other hand, eight patients with pSS who satisfied only the ACR-EULAR criteria but none of other three sets of criteria might cause the high sensitivity of the ACR-EULAR criteria. These eight patients with pSS had positive FS (5/8 cases) or positive anti-SS-A/Ro (3/8 cases), accompanied with decreased salivary (7/8 cases) or lacrimal (1/8 cases) volume. Collectively, these 19 cases (8 patients with pSS and 11 non-pSS cases) that satisfied only the ACR-EULAR criteria but none of other three sets of criteria seem to lead to the disagreement between the ACR-EULAR criteria and other three sets of criteria. All of these 19 cases had positive FS (11/19 cases) or positive anti-SS-A/Ro (8/19 cases) accompanied by decreased salivary (18/19 cases) or lacrimal (1/19 cases) volume. Thus these 19 patients, even including clinically judged 11 non-pSS cases, seem to have a high probability of suffering from 'true pSS'. This means that the ACR-EULAR criteria might allow to correctly select patients with pSS who are misclassified by other criteria sets. Importantly, the highest sensitivity of the ACR-EULAR criteria might offer some advantages considering that more patients with true pSS could be selected for clinical and therapeutic trials. However, on the other hand, the lowest

A

Case	3 points		1 point			Total score
	FS-LSG	SS-A/Ro	Ocular staining	Schirmer	Saliva	
1	■				■	4
2	■				■	4
3	■				■	4
4	■				■	4
5	■				■	4
6		■			■	4
7		■			■	4
8		■		■		4
Positivity (%)	62.5	37.5	0	12.5	87.5	

B

Case	3 points		1 point			Total score
	FS-LSG	SS-A/Ro	Ocular staining	Schirmer	Saliva	
1	■				■	4
2		■			■	4
3	■				■	4
4	■				■	4
5		■			■	4
6		■			■	4
7	■				■	4
8	■				■	4
9		■			■	4
10	■				■	4
11		■			■	4
Positivity (%)	54.5	45.5	0	0	100.0	

Figure 1 Satisfaction for each item adopted in the American College of Rheumatology (ACR)-European League Against Rheumatism (EULAR) classification criteria for pSS (ACR-EULAR) among 19 cases that satisfied only the ACR-EULAR criteria. (A) Eight patients with primary Sjögren's syndrome (pSS) who only satisfied the ACR-EULAR criteria had positive Focus Score (FS) (62.5%, 5/8 cases) or pSS-A/Ro (37.5%, 3/8 cases), together with decreased salivary (87.5%, 7/8 cases) or lacrimal (12.5%, 1/8 cases) secretion, resulting in Total Score 4. (B) Eleven non-pSS cases who only satisfied the ACR-EULAR criteria had positive FS (54.5%, 6/11 cases) or positive SS-A/Ro (45.5%, 5/11 cases), together with decreased salivary secretion (100%, 11/11 cases), resulting in Total Score 4. White boxes: non-satisfaction, shadowed boxes: satisfaction (dark shadow: 3 points, light shadow: 1 point). FS-LSG, FS ≥1 foci/4 mm^2 in labial salivary gland; SS-A/Ro, positive anti-SS-A/Ro antibody; Ocular staining, Van Bijsterveld Score ≥4 in Rose Bengal test, lissamine green test or fluorescein staining test; Schirmer, Schirmer's test ≤5 mm/5 min; Saliva, unstimulated whole saliva (UWS) ≤0.1 mL/min, gum test ≤10 mL/10 min or Saxon test ≤2 g/2 min.

specificity of the ACR-EULAR criteria for Japanese patients with pSS was confirmed in both whole analysis and subanalysis using 383 cases that were examined for UWS, whereas the specificity was higher (76.7%) in subanalysis than in whole analysis (72.1%). A high specificity is the most critical aspect because this prevents subjects without pSS from entering clinical studies or trials. Therefore, if we apply the ACR-EULAR criteria to Japanese clinical studies targeted on pSS, we have to pay enough attention to this low specificity.

The 2016 ACR-EULAR criteria focused on pSS only but not on secondary SS, which is associated with other well defined CTDs.[6 7] However, the International SS Criteria Working Group indicated that these criteria could also be applicable for secondary SS, and recommended further studies for secondary SS to confirm this.[6 7] Thus, we also need to compare the performance of these four sets of criteria, including the ACR-EULAR, in the diagnosis of secondary SS because we targeted only patients with pSS or suspected pSS who were free of other CTDs.

The present study has certain limitations. First, we applied the diagnosis by the physician in charge as the 'gold standard'. However, it is better to decide disease case or non-case status by expert clinical judgement based on clinical vignettes for the 'gold standard' diagnosis, which has been employed by newly developed classification criteria, such as the 2016 ACR-EULAR criteria for pSS[6 7] and the proposed new classification criteria for systemic lupus erythematosus (SLE) by the Systemic Lupus International Collaborating Clinics.[8] Moreover, since this study was entirely carried out in Japan, it might be predicted that the physicians who commonly used JPN criteria could be strongly influenced in their mind by this conceptual habit when they clinically defined cases as having pSS or not. Second, the methods adopted for ocular staining and salivary tests varied among the participating institutions, forcing us to modify certain items in some criteria as described in the Patients and methods section. For these reasons, we need a more sophisticated validation cohort study, using expert clinical judgement based on clinical vignettes as the 'gold standard' and integrated methodology for ocular staining and salivary measurement that fits completely with the items adopted in the criteria.

In addition to the classification criteria for clinical studies, we also need diagnostic criteria for daily clinical practice. The JPN criteria were established for the diagnosis of SS including both pSS and secondary SS, and are always used in daily clinical

practice in Japan. Moreover, we reported previously that the JPN criteria had the highest sensitivity (79.6%) and specificity (90.4%) for all SS, including both primary and secondary SS, among the JPN, AECG and ACR criteria in Japan.[5] However, the JPN criteria adopt sialography, scintigraphy, and gum and Saxon tests for assessment of salivary volume, which are neither adopted in the 2016 ACR-EULAR nor ACR criteria.[3 4 6 7] Furthermore, the JPN criteria use a different cut-off value for ocular staining (Van Bijsterveld Score ≥3) from those used by the ACR-EULAR (Van Bijsterveld Score ≥4 or OSS ≥5), AECG (Van Bijsterveld Score ≥4) and ACR criteria (OSS ≥3).[2–4 6 7] Thus, we need to examine the performance of the JPN criteria using alternative items that are adopted in the ACR-EULAR criteria, such as UWS for gum and Saxon tests, and Van Bijsterveld Score ≥4 or OSS ≥5 for Van Bijsterveld Score ≥3 by a prospective validation cohort study in the near future.

In conclusion, although this study has certain limitations, the results showed that the ACR-EULAR criteria have higher sensitivity and lower specificity in the diagnosis of pSS, compared with the JPN, AECG and ACR criteria. Furthermore, the degree of agreement of the ACR-EULAR criteria with the other three sets of criteria for the diagnosis and denial of pSS was low.

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OPEN ACCESS

EXTENDED REPORT

Long-term efficacy and safety in patients with rheumatoid arthritis continuing on SB4 or switching from reference etanercept to SB4

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ABSTRACT

Objectives SB4 (Benepali, Brenzys) is a biosimilar of reference etanercept (ETN). In a randomised, double-blind, 52-week study, SB4 demonstrated comparable efficacy and safety to ETN in patients with rheumatoid arthritis (RA). The open-label extension period evaluated long-term efficacy, safety and immunogenicity when continuing SB4 versus switching from ETN to SB4.

Methods In the randomised, double-blind phase, patients received weekly subcutaneous administration of 50 mg SB4 or ETN with background methotrexate for up to 52 weeks. Patients in the Czech Republic and Poland who completed the 52-week visit were enrolled in the open-label extension period and received SB4 for 48 additional weeks. Efficacy, safety and immunogenicity were assessed up to week 100.

Results Of 245 patients entering the extension period, 126 continued to receive SB4 (SB4/SB4) and 119 switched to SB4 (ETN/SB4). American College of Rheumatology (ACR) response rates were sustained and comparable between SB4/SB4 and ETN/SB4 with ACR20 response rates at week 100 of 77.9% and 79.1%, respectively. Other efficacy results, including radiographic progression, were also comparable between the groups. After week 52, rates of treatment-emergent adverse events were 47.6% (SB4/SB4) and 48.7% (ETN/SB4); one patient/group developed non-neutralising antidrug antibodies. No cases of active tuberculosis or injection-site reactions were reported during the extension period. One patient (SB4/SB4) died of hepatic cancer.

Conclusions SB4 was effective and well tolerated over 2 years in patients with RA. Efficacy, safety and immunogenicity were comparable between the SB4/SB4 and ETN/SB4 groups, showing no risk associated with switching patients from ETN to SB4.

Trial registration number NCT01895309; 2012-005026-30

INTRODUCTION

The tumour necrosis factor inhibitor etanercept was the first approved biologic disease-modifying antirheumatic drug and allowed for a major advance in the treatment of rheumatoid arthritis (RA).[1] Eighteen years since its approval, etanercept continues to play a key role in RA management, having demonstrated efficacy and a manageable safety profile in both clinical trial and real-world settings.[1] Other current indications for etanercept include juvenile idiopathic arthritis, psoriatic arthritis, ankylosing spondylitis, non-radiographic axial spondyloarthritis (European Union only), plaque psoriasis and paediatric psoriasis (USA only).[2 3]

SB4 (Benepali, Samsung Bioepis UK Limited, Surrey, UK; Brenzys, Samsung Bioepis, Republic of Korea) is a biosimilar of reference etanercept (ETN). The structural, physicochemical and biological quality attributes of SB4 have been shown to be highly similar to ETN in a comprehensive comparability exercise designed as part of the European Medicines Agency's rigorous approval pathway.[4] A phase 1 study in healthy subjects demonstrated pharmacokinetic equivalence between SB4 and ETN[5] ; a phase 3 study (NCT01895309; EudraCT 2012-005026-30) in patients with moderate to severe RA despite treatment with methotrexate (MTX) demonstrated equivalent efficacy in terms of American College of Rheumatology 20% response rate (ACR20) at the 24-week interim analysis (SB4, 78.1%; ETN, 80.3%)[6] and at week 52 (SB4, 80.8%; ETN, 81.5%).[7] Safety was generally comparable between SB4 and ETN.[6 7]

SB4 has been approved for treatment of RA, psoriatic arthritis, ankylosing spondylitis, non-radiographic axial spondyloarthritis and plaque psoriasis in the European Union.[8 9] However, an important consideration for prescribing physicians is whether switching from ETN to SB4, which may occur in clinical practice, can be achieved without detriment to safety and efficacy. We analysed data from the open-label extension period of the phase 3 study to evaluate the efficacy, safety and immunogenicity of continuing SB4 (SB4/SB4) versus switching from ETN to SB4 (ETN/SB4). Long-term safety and efficacy were assessed up to week 100.

METHODS

Study design and patients

Patients with moderate to severe RA despite treatment with MTX were eligible to enrol in this phase 3, randomised, double-blind, multicentre study, which included an open-label extension period. Detailed patient inclusion/exclusion criteria were previously published.[6] During the double-blind period, patients were randomised 1:1 to receive subcutaneous SB4 50 mg or ETN 50 mg once weekly for 52 weeks. Patients in the Czech Republic or Poland who completed the scheduled 52-week visit were enrolled in the open-label, single-arm

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extension period. During the extension period, patients from the SB4 group continued to receive SB4 (SB4/SB4), and patients from the ETN group switched to SB4 50 mg (ETN/SB4) once weekly for an additional 48 weeks. All patients took a stable dose of MTX (10–25 mg/week) from 4 weeks before screening until the end-of-treatment visit for the extension period. For patients who entered the extension period, efficacy was assessed at weeks 52, 76 and 100, and safety was assessed at all visits during treatment and at 4 weeks after treatment (or after the early termination visit).

Endpoints

Efficacy endpoints for the extension period included ACR20/50/70 response (≥20%/50%/70% improvement, respectively, from baseline in ACR response criteria), European League Against Rheumatism (EULAR) response and disease activity score based on a 28-joint count (DAS28). Physical function was assessed using the Health Assessment Questionnaire-Disability Index (HAQ-DI). For patients who entered the extension period, radiographs of the hands and feet obtained at weeks 0, 52 and 100 were evaluated by a single reader to determine the modified Total Sharp Score (mTSS), which is the sum of the joint erosion and joint space narrowing (JSN) scores.[10] Post hoc assessments included the proportions of patients achieving low disease activity (LDA) and remission based on the Simplified Disease Activity Index (SDAI), Clinical Disease Activity Index (CDAI) and DAS28 and the proportions achieving Boolean-based remission (defined as ≤1 swollen and ≤1 tender joint, C-reactive protein ≤1 mg/dL and patient global visual analogue scale score ≤1 using a 0–10 scale). Safety endpoints included the incidence of treatment-emergent adverse events (TEAEs) and adverse events (AEs) of special interest (serious infections and active tuberculosis). Immunogenicity was assessed by determining the incidence of antidrug antibodies (ADAs) and neutralising antibodies; ADAs were detected in serum samples using an electrochemiluminescence bridging assay (Meso Scale Discovery, Maryland, USA), double-antigen format with acid dissociation and neutralising antibodies were measured using a competitive ligand-binding assay.[6]

Statistical analysis

All data were analysed descriptively. Efficacy and safety data were analysed in the extended population, which comprised all patients who provided informed consent for the open-label extension period and received ≥1 dose of study medication in the open-label extension period. Efficacy data obtained up to week 52 were analysed retrospectively in this population. No imputation was made for missing data. Analyses were performed using SAS software, V.9.2 or higher (SAS Institute, Cary, North Carolina, USA).

RESULTS

Patients

A total of 245 patients, including 126 who continued on SB4 and 119 who switched to SB4 from ETN, enrolled in the extension period. All patients received ≥1 dose of study drug during the extension period and were included in this analysis. Patient disposition is shown in figure 1; 94.7% of patients (232/245) who entered the extension period completed 100 weeks of treatment, with 5.6% of patients in the SB4/SB4 group and 5.0% of patients in the ETN/SB4 group withdrawing before week 100. Patient demographic and clinical characteristics were well balanced between the two groups (table 1).

Efficacy

ACR responses were comparable between the SB4/SB4 and ETN/SB4 groups and were maintained from weeks 52 through 100, with 79.2%/52.0%/38.4% and 82.4%/53.8%/32.8% of patients achieving ACR20/50/70 in each group, respectively, at week 52%

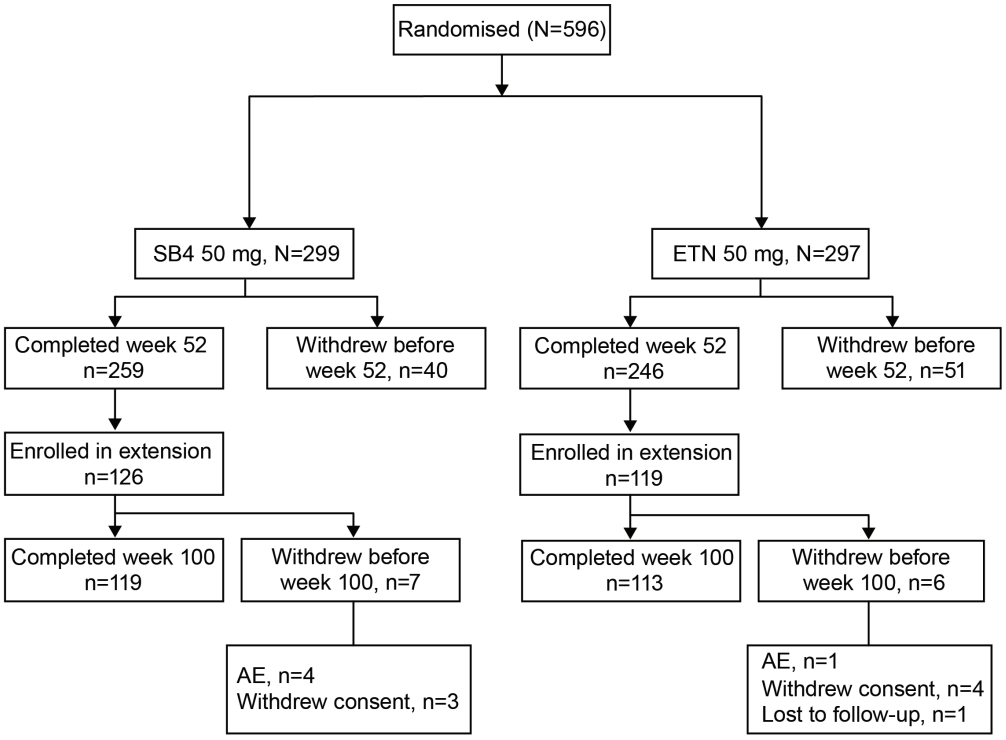

Figure 1 Patient disposition. AE, adverse event; ETN, reference etanercept.

and 77.9%/59.8%/42.6% and 79.1%/60.9%/41.7% of patients achieving ACR20/50/70 in each group, respectively, at week 100 (figure 2). ACR responses were also comparable between the two groups in the retrospective analysis of this population during the initial 52-week treatment period. Other efficacy results at week 100 are shown in table 2. At this time point, the proportion of patients who had moderate or good EULAR responses; the proportion who achieved LDA and remission based on DAS28, SDAI or CDAI criteria and the proportion who achieved Boolean-based remission were comparable between the SB4/SB4 and ETN/SB4 groups. Further, throughout the study, DAS28, SDAI, CDAI and HAQ-DI scores were also comparable between the two groups (see figure in the online supplementary material 1). The main factor driving the improvement in DAS28 score was the reduction in swollen and tender joint counts; these components demonstrated the largest percentage improvements from baseline during the extension period. At week 100, radiographic progression was comparable and minimal (figure 3), with mean (SD) change from baseline mTSS values of 0.48 (4.053) for the SB4/SB4 group and 1.00 (5.563) for the ETN/SB4 group (table 2). Summary of structural joint damage for each visit can be found in table S1 in the online supplementary material 1.

Table 1 Patient baseline demographics and disease characteristics at baseline and week 52 (extended population)

Variable	SB4/SB4 (n=126)	ETN/SB4 (n=119)
Age, years	49.9 (12.05)	52.1 (10.91)
Women, n (%)	107 (84.9)	100 (84.0)
White, n (%)	126 (100.0)	118 (99.2)
BMI, kg/m^2	26.7 (5.80)	26.1 (5.05)
Disease duration, years	5.7 (3.94)	5.8 (4.18)
Duration of MTX use, months	46.0 (35.63)	43.9 (39.81)
Weekly dose of MTX, mg	16.9 (4.92)	16.5 (4.91)
Swollen joint count (0–66)		
Baseline	14.4 (7.25)	14.4 (7.74)
Week 52	2.9 (4.84)	2.8 (4.30)
Tender joint count (0–68)		
Baseline	21.0 (9.96)	21.4 (11.08)
Week 52	5.0 (7.11)	5.6 (7.86)
Physician VAS (0–100)		
Baseline	62.4 (16.35)	63.6 (15.25)
Week 52	16.8 (14.47)	18.8 (15.27)
Patient VAS (0–100)		
Baseline	58.9 (19.75)	61.5 (18.08)
Week 52	24.9 (20.97)	26.8 (19.62)
Patient pain VAS (0–100)		
Baseline	59.0 (21.38)	60.5 (20.22)
Week 52	25.8 (21.86)	27.0 (21.32)
HAQ-DI (0–3)		
Baseline	1.38 (0.555)	1.45 (0.597)
Week 52	0.68 (0.585)	0.74 (0.651)
DAS28		
Baseline	6.22 (0.908)	6.26 (0.877)
Week 52	3.40 (1.179)	3.49 (1.119)
SDAI		
Baseline	37.01 (12.037)	37.65 (12.052)
Week 52	10.04 (8.589)	10.38 (8.713)
CDAI		
Baseline	35.85 (11.586)	36.45 (11.672)
Week 52	9.41 (8.249)	10.01 (8.670)
CRP, mg/L		
Baseline	11.5 (15.71)	12.0 (16.35)
Week 52	6.2 (15.84)	3.8 (5.47)
ESR, mm/h		
Baseline	41.9 (23.26)	41.7 (19.53)
Week 52	24.5 (18.63)	22.2 (16.21)
Rheumatoid factor positive, n (%)	99 (78.6)	89 (74.8)

Values represent mean (SD) unless otherwise specified.
BMI, body mass index; CDAI, Clinical Disease Activity Index; CRP, C-reactive protein; DAS28, disease activity score based on a 28-joint count; ESR, erythrocyte sedimentation rate; ETN, reference etanercept; HAQ-DI, Health Assessment Questionnaire-Disability Index; MTX, methotrexate; SDAI, Simplified Disease Activity Index; VAS, visual analogue scale.

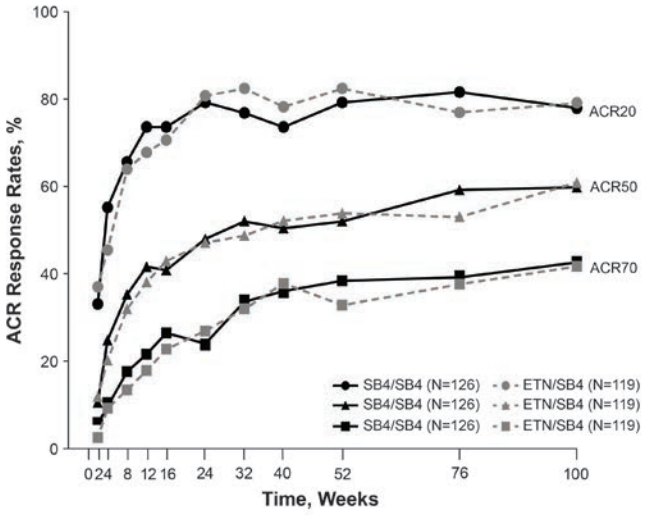

Figure 2 American College of Rheumatology (ACR) response rates up to week 100 (extended population). ACR20/50/70=American College of Rheumatology 20%/50%/70% response criteria; ETN, reference etanercept.

Safety

Safety after week 52 was generally comparable between the SB4/SB4 and ETN/SB4 groups (table 3). This extension study was not adequately powered to showsimilar safety and imbalance might be expected as shown in the incidence of serious TEAEs, RA, viral infection, laryngitis and hypertension. Serious infection was reported in one patient in each treatment group, and there were no reports of active tuberculosis. Also during the extension period, no injection-site reactions were reported. One patient in the SB4/SB4 group died of hepatic cancer, which was considered to be related to the study drug. One patient in each treatment group developed non-neutralising ADAs after week 52 (see table S2 in the online supplementary material 1). Both patients had a low titre, and the ADAs did not affect efficacy. The patient in the SB4/SB4 group tested positive at week 100 with a titre of 1 and achieved an ACR50 response at week 100. The patient from the ETN/SB4 group tested positive at week 76 with a titre of <1 and achieved an ACR70 response at week 100.

DISCUSSION

This open-label extension period of a phase 3, randomised, double-blind study evaluated the long-term efficacy, safety and immunogenicity of SB4 in patients with moderate to severe RA despite MTX treatment and compared outcomes between patients who continued SB4 (n=126) and those who switched from ETN to SB4 (n=119). Results showed SB4 to be effective

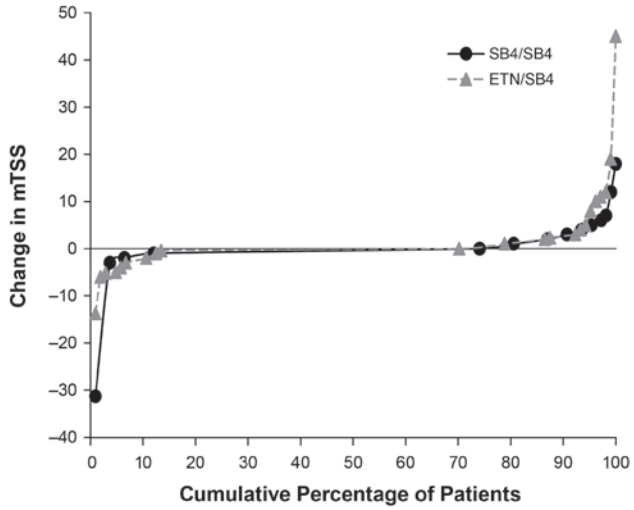

Figure 3 Cumulative probability of mTSS change from baseline at week 100 (extended population). Data based on patients with available radiographic assessment results at each visit. ETN, reference etanercept; mTSS, modified Total Sharp Score.

Table 2 Efficacy results at week 100 (extended population)

	SB4/SB4 (n=126)	ETN/SB4 (n=119)
EULAR response, n/N* (%)		
Good	59/121 (48.8)	63/115 (54.8)
Moderate	54/121 (44.6)	40/115 (34.8)
No response	8/121 (6.6)	12/115 (10.4)
DAS28		
Improvement from baseline, mean (SD)	2.9 (1.5)	3.0 (1.5)
Disease activity, n/N* (%)		
Low (≤3.2)	60/122 (49.2)	63/115 (54.8)
Remission (<2.6)	37/122 (30.3)	40/115 (34.8)
SDAI score		
Improvement from baseline, mean (SD)	27.4 (15.5)	28.7 (14.6)
Disease activity, n/N* (%)		
Low (>3.3 and≤11)	41/123 (33.3)	44/115 (38.3)
Remission (≤3.3)	38/123 (30.9)	39/115 (33.9)
CDAI score		
Improvement from baseline, mean (SD)	26.8 (15.0)	27.9 (14.1)
Disease activity, n/N* (%)		
Low (>2.8 and≤10)	38/123 (30.9)	46/115 (40.0)
Remission (≤2.8)	40/123 (32.5)	33/115 (28.7)
Boolean-based remission, n/N* (%)	31/123 (25.2)	23/115 (20.0)
Radiographic results†		
Change from baseline in JSN score, mean (SD)	0.19 (1.98)	0.39 (2.86)
Change from baseline in joint erosion score, mean (SD)	0.28 (2.57)	0.61 (3.08)
Change from baseline in mTSS, mean (SD)	0.48 (4.05)	1.0 (5.56)

*Number of patients with available data at each time point.
†Based on number of patients who completed week 100 visit with available radiographic assessment results at weeks 0 and 100 (SB4/SB4, n=108; ETN/SB4, n=104).
CDAI, Clinical Disease Activity Index; DAS28, disease activity score based on a 28-joint count; ETN, reference etanercept; EULAR, European League Against Rheumatism; JSN, joint space narrowing; mTSS, modified Total Sharp Score; SDAI, Simplified Disease Activity Index.

and well tolerated over 2 years. In patients who switched from ETN to SB4, comparable efficacy to the SB4/SB4 group was observed, with no new safety signals identified.

Among the patients entering the extension period, 94.4% in the SB4/SB4 group and 95.0% in the ETN/SB4 group completed an additional 48 weeks of SB4 treatment. The discontinuation rate due to lack of efficacy or TEAEs was very low, which suggests the long-term tolerability of SB4 treatment.

Efficacy outcomes in the extended population were comparable between the SB4/SB4 and ETN/SB4 groups at all visits up to week 100, sustained from weeks 52 to 100 and unaffected by switching. Comparable inhibition of radiographic progression was previously reported after 52 weeks of treatment with SB4 or ETN (mean change in mTSS: 0.45 for SB4 vs 0.74 for ETN).[7] In both groups, continued inhibition of radiographic progression was observed with an additional year of SB4 treatment, with mean changes from baseline in joint space narrowing and joint erosion of <1. This is consistent with historical results from randomised studies of etanercept with or without MTX in patients with RA.[11–13] Two-year radiographic findings in patients with early RA continuing ETN+MTX therapy from year 1 showed a mean Sharp/van der Heijde score change of −0.02 and an improvement in mean 28-swollen joint count from 1.7 to 1.3.[11] Similarly, 2-year data from the Canadian Methotrexate and Etanercept Outcome study showed that patients continuing ETN+MTX therapy after the first 6 months had mean changes from baseline in mTSS and JSN of 0.0 at month 24 and those switching to ETN monotherapy had mean changes from baseline in mTSS and JSN of <1.[12] Lastly, the Trial of Etanercept and Methotrexate with Radiographic Patient Outcomes (TEMPO) demonstrated mean changes from baseline in mTSS, joint erosion scores and JSN of <1 at years, 1, 2 and 3 of treatment with ETN+MTX.[13]

In the extension period, SB4 demonstrated a safety profile similar to that observed in the pivotal etanercept trials.[11–13] There were no reports of active tuberculosis or injection-site reactions. One patient in each group reported a serious

Table 3 Safety after week 52 (extended population)

n (%)	SB4/SB4 (n=126)	ETN/SB4 (n=119)
≥1 TEAE	60 (47.6)	58 (48.7)
Frequently reported TEAEs (≥3%)		
Upper respiratory tract infection	10 (7.9)	9 (7.6)
Pharyngitis	9 (7.1)	5 (4.2)
Rheumatoid arthritis	7 (5.6)	3 (2.5)
Bronchitis	6 (4.8)	7 (5.9)
Nasopharyngitis	6 (4.8)	5 (4.2)
Viral infection	4 (3.2)	1 (0.8)
Laryngitis	4 (3.2)	0 (0.0)
Hypertension	1 (0.8)	5 (4.2)
≥1 serious TEAE	6 (4.8)	2 (1.7)
TEAE leading to study drug discontinuation	4 (3.2)	2 (1.7)
Serious infection	1 (0.8)	1 (0.8)
Active tuberculosis	0 (0.0)	0 (0.0)
Injection-site reaction*	0 (0.0)	0 (0.0)
Malignancy†	1 (0.8)	0 (0.0)
Death†	1 (0.8)	0 (0.0)

*TEAE with high-level group term of administration site reaction.
†Hepatic cancer, which was considered related to study drug.
ETN, reference etanercept; TEAE, treatment emergent adverse event.

infection and one patient in the SB4/SB4 group died from hepatic cancer. After week 52, one patient in each group developed non-neutralising ADAs. The low incidence of non-neutralising ADAs observed in the study was expected given the low rates reported in short-term and long-term studies of etanercept-treated patients with RA (0%–6%).[14–16] The ADAs developed prior to switching did not affect the efficacy or safety of SB4 in the ETN/SB4 group.

Results from this extended-period switching study showed maintenance of response after switching from ETN to SB4 with no newly identified safety issues (eg, no increase in immunogenicity or immune-related TEAEs of anaphylaxis, hypersensitivity or injection-site reactions). In extensions of PLANETRA (Program evaLuating the Autoimmune Disease iNvEstigational Drug cT-p13 in RA Patients)[17] and PLANETAS (Program evaLuating the Autoimmune Disease iNvEstigational Drug cT-p13 in AS patients)[18] which had similar study designs with the present study, switching from reference infliximab to the biosimilar infliximab CT-P13 was not associated with diminished efficacy or change in safety profile. These results are further corroborated by findings from the randomised, non-inferiority NOR-SWITCH study which demonstrated that switching to CT-P13 is not inferior to continued treatment with reference infliximab.[19] In addition, data from the DANBIO registry where a nationwide switch took place, disease activity was not affected by the non-medical switch from the reference infliximab or ETN to CT-P13 or SB4, respectively.[20 21] Observations from these studies provide data relevant to clinical practice and support switching of reference products to biosimilars for non-medical reasons.

A retrospective analysis of our data was conducted for any potential anaphylaxis cases using related AEs (eg, pruritus, flushing, dyspnoea, hypotonia, syncope, incontinence, vomiting) and blood pressure (systolic blood pressure <90 mm Hg or >30% decrease from baseline), as defined in the National Institute of Allergy and Infectious Diseases/Food Allergy Anaphylaxis Network criteria.[22] No cases of potential anaphylaxis were identified based on this analysis.

The open-label nature of the extension period is a study limitation. Because patients were required to have completed the 52-week visit of the randomised, double-blind period in order to enrol in the extension, there was the potential for selection bias. However, baseline demographic and clinical characteristics were well balanced between the treatment groups and were representative of those in the core study. Moreover, disease activity at week 52 for patients who enrolled in the extension period was comparable with that of patients who did not enrol in the extension period (see table S3 in the online supplementary material 1), suggesting no selection bias towards patients who responded well to treatment. This switching study was designed to evaluate approximately 100 patients in each group to allow detection of an increase in the risk for injection site reactions to 1% or more. Therefore, the two countries with the largest number of enrolled patients (Poland and the Czech Republic) were selected to participate in the extension period. Although the extension period was not designed to compare equivalence statistically, it provides valuable data on switching from ETN to SB4 in patients with RA.

CONCLUSIONS

SB4 was well tolerated and effective over 2 years in patients with RA. Switching from ETN to SB4 was not associated with treatment-emergent issues such as loss of efficacy or increases in TEAEs or immunogenicity. Postmarketing surveillance and registry studies are ongoing to monitor the efficacy and safety of SB4 in various indications.

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Patient consent Obtained.

Ethics approval This study was conducted in accordance with the Declaration of Helsinki and International Council for Harmonisation Good Clinical practice guidelines. Protocols were reviewed and approved by the independent ethics committee or institutional review board for each study centre. All patients provided written informed consent.

Provenance and peer review Not commissioned; externally peer reviewed.

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EXTENDED REPORT

Epidemiology and burden of systemic lupus erythematosus in a Southern European population: data from the community-based lupus registry of Crete, Greece

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ABSTRACT

Objectives Several population-based studies on systemic lupus erythematosus (SLE) have been reported, yet community-based, individual-case validated, comprehensive reports are missing. We studied the SLE epidemiology and burden on the island of Crete during 1999–2013.

Methods Multisource case-finding included patients ≥15 years old. Cases were ascertained by the ACR 1997, SLICC 2012 criteria and rheumatologist diagnosis, and validated through synthesis of medical charts, administrative and patient-generated data.

Results Overall age-adjusted/sex-adjusted incidence was 7.4 (95% CI 6.8 to 7.9) per 100 000 persons/year, with stabilising trends in women but increasing in men, and average (±SD) age of diagnosis at 43 (±15) years. Adjusted and crude prevalence (December 2013) was 123.4 (113.9 to 132.9) and 143 (133 to 154)/10^5 (165/10^5 in urban vs 123/10^5 in rural regions, p<0.001), respectively. Age-adjusted/sex-adjusted nephritis incidence was 0.6 (0.4 to 0.8) with stable trends, whereas that of neuropsychiatric SLE was 0.5 (0.4 to 0.7) per 100 000 persons/year and increasing. Although half of prevalent cases had mild manifestations, 30.5% developed organ damage after 7.2 (±6.6) years of disease duration, with the neuropsychiatric domain most frequently afflicted, and 4.4% of patients with nephritis developed end-stage renal disease. The ACR 1997 and SLICC 2012 classification criteria showed high concordance (87%), yet physician-based diagnosis occurred earlier than criteria-based in about 20% of cases.

Conclusions By the use of a comprehensive methodology, we describe the full spectrum of SLE from the community to tertiary care, with almost half of the cases having mild disease, yet with significant damage accrual. SLE is not rare, affects predominantly middle-aged women and is increasingly recognised in men. Neuropsychiatric disease is an emerging frontier in lupus prevention and care.

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INTRODUCTION

Systemic lupus erythematosus (SLE) is a complex autoimmune disease with chronic relapsing-remitting nature.[1] Clinical-epidemiological research has advanced our knowledge; however, most reports are based on tertiary care data, which often provide conflicting or non-generalisable results.[2 3] Updated, comprehensive information at the community level may contribute to realising the disease's unmet needs and unravel the role of genetic and environmental factors.[4]

To address the need for accurate data on the epidemiology of the disease, we established the Lupus Registry '*Leto*'. The main objectives of the study were (1) to obtain population-based estimates of SLE incidence and prevalence in individuals residing in Crete during 1999–2013, and (2) to describe the main clinical features, including trends of severe disease manifestations (lupus nephritis (LN), neuropsychiatric lupus) and outcomes (organ damage).

METHODS

Source population and setting

Crete, the fifth largest southernmost island in the Mediterranean, provides an advantageous setting to study complex diseases such as SLE. First, it is geographically isolated with a relatively closed, genetically homogeneous population of approximately 0.65 million and low migration/translocation rates. Second, it comprises both urban (>15 000, 39% of the inhabitants) and rural (61% of the inhabitants) areas (2011 National Census, http://www.statistics.gr/en/statistics/pop). Third, the healthcare system is mixed public/private and patients can visit a specialist without a general practitioner's referral, but typically even patients with mild SLE are not followed exclusively at primary care. There is a single rheumatology clinic, at the University Hospital of Iraklio, with expertise in SLE since 1990, which serves as referral centre and has strong connections to primary/secondary care units (including private rheumatologists) involved in lupus diagnosis and care, thus resulting in low rate of patients seeking medical care outside Crete.

Case-finding and patient recruitment

This is an ongoing programme that started in 2012. The present study involved an initial retrospective research of potential cases from 1990 until 2011 (irrespective of the year of their diagnosis), coupled

with a prospective active surveillance (2012–2015). To ensure data completeness, we included in the study incident and prevalent SLE cases from 1999 to 2013 (thus extending our surveillance period by another 2 years).

Multiple case-finding sources were used (online supplementary figure S1). Our primary source was the medical charts of the rheumatology clinic. More than 10 000 paper records were screened for SLE and related diagnoses ('incomplete lupus', 'possible lupus', 'lupus-like' and 'undifferentiated connective tissue disease'). Second, the programme was communicated to nephrology and dermatology departments across the island, so that they provided access to data (eg, biopsies archives) and referred patients who had never been evaluated by us. Third, we communicated with all private rheumatologists to possibly detect milder cases followed at the community. Furthermore, the Arthritis Foundation of Crete endorsed a campaign to inform patients and the public.

Patients who were identified were recruited (on their visit at the clinic, referred by the collaborative network of physicians or by email/telephone in case of no regular follow-up). On enrolment to the registry, they were administered with a structured questionnaire after signing informed consent. Interviews were performed by trained personnel.

Complementary data were retrieved from hospital databases (discharges, laboratory tests) queried for relevant International Classification of Diseases-9 codes and the National Renal Data System for end-stage renal disease (ESRD). These sources also provided mortality data.

Database registry establishment, variables and information synthesis

For each potential case, dedicated personnel reviewed all available paper and electronic files. The ACR 1997[5] and Systemic Lupus International Collaborating Clinics (SLICC) 2012[6] classification criteria, validated activity (SLE Disease Activity Index 2000[7]) and organ damage (SLICC/ACR Damage Index (SDI)[8]) items were recorded from the medical charts, and the respective indexes were calculated. SLE was characterised as mild, moderate or severe based on the British Isles Lupus Assessment Group-defined[9] severity of disease manifestations, medications received and physician's global assessment. LN was defined according to kidney biopsy and/or the classification criteria. The SDI definition of ESRD was used.[8] Diagnosis of neuropsychiatric SLE (NPSLE) (ie, attributed to the disease) was according to the ACR definitions,[10] following multidisciplinary approach and validated with attribution models.[11 12] Time-relevant data included dates of SLE diagnosis, any previous diagnoses and dates for major clinical features and score items described above. From the questionnaires, we gathered detailed data on demographics, residence, family and personal history, disease manifestations, body mass index (BMI) and tobacco use. All data/variables were entered into a database to enable cross-checking and information synthesis.

SLE definitions and case ascertainment (validation)

Primary analyses were based on cases that fulfilled the ACR 1997 criteria during the period 1999–2013. Secondary analyses used rheumatologist-based and SLICC 2012 criteria-based diagnoses. Cases were counted as incident at the year the diagnosis clearly reported for the first time in patient's medical records and/or the fourth criterion was fulfilled. To count a case (incident or prevalent) for a specific calendar year, a patient had to reside in Crete at least 1 year before and be over 15 years old. Patients who died still counted as prevalent in the same year of death but not in the ensuing year(s). Drug-induced and cutaneous-only lupus were excluded. The relevant flow chart is shown in online supplementary figure S1.

Statistical analysis (see also online supplementary material[13–15])

Age-specific, sex-specific and region-specific denominators were based on National Census (2001, 2011). Interim population estimations generated by the Hellenic Statistical Authority were publicly distributed (Thessaly University, http://www.e-demography.gr). Crude and stratum-specific average annual incidence and prevalence with 95% CIs were calculated. Age-standardised and gender-standardised rates for SLE, LN and NPSLE were calculated with the direct method using the European Standard Population as a reference.

Study approval

The study was approved by the Ethics Committee of the University Hospital of Iraklio.

RESULTS

Increasing incidence of SLE during the period 1999–2010

The overall crude and age-adjusted/sex-adjusted incidence rate of SLE (ACR 1997-based) in Crete during 1999–2013 was 8.6 (95% CI 8.0 to 9.0) and 7.4 (95% CI 6.8 to 7.9) per 100 000 persons/year, respectively. The incidence female-to-male ratio was 13:1. There was an increase in SLE incidence during the years 1999–2010, which stabilised afterwards (figure 1A). This trend was observed in both genders, but in men the increase continued until the end of the study period (online supplementary table S1).

Incidence of severe SLE: stable rates of LN but increasing trends of NPSLE

We next focused on severe forms of SLE, namely LN and NPSLE. Overall age-adjusted/sex-adjusted incidence of LN during 1999–2013 was 0.6 (95% CI 0.4 to 0.8) per 100 000 persons/year, corresponding to incidence rates of 1.0 (95% CI 0.7 to 1.3) and 0.2 (95% CI 0.1 to 0.4) per 100 000 persons/year in women and men, respectively. Rates of incident nephritis remained stable (figure 1B). Adjusted NPSLE (cerebrovascular disease, seizures and cognitive dysfunction being the most frequent manifestations) incidence rates in the total, female and male population were 0.5 (95% CI 0.4 to 0.7), 0.8 (95% CI 0.5 to 1.1) and 0.3 (95% CI 0.1 to 0.4), respectively. Temporal trends of incident NPSLE resembled those of SLE, that is, increasing during 1999–2010 and remaining stable afterwards (figure 1C). Detailed rates per calendar year are presented in online supplementary table S1. The female-to-male ratio of incident nephritis and NPSLE cases was 4.2:1 and 3:1, respectively.

Earlier onset of LN and NPSLE in male than female patients

The mean (±SD) age at the time of SLE diagnosis was 43 (±15) years (range 9–81), with a peak at the age group 45–54 years for both men and women (figure 2A). LN occurred earlier in men than in women, with most cases diagnosed at 15–24 vs 45–54 years, respectively (figure 2B). In NPSLE, the peak age of diagnosis was also lower in men than in women (35–44 vs 45–54 years) (figure 2C).

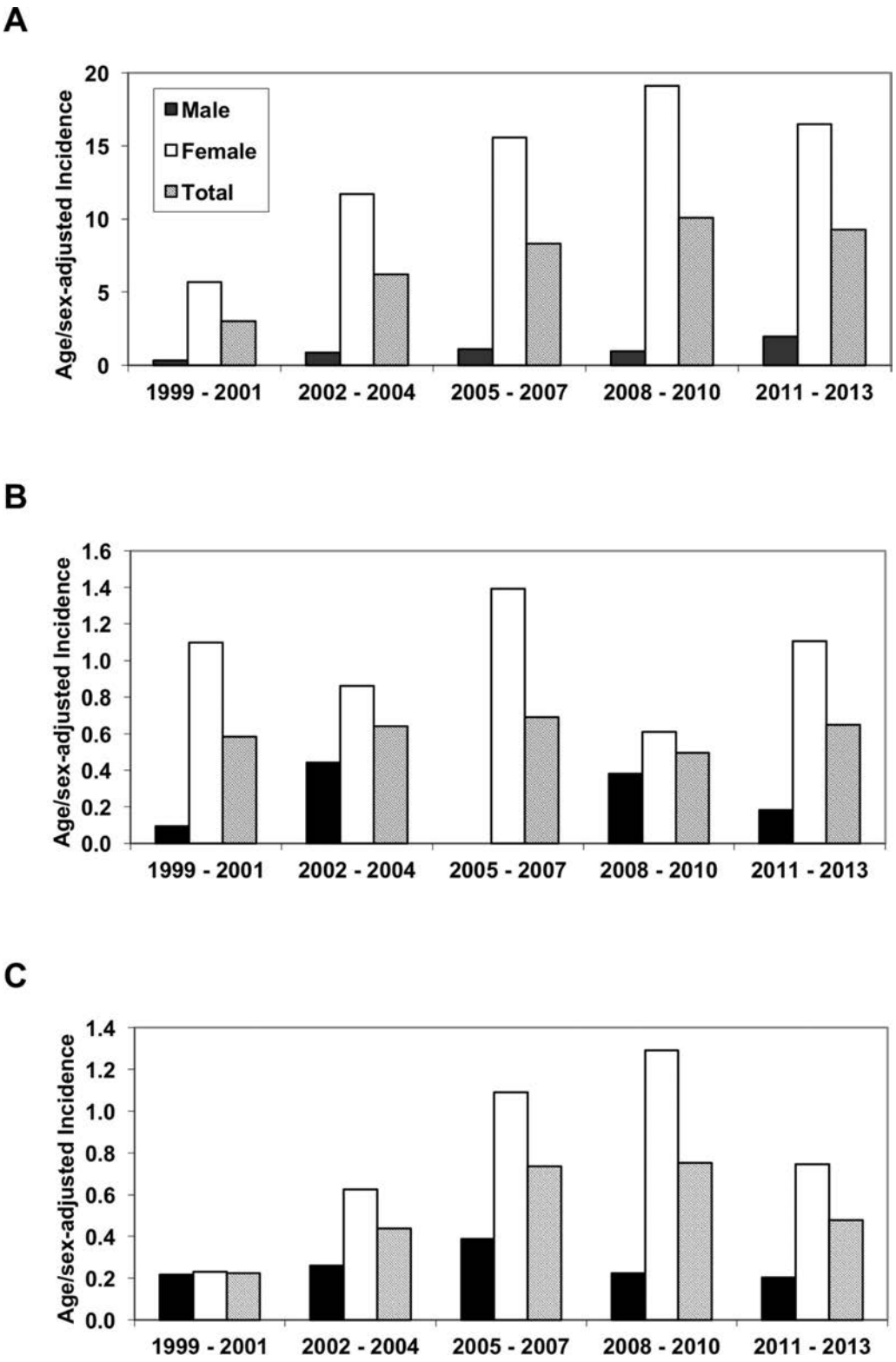

Figure 1 Incidence of SLE, lupus nephritis and NPSLE according to 3-year intervals during the period 1999–2013. (A) Age-adjusted and sex-adjusted incidence of SLE (ACR 1997 definition) per 100 000 persons/year. (B) Age-adjusted and sex-adjusted incidence of lupus nephritis per 100 000 persons/year. (C) Age-adjusted and sex-adjusted incidence of NPSLE per 100 000 persons/year. ACR, American College of Rheumatology; NPSLE, neuropsychiatric SLE; SLE, systemic lupus erythematosus.

Increasing prevalence of SLE, LN and NPSLE

There was a steady increase in crude SLE prevalence (ACR 1997-defined) from 22 (95% CI 18 to 26) in 1999 to 143 (95% CI 133 to 154) per 100 000 individuals aged ≥15 years old in 2013. The age-adjusted/sex-adjusted prevalence was 18.7 (95% CI 14.8 to 22.6) in 1999 and 123.4 (95% CI 113.9 to 132.9) per 100 000 in 2013. The increasing trend was noted in both genders (online supplementary table S2).

Crude prevalence of LN in 2013 was 14.4 (95% CI 11.1 to 17.6) per 100 000, which corresponds to prevalence of 24.1 (95% CI

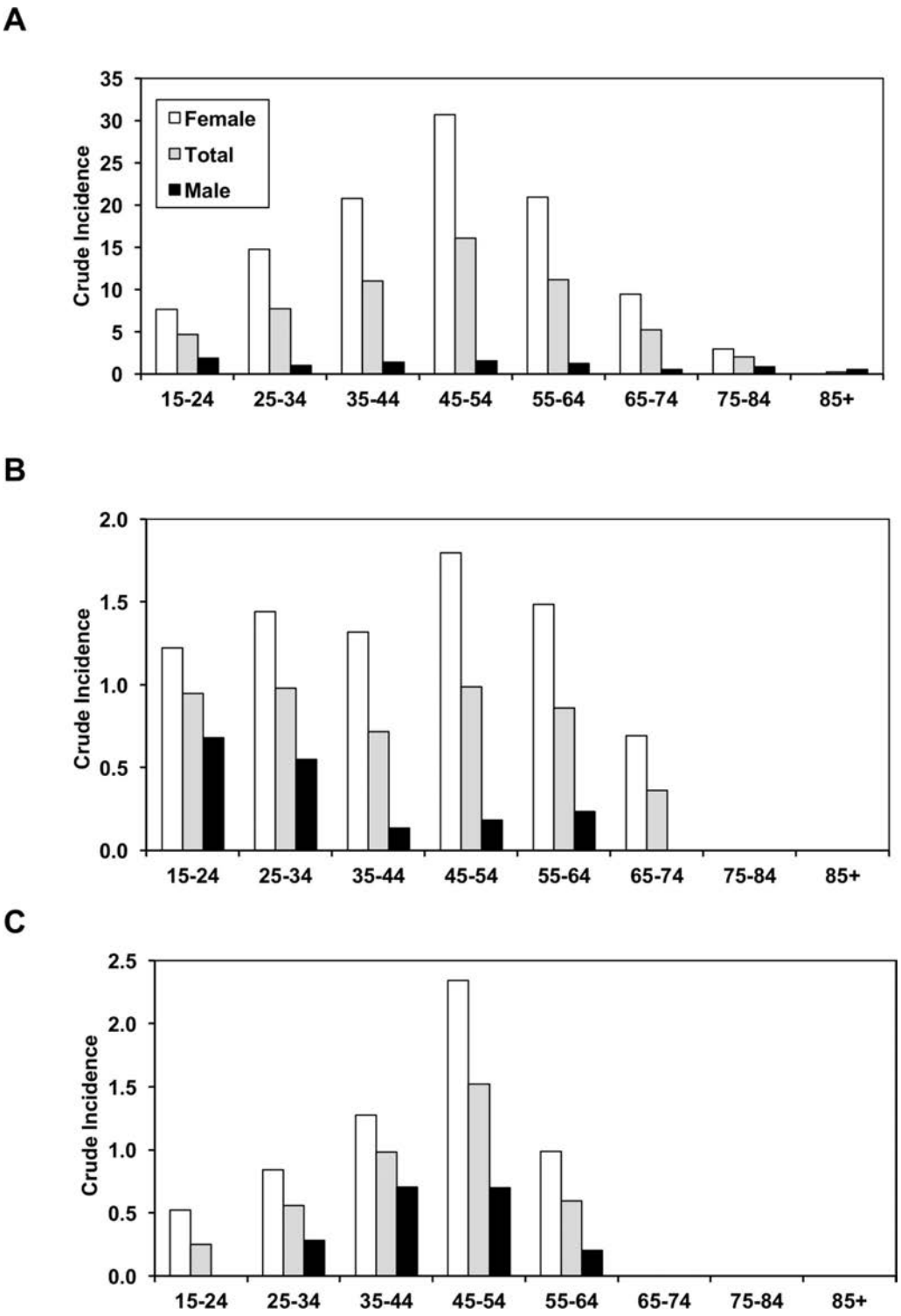

Figure 2 Incidence of SLE, lupus nephritis and NPSLE across different age groups (period 1999–2013). (A) Crude incidence rates (per 100 000 persons/year) of SLE. (B) Crude incidence rates (per 100 000 persons/year) of lupus nephritis. (C) Crude incidence rates (per 100 000 persons/year) of NPSLE. NPSLE, neuropsychiatric SLE; SLE, systemic lupus erythematosus.

18.1 to 29.9) and 4.3 (95% CI 1.8 to 6.8) (per 100 000) in women and men, respectively. We examined the possibility of missing LN cases and found that the number of prevalent cases (n=90) approximated the median number of expected patients with LN according to capture–recapture for all sources (89.5, 95%CI 73.0 to 105.9) and the Bayesian model-derived estimate of prevalent LN cases (86, 95%CI 83 to 89). As for NPSLE, crude prevalence in the total, female and male population was 9.7 (95% CI 7.0 to 12.3), 14.9 (95% CI 10.3 to 19.5) and 4.3 (95% CI 1.8 to 6.9) per 100 000, respectively. Both prevalence of nephritis and NPSLE increased during the study period (twofold and sixfold, respectively).

Similar trends in SLE incidence and prevalence using different case definitions

To validate our findings, we estimated the disease occurrence using alternative case definitions. Time trends in SLE incidence were similar according to ACR 1997, SLICC 2012 classification

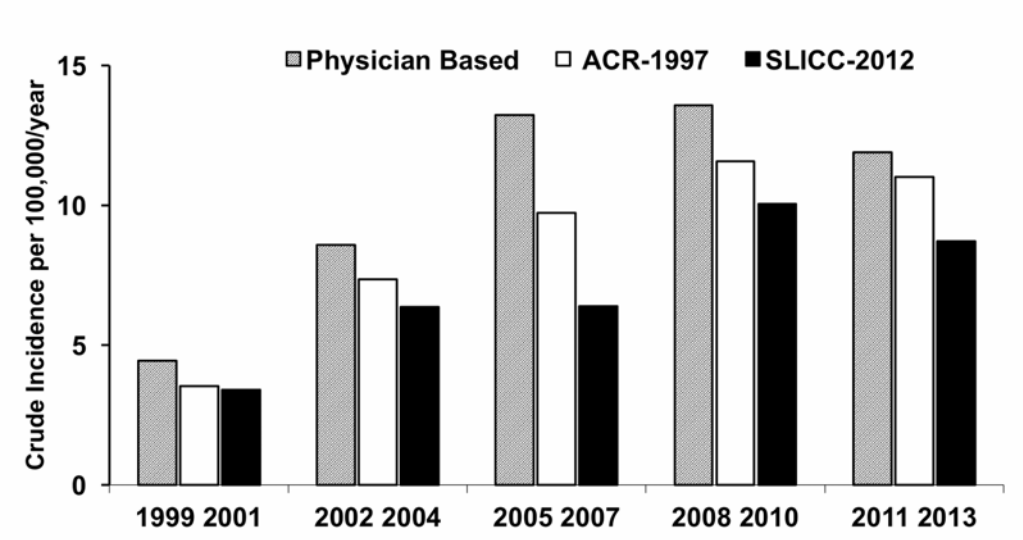

Figure 3 Incidence of SLE (per 100 000 persons/year) according to 3-year intervals (period 1999–2013) based on three case definitions (ACR 1997 criteria definition, rheumatologist-based definition and SLICC 2012 criteria definition). ACR, American College of Rheumatology; SLE, systemic lupus erythematosus; SLICC, Systemic Lupus International Collaborating Clinics.

criteria and physician diagnosis (figure 3). Incident cases were defined by rheumatologist diagnosis on average 3 months earlier than with the ACR 1997 criteria, and by the ACR 1997 criteria on average 3 months earlier than the SLICC 2012 criteria. Within cases who fulfilled both the ACR 1997 criteria and rheumatologist-based diagnosis, 68% had the diagnosis by these two definitions in the same year; in 22%, clinical diagnosis preceded that of ACR 1997 criteria and in the remaining 10% the ACR-based diagnosis preceded the clinical one. The respective percentages were 70%, 19% and 11% for the comparison of SLICC 2012 criteria against rheumatologist-based diagnosis, and 87%, 5% and 8% for the comparison of ACR 1997 against SLICC 2012 criteria. By use of any of the three definitions, SLE prevalence estimates demonstrated a steady increase during 1999–2013 (figure 4).

Demographic and clinical features of prevalent cases: higher disease severity in male patients

The ACR 1997-based prevalent population (December 2013) comprised 750 patients and was sociodemographically homogeneous: 97% Greek, 93% women, 81% married, 70% with <12 years of education and 80% with Cretan descent (defined as past three generations). The mean disease duration was 7.2 (±6.6) years. Of the patients, 68.4% had disease duration longer than 5 years. Current smoking and obesity (BMI >30 kg/m^2) were each found in 30% of the patients.

The most frequent clinical features (cumulative incidence of ACR 1997 criteria) were arthritis and mucocutaneous manifestations (figure 5). The following manifestations occurred more frequently in men versus women: serositis (28% vs 14%, p<0.001), renal involvement (26.4% vs 11.8%, p<0.001), neurological manifestations (13.3% vs 3.3%, p<0.001) and haematological abnormalities (47.2% vs 28.0%, p<0.001). Based on the severity of manifestations and the use of lupus treatments, the disease was classified as mild, moderate and severe in 50%, 33% and 17% of all prevalent SLE cases. Of the patients, 14.8% had received azathioprine or mycophenolate, 9% cyclophosphamide and 3.6% rituximab. In total, 34% of the moderate/severe cases had received potent immunosuppressive drugs. Significantly more men than women displayed moderate (30.2% vs 25.8%) or severe (34.0% vs 13.5%) forms of SLE (p<0.001 for both comparisons).

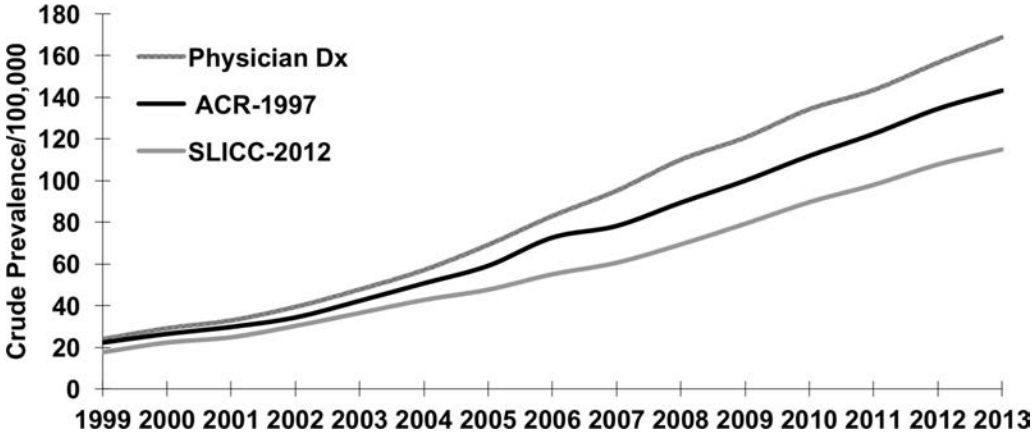

Figure 4 Annual prevalence of SLE (per 100 000 persons/year) during the period 1999–2013 based on three case definitions (ACR 1997 criteria definition, rheumatologist-based definition and SLICC 2012 criteria definition). ACR, American College of Rheumatology; SLE, systemic lupus erythematosus; SLICC, Systemic Lupus International Collaborating Clinics.

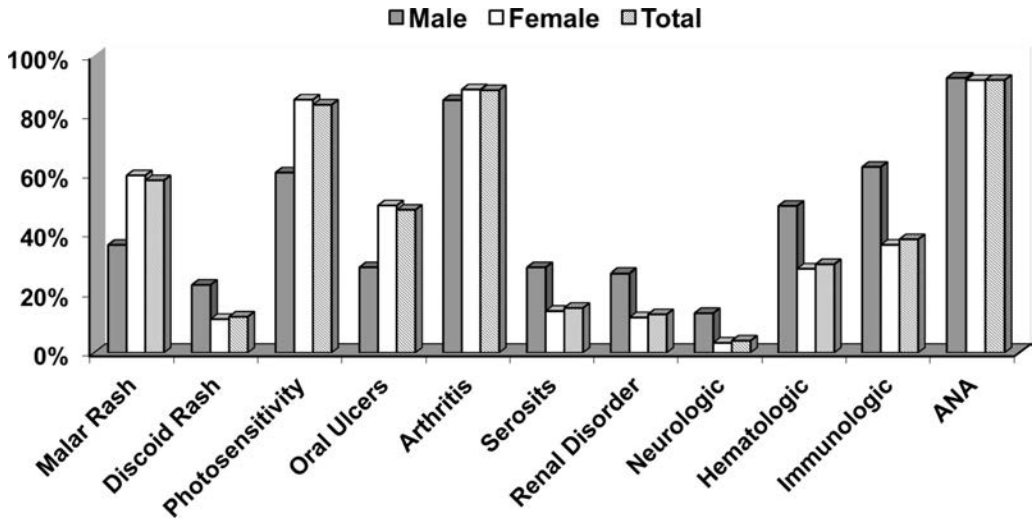

Figure 5 Clinical and serological manifestations in the 750 prevalent SLE cases (year 2013) (ACR 1997 criteria definition). ACR, American College of Rheumatology; SLE, systemic lupus erythematosus.

Significant organ damage develops early in the disease course

Data for organ damage, assessed by the SDI (score range 0–46; 0=no damage, 1–2=moderate damage, ≥3= severe damage),[16 17] were available in 613 patients. On the year of diagnosis, 84.0% of patients with SLE were free of damage, whereas 12.6%, 2.9% and 0.5% had SDI scores of 1, 2 and 3, respectively. Three years after diagnosis, the respective percentages were 76.7%, 18.3%, 3.8% and 1.3% (figure 6A). At last follow-up, 30.5% of patients with prevalent SLE had organ damage (figure 6B). Men had more damage than women: 28% vs 18% ($p<0.003$) on the year of diagnosis, 34% vs 22% ($p<0.005$) after 3 years and 38% vs 30% ($p<0.005$) at last follow-up. The most frequent component of the SDI was the neuropsychiatric items (cognitive impairment, seizures, cerebrovascular accident), followed by the musculoskeletal and malignancy items (figure 6C). Within the subgroup of patients with LN, 4.4% developed ESRD.

DISCUSSION

By employing a comprehensive methodology, from primary to tertiary care, we describe high SLE occurrence in Crete. Our incidence is higher than estimates from Nordic countries and Europe,[18] although lower than ethnic minorities' rates in the UK,[19] USA[20] and elsewhere.[3 21] The observed adjusted incidence rates ($7.4/10^5$ persons/year) exceed those from previous decade in Greece ($1.90/10^5$ persons/year, 1982–2001).[22] Likewise, our prevalence estimates are higher than those previously reported in Greece (50[23]–110[24] per 100 000). Together, our findings concord with recent estimates suggesting that SLE should no longer be considered a rare disease (average threshold of 40 cases/10^5[25]).

We found an increase in SLE incidence in our region during 1999–2010, which stabilised thereafter. Relevant reports worldwide[21] are conflicting and suggest increasing trends in USA[26 27] and Greece,[22] decreasing in Spain[28] and UK,[29] or stable in Norway and Denmark.[30 31] Increases during the previous decades (1950–1990) were attributed to wider use and improvements in antinuclear antibodies (ANA) testing and diagnosis of milder cases.[27]

In our study, the observed rise might be—at least partially—explained by better disease awareness and recognition. Incomplete case-finding or ascertainment during the early years might also be possible, thus leading to 'inflation' of cases. To address this, we compared disease severity, use of potent immunosuppressive/biological drugs and organ damage in incident cases across three consecutive periods (1999–2003, 2004–2008 and 2009–2013) and found no differences in ratios (online supplementary table S3).

The possible effect of environmental factors on the increase cannot be excluded. In Crete, there was a profound urbanisation during the previous decades both in terms of people migrating to larger cities and also in lifestyle changes. Circumstantial evidence suggests increased or increasing prevalence among adults in the general population of Crete in factors such as westernised diet and lifestyle,[32] vitamin D deficiency (severe in 21%[33]) and smoking (44% among parents of preschool children[34]), which all could contribute to SLE increase. Notably, and in agreement with other studies,[22 35] SLE was more prevalent in urban than rural regions, a result that deserves more detailed investigation.

The increasing prevalence of SLE in our region concords with worldwide trends.[21] Besides increased incidence, this may be explained also by improved survival. Unfortunately, detailed mortality data were not available especially for the first decade of the observation period, and this is a limitation. Nonetheless, during the more recent 5 years for which we had more accurate data, we had only nine deaths, suggesting a low risk of survival bias.

Our incidence of LN (0.6 per 100 000/year) is higher than those in the UK,[36] Denmark[37] and Norway[38] (ranging from 0.40 to 0.45 per 100 000/year). Accordingly, prevalence of LN in Crete in 2013 (14.4 per 100 000) is higher than in the UK (5.6 per 100 000 white individuals in 2001)[36] and Denmark (6.4 per 100 000 in 2011),[37] but lower than in white US Medicaid-enrolled adults (15.8 per 100 000).[20] Regarding NPSLE, epidemiological studies are scarce and results are variable.[39] Herein, we provide, for the first time, sex-adjusted/age-adjusted incidence and prevalence estimates for NPSLE, and demonstrate increasing trends during 1999–2010, possibly due to better awareness and increasing use of neuroimaging.

By using rheumatologist diagnosis, the ACR 1997 and SLICC 2012 criteria for case definition, we noted concordant time trends in SLE (figures 3–4). There were differences in the timing of diagnosis with physician-based preceding criteria-based

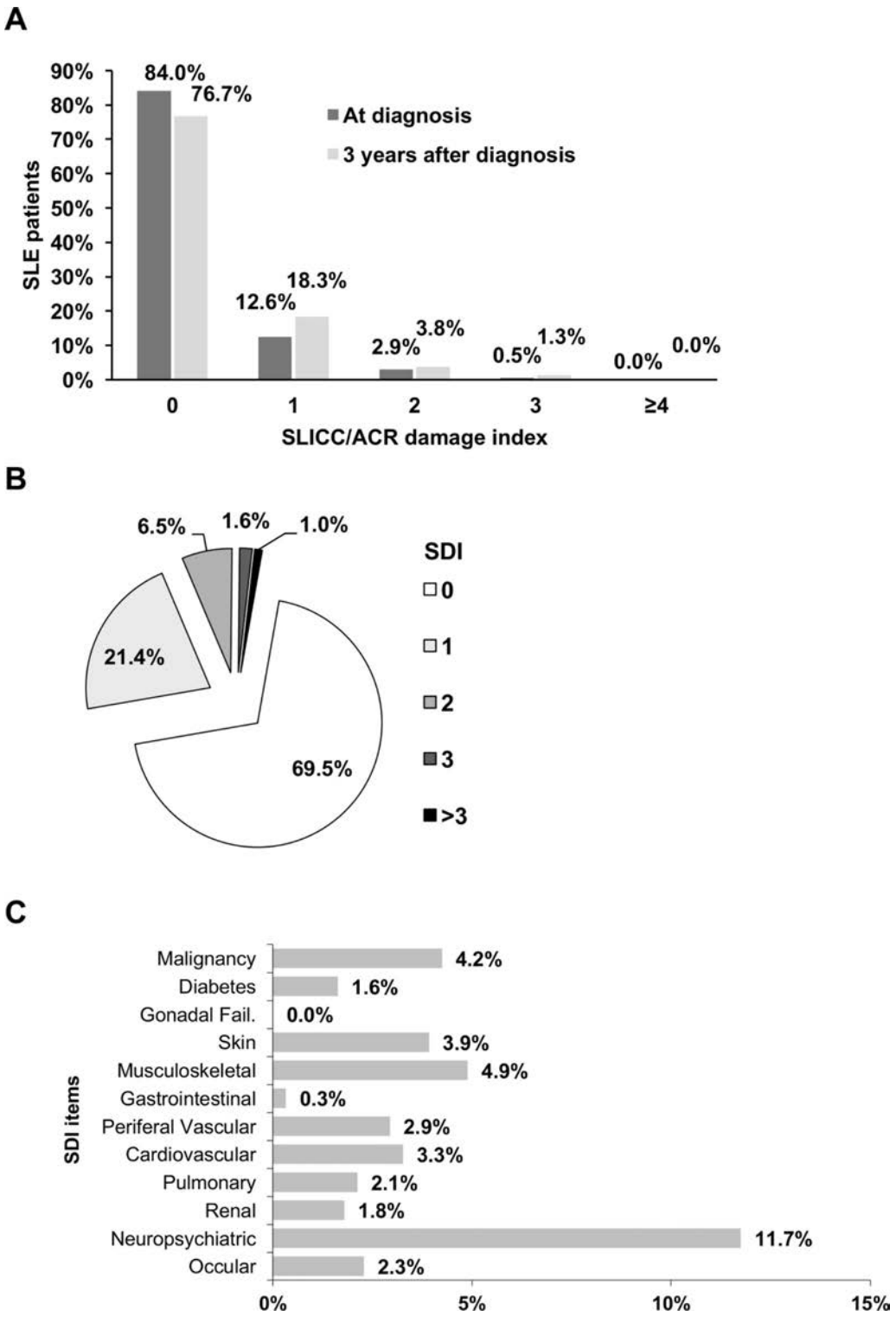

Figure 6 Non-reversible organ damage in patients with prevalent SLE. (A) Frequency of organ damage (assessed by the SDI) in prevalent SLE cases at the time of diagnosis and after 3 years. Results are from 613 patients with available data at both time points. No deaths occurred during this time period. (B) Frequency of organ damage (SDI) in prevalent SLE cases at last follow-up. (C) Individual damaged domains (SDI) in prevalent SLE at last follow-up. ACR, American College of Rheumatology; SDI, SLICC/ACR Damage Index; SLE, systemic lupus erythematosus; SLICC, Systemic Lupus International Collaborating Clinics.

diagnosis in about 20% of cases. At the end of 2013, fewer patients with SLE had been classified with the SLICC 2012 than with the ACR 1997 criteria. Preliminary data suggest that this is largely due to lack of inclusion of photosensitivity and of malar rash in the former, which agrees with a previous report.[40]

Although direct comparisons are difficult, one could argue SLE in our region has some characteristics suggestive of less severe disease as compared with North/Latin America or other parts of Europe.[41 42] This can be extrapolated by the lower prevalence of nephritis (13%), NPSLE (7.8%), anti-DNA

autoantibodies (23% by *Crithidia luciliae*), organ damage (30.5%) and the increased prevalence (50%) of mild disease forms. In accordance, ESRD rate was 4.4%, which is lower than elsewhere (typically 10%–15% after 5 years).[43–45] Alamanos *et al* also reported a milder SLE profile in northern Greece due to lower prevalence of nephritis (15% at diagnosis) and lower standardised mortality ratio.[22 46] These findings could be attributed to genetic/ethnicity factors, although differences in the methodology, particularly the fact that our study is representative of SLE at the community, may be important. In addition, a theoretical risk for survival bias could have influenced the prevalence of severe cases, but we do not consider this significant, in view of the small number of observed deaths and the stable ratio of mild/severe disease over the study period (3.80, 4.17 and 3.85 across the three consecutive 5-year periods; online supplementary table S3).

Our study has several strengths; it included multiple sources to ensure data completeness and reliability.[47 48] Demographics (including residence history) were determined from self-reports and not exclusively from administrative data.[4] Case validation was performed through chart review and in-person interviews,[4] further contributing to data reliability and integrity.[49] The use of alternative case definitions facilitated disease ascertainment. Although this is a referral centre study, we adopted a community-based method, which avoids selection biases.[50]

One of the study limitations is that capture–recapture methods were not used in the total SLE population. LN cases followed exclusively in nephrology departments may have received care outside our capture area hospitals and are more likely to have been missed. Accordingly, we used capture–recapture analysis in this particular group, showing no missing cases. Although our study was regional, Crete is inhabited by 6.5% of the total Greek population, and due to the homogeneity of the ethnicity/race and the infrequency of extreme socioeconomic differences our estimate might approach the national estimate.

In conclusion, our project offers robust, updated estimates of SLE occurrence and burden. Alike other studies, we document that SLE frequency may be higher than previously considered. These results confirm that SLE is not rare, affecting also older ages and being increasingly recognised in men. Our data corroborate previous findings on the increased burden of NPSLE among whites, which represents an unmet need. Despite milder phenotype in the community as compared with tertiary centres, a considerable proportion of patients develop severe disease requiring immunosuppressive therapy and accrue organ damage, emphasising the need for optimisation of early diagnosis and management.

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Contributors IG conducted the study, performed research and face interviews; AF, AR, CA and AP examined and interviewed patients; MT and IT provided data on lupus nephritis patients; GS established and maintained the SLE database; EK arranged patients visits and delivered questionnaires; LC supervised the methodology; AB performed the statistical analysis; PS examined patients and supervised the study; GKB and DTB conceived the study and supervised the study; GKB and IG drafted the manuscript.

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EXTENDED REPORT

Sirukumab for rheumatoid arthritis: the phase III SIRROUND-D study

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ABSTRACT

Objectives Interleukin-6 (IL-6) is implicated in rheumatoid arthritis (RA) pathophysiology. Unlike IL-6 receptor inhibitors, sirukumab is a human monoclonal antibody that selectively binds to the IL-6 cytokine. The phase III, multicentre, randomised, double-blind, placebo-controlled, parallel-group SIRROUND-D study (ClinicalTrials.gov identifier NCT01604343) evaluated the efficacy and safety of sirukumab in patients with active RA refractory to disease-modifying antirheumatic drugs.

Methods Patients were randomised 1:1:1 to treatment with sirukumab 100 mg every 2 weeks, 50 mg every 4 weeks or placebo every 2 weeks subcutaneously. Results through week 52 are reported.

Results Of 1670 randomised patients, significantly more patients achieved American College of Rheumatology 20% (ACR20) response at week 16 (coprimary endpoint) with sirukumab 100 mg every 2 weeks (53.5%) or 50 mg every 4 weeks (54.8%) versus placebo (26.4%; both p<0.001). Mean (SD) change from baseline in modified Sharp/van der Heijde score at week 52 (coprimary endpoint) was significantly lower with sirukumab (100 mg every 2 weeks: 0.46 (3.26); 50 mg every 4 weeks: 0.50 (2.96)) versus placebo (3.69 (9.25); both p<0.001). All major secondary endpoints (week 24 Health Assessment Questionnaire–Disability Index change from baseline, ACR50 response, 28-joint Disease Activity Score based on C reactive protein and major clinical response (ACR70 for six continuous months by week 52)) were met. The most common adverse events with sirukumab were elevated liver enzymes, upper respiratory tract infection, injection site erythema and nasopharyngitis.

Conclusions Sirukumab 100 mg every 2 weeks and 50 mg every 4 weeks led to significant reductions in RA symptoms, inhibition of structural damage progression and physical function and quality of life improvements, with an expected safety profile.

Trial registration number NCT01604343; Results.

INTRODUCTION

Patients with rheumatoid arthritis (RA) often have increased levels of interleukin (IL)-6 in serum and the synovial compartment where its levels are correlated to local disease activity.[1–3] In the RA synovium, both tumour necrosis factor (TNF) and IL-1 can stimulate IL-6 production by multiple cell types.[4] Local concentrations of IL-6 may stimulate leucocyte recruitment to the joint, promote osteoclast maturation and activation, suppress chondrocytes and stimulate synovial proliferation, summarily contributing to joint damage.[5] Systemically, elevated IL-6 levels in patients with RA may induce haepatic production of acute-phase proteins[6] and likely increase hepcidin and the development of anaemia of chronic inflammation.[7] Elevated IL-6 may also be responsible for autoimmune features in RA, such as autoreactive T cell activation and hypergammaglobulinaemia.[8] Therefore, IL-6 is an attractive target for the treatment of RA.

In patients with active RA and inadequate response to disease-modifying antirheumatic drug (DMARD) therapy, inhibition of the IL-6 receptor with the monoclonal antibody (mAb) tocilizumab reduced joint swelling and tenderness, improved physical function and reduced the rate of radiographic progression.[9–12] Another anti–IL-6 receptor mAb, sarilumab, demonstrated similar efficacy in patients with RA and inadequate response to methotrexate (MTX).[13] Although the clinical relevance of a different mechanism of targeting the IL-6 pathway is not fully understood, sirukumab is a human mAb that selectively binds to the IL-6 cytokine with high affinity. Sirukumab was shown to significantly improve signs and symptoms (eg, American College of Rheumatology 20% (ACR20) response at week 16), functionality and quality of life versus placebo in a difficult-to-treat population of RA patients refractory to anti-TNF and other biologicals.[14] Two other antibodies to IL-6, clazakizumab and olokizumab, have demonstrated activity in phase II studies of RA patients with an inadequate response to MTX or failure to anti-TNF therapy, respectively.[15 16] The SIRROUND-D study (ClinicalTrials.gov identifier NCT01604343) was designed to assess efficacy and safety of subcutaneous (SC) sirukumab in patients with active RA despite DMARD therapy over 52 weeks.

METHODS

Patients

Patients from 18 countries (USA, Canada, Mexico, Colombia, Chile, South Africa, Lithuania, Poland, Russia, Ukraine, Serbia, Croatia, Bulgaria, Romania, Japan, South Korea, Taiwan and Malaysia) were enrolled and monitored between July 2012 and September 2015. Eligible patients were aged ≥18 years, had moderately to severely active RA and were refractory to single-agent or combination DMARD therapy including MTX or sulfasalazine, based on lack of benefit after ≥12 weeks. Patients needed ≥6/68 tender joints and ≥6/66 swollen joints at screening and baseline; C reactive protein (CRP) ≥8.0 mg/L; and ≥1 of the following

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three criteria to be met prior to treatment: (A) anticitrullinated peptide antibody-positive (measured by anticyclic citrullinated peptide antibody test) at screening; (B) rheumatoid factor positive at screening; or (C) documented history of radiographic evidence of erosive RA in the hands and/or feet. Patients using non-biological DMARDs must have been on a stable dose for ≥4 weeks prior to receiving study drug. Patients not currently using DMARDs must not have received DMARDs for ≥4 weeks prior to receiving study drug. Patients who previously were treated with biologicals were permitted, as long as they had not failed anti-TNF or tocilizumab for safety or efficacy reasons and had not received biologicals within the past 3 months (6 weeks for etanercept or yisaipu and 4 weeks for anakinra). Patients with a history of or current serious infection (including tuberculosis) were excluded.

Study design

This global, phase III, multicentre, randomised, double-blind, placebo-controlled, parallel-group study randomised patients 1:1:1 at week 0 to sirukumab 100 mg SC every 2 weeks, sirukumab 50 mg SC every 4 weeks or placebo SC every 2 weeks (see online supplementary figure S1). These doses were previously studied in a phase IIb dose-ranging study.[17] Patients were stratified by baseline MTX use (none, up to 12.5 mg/week or ≥12.5 mg/week). Patients on placebo demonstrating <20% improvement from baseline in both swollen and tender joint counts at week 18 (early escape (EE)) or week 40 (late escape (LE)), or still on study treatment at week 52 (crossover), were rerandomised 1:1 to receive blinded treatment with one of the two sirukumab doses through week 104. A 16-week safety follow-up phase occurred after the final dose, making the total study duration 120 weeks excluding the screening period of up to 6 weeks. The study protocol was approved by the relevant institutional review boards or ethics committees, and all patients gave written informed consent. Data were collected by the investigators and analysed by the study sponsor.

Assessments

All analyses were prespecified unless otherwise noted; all randomised patients were included in population summaries and efficacy analyses, and all patients treated with ≥1 dose of study agent were included in safety analyses. The coprimary efficacy endpoints were proportion of patients who achieved an ACR20 response at week 16 and change from baseline in modified Sharp/van der Heijde score (SHS) at week 52. Radiographs of the hands and feet were taken at baseline, week 18 (for patients meeting EE criteria), week 24 (for patients who did not meet EE criteria) and week 52. Major secondary endpoints included change from baseline in Health Assessment Questionnaire–Disability Index (HAQ-DI) score at week 24, proportion of patients achieving an ACR50 response at week 24, proportion of patients with the 28-joint Disease Activity Score based on CRP (DAS28 (CRP)) <2.6 at week 24 and proportion of patients achieving major clinical response (defined as ACR70 response for six continuous months) by week 52. Additional endpoints included physical component summary (PCS) and mental component summary (MCS) of the patient-reported 36-item Short Form survey (SF-36) and proportions of patients achieving clinical disease activity index (CDAI) low disease activity (≤10.0) at week 24 and CDAI remission (≤2.8) at weeks 24 and 52 (analyses of CDAI low disease activity and remission were post hoc). Efficacy endpoints were also assessed over

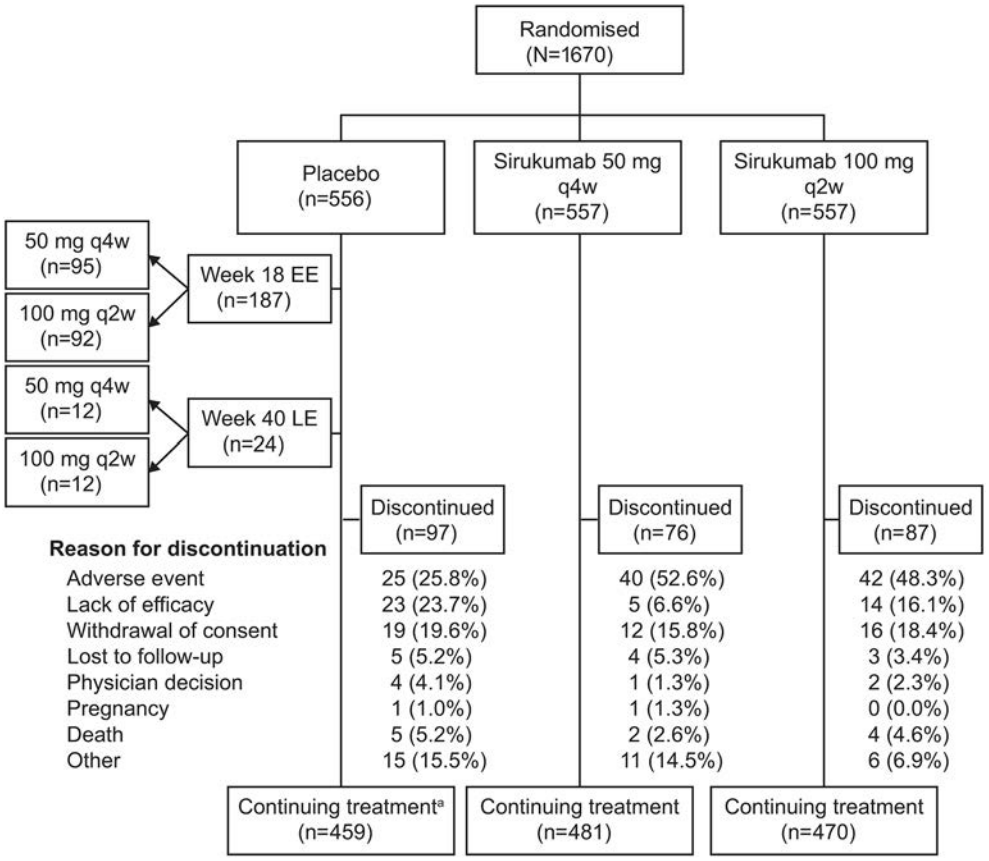

Figure 1 Patient disposition through week 52. EE, early escape; LE, late escape; q2w, every 2 weeks; q4w, every 4 weeks. [a]Includes placebo patients who escaped (EE/LE) to sirukumab 50 mg q4w or 100 mg q2w.

Table 1 Demographic and baseline characteristics

Characteristic	Placebo (n=556)	Sirukumab 50 mg q4w (n=557)	Sirukumab 100 mg q2w (n=557)	Total (n=1670)
Female sex, n (%)	436 (78.4)	447 (80.3)	452 (81.1)	1335 (79.9)
Age, years	52.9 (11.9)	52.9 (11.8)	53.0 (11.3)	52.9 (11.7)
Race, n (%)				
White	403 (72.5)	397 (71.3)	408 (73.2)	1208 (72.3)
Asian	88 (15.8)	89 (16.0)	95 (17.1)	272 (16.3)
Black or African-American	16 (2.9)	15 (2.7)	10 (1.8)	41 (2.5)
American Indian or Alaska Native	6 (1.1)	4 (0.7)	4 (0.7)	14 (0.8)
Other*	39 (7.0)	49 (8.8)	38 (6.8)	126 (7.5)
Not reported/unknown	4 (0.7)	3 (0.5)	2 (0.4)	9 (0.5)
Region, n (%)				
Eastern Europe	271 (48.7)	263 (47.2)	273 (49.0)	807 (48.3)
North America	85 (15.3)	96 (17.2)	91 (16.3)	272 (16.3)
Asia-Pacific	87 (15.6)	89 (16.0)	91 (16.3)	267 (16.0)
Latin America	73 (13.1)	75 (13.5)	76 (13.6)	224 (13.4)
South Africa	40 (7.2)	34 (6.1)	26 (4.7)	100 (6.0)
Weight, kg	72.7 (17.4)	72.3 (18.6)	71.6 (17.1)	72.2 (17.7)
Disease duration, years	8.3 (7.0)	8.7 (7.5)	8.8 (7.6)	8.6 (7.4)
BMI, kg/m^2	27.4 (6.0)	27.3 (6.4)	27.2 (6.0)	27.3 (6.2)
CRP, mg/L	25 (34)	24 (26)	24 (26)	24 (29)
RF positive, n (%)	444 (79.9)	433 (77.7)	468 (84.0)†	1345 (80.5)
Anti-CCP positive, n (%)	467 (84.0)	476 (85.5)	484 (86.9)	1427 (85.4)
SHS	41.9 (46.7)	41.8 (45.4)	42.5 (49.3)	42.1 (47.1)
HAQ-DI score, range: 0–3	1.6 (0.7)	1.5 (0.6)	1.5 (0.7)	1.5 (0.6)
DAS28 (CRP)	5.9 (0.9)	5.9 (0.9)	5.8 (0.9)‡	5.9 (0.9)
Prior medication use§				
1 DMARD	183 (32.9)	179 (32.1)	173 (31.1)	535 (32.0)
≥2 DMARDs	373 (67.1)	378 (67.9)	384 (68.9)	1135 (68.0)
MTX	547 (98.4)	550 (98.7)	548 (98.4)	1645 (98.5)
Sulfasalazine	174 (31.4)	167 (30.0)	152 (27.3)	493 (29.5)
Systemic corticosteroids	422 (75.9)	407 (73.1)	418 (75.0)	1247 (74.7)
Baseline medication use, n (%)				
DMARDs	508 (91.4)	517 (92.8)	511 (91.7)	1536 (92.0)
NSAIDs	434 (78.1)	420 (75.4)	454 (81.5)¶	1308 (78.3)
Corticosteroids	341 (61.3)	331 (59.4)	360 (64.6)	1032 (61.8)

Values are mean (SD) unless otherwise indicated.
Differences in demographic and baseline characteristics among groups were not significant, except where noted.
*No Native Hawaiian or other Pacific Islanders were reported in any treatment group.
†p=0.01 versus sirukumab 50 mg q4w based on χ^2 test.
‡p=0.02 versus placebo based on t-test.
§All randomised patients took ≥1 DMARD.
¶p=0.01 versus sirukumab 50 mg q4w based on χ^2 test.
q2w, every 2 weeks; q4w, every 4 weeks; BMI, body mass index; CCP, cyclic citrullinated peptide; CRP, C reactive protein; DAS28 (CRP), 28-joint Disease Activity Score based on C reactive protein; DMARD, disease-modifying antirheumatic drug; HAQ-DI, Health Assessment Questionnaire–Disability Index; MTX, methotrexate; NSAID, nonsteroidal anti-inflammatory drug; RF, rheumatoid factor; SHS, Sharp/van der Heijde score.

time. Safety assessments included monitoring of adverse events (AEs), standard clinical laboratory tests, vital signs evaluations and physical examinations. Serum samples were analysed for antibodies to sirukumab using a validated drug-tolerant electrochemiluminescent immunoassay method on the Meso Scale Discovery platform.

Statistical methods

A sample size of 550 patients per treatment group would provide approximately 98%–99% power to detect a treatment difference of 11%–17% in the proportion of patients who achieved ACR20 response at week 16 and approximately 98% power to detect a treatment difference of 1.0 in the mean change from baseline SHS at week 52.

The coprimary efficacy endpoints were tested in the following predefined order: (1) sirukumab 100 mg every 2 weeks versus placebo in week 16 ACR20 response; (2) sirukumab 100 mg every 2 weeks versus placebo in week 52 SHS change from baseline; (3) sirukumab 50 mg every 4 weeks versus placebo in week 16 ACR20 response; (4) sirukumab 50 mg every 4 weeks versus placebo in week 52 SHS change from baseline. If a given comparison was not significant at α=0.05 (two sided), the remaining treatment group comparisons were to be considered as supportive analyses. For week 16 ACR20, Cochran-Mantel-Haenszel tests

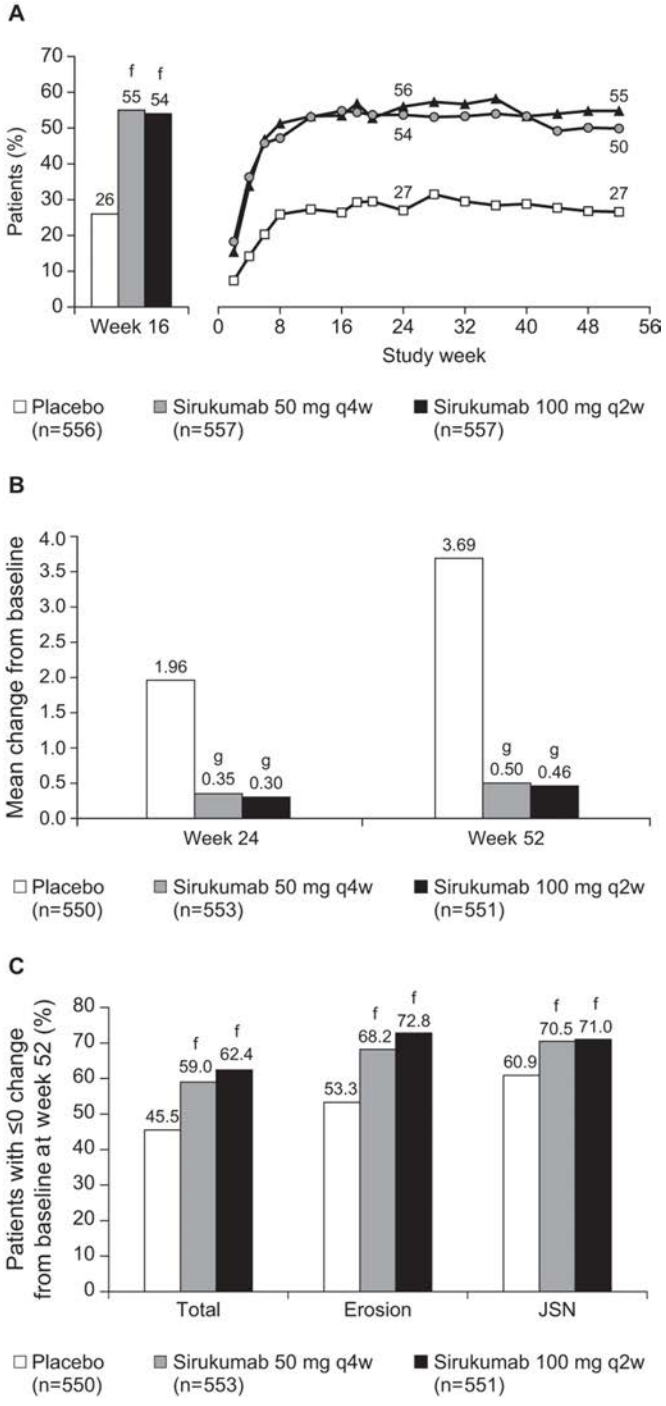

Figure 2 (A) Proportions of patients with an ACR20 response at week 16[a] and ACR20 response over time.[b,c,d] (B) Change from baseline in SHS results at weeks 24 and 52.[e] (C) Proportions of patients with no radiographic progression from baseline to week 52.[e] EE, early escape; JSN, joint space narrowing; LE, late escape; NR, non-responder; q2w, every 2 weeks; q4w, every 4 weeks; SHS, Sharp/van der Heijde score; TF, treatment failure. [a]Based on imputed values by missing data (NR)/TF(NR). [b]Based on imputed values by missing data (NR)/TF(NR)/EE(NR)/LE(NR). [c]p<0.001 for both doses of sirukumab versus placebo across all timepoints based on Cochran-Mantel-Haenszel test. [d]Not significant for sirukumab 50 mg q4w versus sirukumab 100 mg q2w across all timepoints based on Cochran-Mantel-Haenszel test. [e]Based on imputed values by EE rules and then missing data rules. [f]p<0.001 versus placebo based on Cochran-Mantel-Haenszel test. [g]p<0.001 versus placebo based on van der Waerden analysis of variance.

stratified by baseline MTX use (none, up to 12.5 or ≥12.5 mg/week) were used for treatment comparisons. The last observation carried forward method was used for imputing missing ACR components if a patient had data for ≥1 ACR component at week 16. Patients were considered ACR20 non-responders if they did not have data for any ACR component at week 16 or if they met any of the following treatment failure criteria prior to week 16: initiated treatment with DMARDs, systemic immunosuppressives or biologicals for RA; increased their dose of MTX; initiated or increased oral corticosteroid treatment or received intravenous or intramuscular corticosteroids for RA; or discontinued study agent. For week 52 SHS change from baseline analyses, analysis of variance tests stratified by baseline MTX use on the van der Waerden normal scores were used for treatment comparisons. Missing SHS values at week 52 were imputed by a linear extrapolation of non-missing values before week 52. For patients who met EE criteria in the placebo treatment group, SHS value at week 52 was replaced by the imputed value from a linear extrapolation of non-missing values prior to escape.

RESULTS

Patients

Overall, 1670 patients across 185 sites were randomised, administered ≥1 dose of study agent and included in efficacy (non-radiographic) and safety analyses (figure 1). Radiographic efficacy analyses included 1654 patients with non-missing baseline SHS. Demographic and baseline characteristics were generally well-balanced across all treatment groups (table 1). Of note, 583 (34.9%) of the enrolled patients had previously received ≥1 biological therapy (see online supplementary table S1).

Efficacy

Both coprimary endpoints were met. The proportion of patients with ACR20 responses was significantly greater for both sirukumab doses compared with placebo at week 16 (both p<0.001; figure 2A). Differences in proportions of patients achieving ACR20 were observed as early as week 2 and sustained through week 52 (both doses p<0.001 vs placebo at weeks 24 and 52). ACR20 response rates in both sirukumab groups were higher compared with placebo, regardless of baseline MTX use (see online supplementary table S2). A summary of percent improvement in ACR components at week 16 is provided (see online supplementary table S3). Significant inhibition of radiographic progression (SHS mean change from baseline) was achieved at week 52 (coprimary endpoint), with differences observed as early as week 24 for sirukumab versus placebo (both doses p<0.001 vs placebo at both timepoints; figure 2B). Significantly higher proportions of patients treated with sirukumab did not show radiographic progression (score change of ≤0 from baseline in SHS total, erosion and joint space narrowing scores) compared with placebo at week 52 (both doses p<0.001 vs placebo; figure 2C). Smaller week 52 SHS mean changes from baseline were observed in both sirukumab groups compared with placebo, regardless of baseline MTX use. The probability plot of the SHS change from baseline at week 52 clearly shows separation between both sirukumab groups and the placebo group and no separation between the sirukumab groups (see online supplementary figure S2).

All major secondary efficacy endpoints demonstrated significant improvements for both sirukumab doses versus placebo (all p≤0.001; table 2). In addition, more patients on sirukumab achieved ACR70 as early as week 8, with treatment differences maintained through week 52 (both doses p<0.001 vs placebo at

Table 2 Results of major and other key secondary endpoints

Endpoint	Placebo (n=556)	Sirukumab 50 mg q4w (n=557)	Sirukumab 100 mg q2w (n=557)
HAQ-DI change from baseline at week 24, mean (SD)*	−0.22 (0.53)	−0.43 (0.58)†	−0.46 (0.57)†
ACR50 at week 16, n (%)‡	60 (10.8)	167 (30.0)§	146 (26.2)§
ACR50 at week 24, n (%)¶	69 (12.4)	168 (30.2)§	185 (33.2)§
ACR50 at week 52, n (%)**	77 (13.8)	169 (30.3)§	198 (35.5)§
ACR70 at week 16, n (%)‡	22 (4.0%)	75 (13.5%)§	75 (13.5%)§
ACR70 at week 24, n (%)¶	19 (3.4%)	83 (14.9%)§	91 (16.3%)§
ACR70 at week 52, n (%)**	30 (5.4%)	92 (16.5%)§	103 (18.5%)§
DAS28 (CRP) <2.6 at week 24, n (%)¶	31 (5.6)	145 (26.0)§	142 (25.5)§
Major clinical response by week 52, n (%)**	10 (1.8)	30 (5.4)§	50 (9.0)§
SF-36 PCS change from baseline at week 52, mean (SD)††	2.42 (6.81)	5.66 (7.74)‡‡	6.16 (7.23)‡‡
SF-36 MCS change from baseline at week 52, mean (SD)††	2.69 (9.57)	5.35 (9.64)‡‡	4.77 (9.80)‡‡

*Based on imputed values by missing data (LOCF)/EE(LOCF).
†p≤0.001 versus placebo based on analysis of covariance.
‡Based on imputed values by missing data (NR)/TF(NR).
§p≤0.01 versus placebo based on Cochran-Mantel-Haenszel test.
¶Based on imputed values by missing data (NR)/TF(NR)/EE(NR).
**Based on imputed values by missing data (NR)/TF(NR)/EE(NR)/LE(NR).
††Based on imputed values by missing data (LOCF)/EE(LOCF)/LE(LOCF).
‡‡p≤0.001 versus placebo based on analysis of variance.
ACR50/70, American College of Rheumatology 50%/70%; DAS28 (CRP), 28-joint Disease Activity Score based on C reactive protein; EE, early escape; HAQ-DI, Health Assessment Questionnaire–Disability Index; LE, late escape; LOCF, last observation carried forward; MCS, mental component summary; NR, non-responder; PCS, physical component summary; q2w, every 2 weeks; q4w, every 4 weeks; SF-36, Short Form-36; TF, treatment failure.

weeks 16, 24 and 52; table 2). ACR90 responses were achieved by a significantly greater proportion of patients on both sirukumab doses versus placebo at weeks 16, 24 and 52 (all p<0.05; not shown). The proportions of patients achieving CDAI low disease activity (≤10.0) for sirukumab 100 mg every 2 weeks and 50 mg every 4 weeks at week 24 were 30.2% and 29.4%, respectively, compared with 15.5% for placebo (both p<0.001 vs placebo); at week 52, proportions were 32.0% and 32.5%, respectively, compared with 15.3% for placebo (both p<0.001 vs placebo). The proportions of patients in CDAI remission (≤2.8) at week 24 for sirukumab 100 mg every 2 weeks and 50 mg every 4 weeks were 8.4% and 7.0%, respectively, compared with 3.1% for placebo (both p≤0.003 vs placebo). At week 52, the proportions of patients in CDAI remission for sirukumab 100 mg every 2 weeks and 50 mg every 4 weeks were 8.3% and 10.1%, respectively, compared with 3.8% for placebo (both p≤0.002 vs placebo).

Significantly greater improvements from baseline in health-related physical and emotional well-being were observed with sirukumab on the patient-reported SF-36 PCS and MCS scores at week 52 (p<0.001 for PCS and MCS, both sirukumab doses vs placebo; table 2). At weeks 24 and 52, greater improvements in all eight individual SF-36 domain scores were achieved with sirukumab compared with placebo (all p≤0.006), and significantly more sirukumab-treated patients achieved clinically meaningful improvements (≥5-point increase) from baseline in PCS and MCS scores compared with placebo (all p≤0.009).

Safety

Safety results were summarised in the 'pure' placebo-controlled period prior to EE (to week 18; see online supplementary table S4) and at the end of the placebo-controlled period (to week 52) for all AEs (table 3) and for specific AEs of interest (see online supplementary table S5). Through week 52, no disproportional increase from week 18 was observed in AE rates, and the overall AE profile was similar to that observed through week 18. The most common AEs (≥5%) through week 52 with sirukumab were elevated liver enzymes, upper respiratory tract infection, bronchitis, nasopharyngitis, injection site erythema and pruritus, leucopaenia, neutropaenia, headache, and hypertension (table 3). No dose relationship was apparent between sirukumab doses and the types or frequency of AEs other than injection site reactions (ISRs) and elevated liver enzymes, which were more frequent with sirukumab 100 mg every 2 weeks than 50 mg every 4 weeks. No ISRs were considered severe in intensity, and four patients (two in each sirukumab dose group) discontinued the study due to mild or moderate ISRs.

Through week 18, serious AEs (SAEs) were reported in 4.7%, 2.9% and 3.1% of patients in the sirukumab 100 mg every 2 weeks, 50 mg every 4 weeks and placebo groups, respectively; through week 52, SAEs were reported in 9.8%, 11.0% and 6.8% of patients, respectively. Serious infections were reported in 0.9%, 0.7% and 0.9% of patients, respectively, through week 18, and were numerically greater in the sirukumab 100 mg every 2 weeks combined (3.3%) and 50 mg every 4 weeks combined (4.1%) groups (including EE patients) compared with the placebo group (1.8%) through week 52. Two gastrointestinal (GI) perforations were reported: one upper GI (gastric) perforation in the placebo group and one lower GI perforation (perforated appendicitis) in a patient randomised to placebo with EE to sirukumab 50 mg every 4 weeks. During the 18-week period prior to EE, mortality rates were the same across the treatment groups, with one death each in the three groups. Through 52 weeks, there was a numerical imbalance in exposure-adjusted mortality rates (supplementary table S5); however, the interpretation of these results is confounded by the loss of randomisation as patients in the placebo group switched to sirukumab at EE and LE timepoints.

Laboratory abnormalities were similar for both sirukumab doses and were numerically higher than placebo; the incidence of grade 3/4 laboratory abnormalities with sirukumab was low for decreased platelets (0.2% grade 3; 0% grade 4), decreased

Table 3 Summary of overall safety through week 52

Variable	Placebo (n=556)	Sirukumab*† 50 mg q4w (n=663)	Sirukumab*† 100 mg q2w (n=662)	Sirukumab*† Combined (n=1325)
Mean duration of follow-up, weeks	36.18	45.76	45.12	45.44
Mean number of study agent administrations	17.45	21.88	21.56	21.72
Patients with ≥1 AE, n (%)	364 (65.5)	528 (79.6)	531 (80.2)	1059 (79.9)
p Value versus placebo		<0.001	<0.001	<0.001
Patients with ≥1 SAE, n (%)	38 (6.8)	73 (11.0)	65 (9.8)	138 (10.4)
p Value versus placebo		0.012	NS	0.015
Patients with ≥1 AE that caused study agent discontinuation, n (%)	18 (3.2)	53 (8.0)	51 (7.7)	104 (7.8)
p Value versus placebo		<0.001	<0.001	<0.001
Patients with ≥1 serious infection, n (%)	10 (1.8)	27 (4.1)	22 (3.3)	49 (3.7)
p Value versus placebo		0.021	NS	0.031
Patients with ≥1 injection site reaction, n (%)	14 (2.5)	71 (10.7)	108 (16.3)	179 (13.5)
p Value versus placebo		<0.001	<0.001	<0.001
Patients with ≥1 MACE, n (%)	2 (0.4)	8 (1.2)	3 (0.5)	11 (0.8)
p Value versus placebo‡		NS	NS	NS
Patients with ≥1 malignancy, n (%)	2 (0.4)	2 (0.3)	5 (0.8)	7 (0.5)
p Value versus placebo‡		NS	NS	NS
Patients with ≥1 GI perforation, n (%)	1 (0.2)	1 (0.2)	0	1 (0.1)
p Value versus placebo‡		NS	NS	NS
Death, n (%)	1 (0.2)	7 (1.1)	3 (0.5)	10 (0.8)
p Value versus placebo‡		NS	NS	NS
Events of ≥5% frequency in any sirukumab group, n (%)				
Alanine aminotransferase increased	25 (4.5)	102 (15.4)	124 (18.7)	226 (17.1)
p Value versus placebo		<0.001	<0.001	<0.001
Aspartate aminotransferase increased	19 (3.4)	58 (8.7)	82 (12.4)	140 (10.6)
p Value versus placebo		<0.001	<0.001	<0.001
Upper respiratory tract infection	63 (11.3)	65 (9.8)	66 (10.0)	131 (9.9)
p Value versus placebo		NS	NS	NS
Injection site erythema	6 (1.1)	50 (7.5)	80 (12.1)	130 (9.8)
p Value versus placebo		<0.001	<0.001	<0.001
Nasopharyngitis	57 (10.3)	62 (9.4)	56 (8.5)	118 (8.9)
p Value versus placebo		NS	NS	NS
Leucopaenia	7 (1.3)	37 (5.6)	37 (5.6)	74 (5.6)
p Value versus placebo		<0.001	<0.001	<0.001
Bronchitis	27 (4.9)	39 (5.9)	31 (4.7)	70 (5.3)
p Value versus placebo		NS	NS	NS
Neutropaenia	5 (0.9)	38 (5.7)	29 (4.4)	67 (5.1)
p Value versus placebo		<0.001	<0.001	<0.001
Hypertension	21 (3.8)	28 (4.2)	33 (5.0)	61 (4.6)
p Value versus placebo		NS	NS	NS
Headache	22 (4.0)	33 (5.0)	26 (3.9)	59 (4.5)
p Value versus placebo		NS	NS	NS
Injection site pruritus	1 (0.2)	11 (1.7)	41 (6.2)	52 (3.9)
p Value versus placebo		0.009	<0.001	<0.001

*Includes patients from the placebo group rerandomised to treatment with sirukumab; thus, patients may be represented in >1 treatment group.
†p Values are nominal and from χ^2 tests, unless otherwise noted.
‡p Values are nominal and from Fisher's exact tests.
AE, adverse event; GI, gastrointestinal; MACE, major adverse cardiovascular event; NS, not significant; q2w, every 2 weeks; q4w, every 4 weeks; SAE, serious adverse event.

neutrophils (4.1% grade 3; 0.2% grade 4), increased alanine aminotransferase (ALT; 3.2% grade 3, 0% grade 4) and increased aspartate aminotransferase (AST; 0.7% grade 3; 0% grade 4). Decreased neutrophil and platelet counts and increased haemoglobin, ALT and AST began at week 2 of sirukumab treatment and were sustained through week 52 (see online supplementary figure S3). Total and low-density lipoprotein cholesterol increased with both doses of sirukumab relative to placebo; however, the total cholesterol:high-density lipoprotein ratio remained below 4.0 for all treatment groups at week 52.

The overall incidence of antibodies to sirukumab through week 52 was 2.4% (16/654), occurring in 1.2% (4/328) of patients receiving sirukumab 100 mg every 2 weeks and 3.7% (12/326) of patients receiving sirukumab 50 mg every 4 weeks. Only one of these 16 patients (in the 50 mg every 4 weeks group) was positive for neutralising antibodies to sirukumab. In patients who were positive for antidrug antibodies, there was no apparent relationship between antibodies to sirukumab and clinical response or ISRs.

DISCUSSION

This phase III, double-blind, randomised clinical trial evaluated the safety and efficacy of sirukumab, an IL-6 cytokine antibody, administered as 100 mg every 2 weeks or 50 mg every 4 weeks to patients with moderate-to-severe active RA refractory to

conventional DMARDs, including MTX. Approximately one-third of enrolled patients in this large, global study were previously treated with biological DMARD therapy (noting that these patients could not have failed for safety or efficacy reasons) and over two-thirds had prior treatment with ≥2 conventional DMARDs.

All clinical efficacy endpoints demonstrated that sirukumab was effective at reducing signs and symptoms of active RA in a robust and rapid manner through 52 weeks. Improvements occurred as early as 2 weeks in patients treated with sirukumab who demonstrated an ACR20 response; responses plateaued at week 12 and were maintained through week 52. The clinical findings were supported by robust effects on structural damage inhibition at week 52. Significant inhibition of radiographic progression was observed with sirukumab as early as week 24 (the first timepoint assessed), and a significantly greater proportion of patients treated with sirukumab showed no progression compared with placebo at week 52. Positive clinical and radiographic effects were consistently associated with significant patient-reported improvements in physical and emotional health and functional status. In a phase IIb trial[17] and in the current phase III study, clinical efficacy was largely similar between the 100 mg every 2 weeks and 50 mg every 4 weeks sirukumab doses, suggesting that the two doses do not differ in their effectiveness.

The safety profile of sirukumab did not raise any new concerns and was consistent with those reported for agents targeting the IL-6 receptor, such as tocilizumab[18 19] and sarilumab.[20] The proportions of patients experiencing AEs and SAEs were relatively similar between treatment groups, and the types of AEs and SAEs were similar through the 52-week study period. The most common AEs were elevated liver enzymes and injection site erythema, which was the only AE that was more frequent with sirukumab 100 mg every 2 weeks compared with 50 mg every 4 weeks. ISRs were all considered mild or moderate in severity and led to few discontinuations. No serious opportunistic infections were reported. Laboratory abnormalities included neutropaenia, thrombocytopaenia and increased levels of liver transaminases and lipids, all of which have been reported as class effects of anti–IL-6 therapies. There was no evidence of a dose response for sirukumab in these laboratory abnormalities, except for liver transaminases.

This study included a population of patients with RA who were refractory to DMARDs and who may or may not have received prior biological therapy. The results of this study are therefore not applicable to the full spectrum of patients with RA, but provide important information on the use of anti–IL-6 therapy as a possible first-line or alternate biological therapy in patients who are no longer responding to conventional DMARDs. Use of sirukumab in patients who cannot tolerate or are no longer responding to biological DMARDs was demonstrated in the SIRROUND-T study.[14] The current study design led to loss of randomisation after the 18-week pure placebo-controlled period and, therefore, longer total exposure in patient-years to sirukumab relative to placebo, which confounded interpretation of safety comparisons between sirukumab-treated and placebo-only patients beyond week 18. The safety of sirukumab continues to be assessed in the long-term extension study.

In conclusion, in patients with active RA refractory to DMARDs, sirukumab 100 mg every 2 weeks and 50 mg every 4 weeks led to significant reductions in signs and symptoms of RA, improvement of physical function, inhibition of structural damage progression and improvement of quality of life. Both sirukumab dose regimens were similarly efficacious, and sirukumab also demonstrated an acceptable safety profile.

Contributors TT, CT, GK, SS, WX, RR, BH and PPT contributed to the study design. TT and KF contributed to patient recruitment and treatment. TT, CT, GK, WX, RR, KF and BH contributed to the conduct of the study. SS and KF collected the data. CT, GK, SS, WX, RR, KF, BH and PPT analysed the data. All authors interpreted the data, contributed to drafting the work or revising it critically for important intellectual content, provided final approval of the version published and agreed to be accountable for all aspects of the work.

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EXTENDED REPORT

Survival in adults and children with systemic lupus erythematosus: a systematic review and Bayesian meta-analysis of studies from 1950 to 2016

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ABSTRACT

Objective To determine trends in survival among adult and paediatric patients with systemic lupus erythematosus (SLE) from 1950 to the present.

Methods We performed a systematic literature review to identify all published cohort studies on survival in patients with SLE. We used Bayesian methods to derive pooled survival estimates separately for adult and paediatric patients, as well as for studies from high-income countries and low/middle-income countries. We pooled contemporaneous studies to obtain trends in survival over time. We also examined trends in major causes of death.

Results We identified 125 studies of adult patients and 51 studies of paediatric patients. Among adults, survival improved gradually from the 1950s to the mid-1990s in both high-income and low/middle-income countries, after which survival plateaued. In 2008–2016, the 5-year, 10-year and 15-year pooled survival estimates in adults from high-income countries were 0.95, 0.89 and 0.82, and in low/middle-income countries were 0.92, 0.85 and 0.79, respectively. Among children, in 2008–2016, the 5-year and 10-year pooled survival estimates from high-income countries were 0.99 and 0.97, while in low/middle-income countries were 0.85 and 0.79, respectively. The proportion of deaths due to SLE decreased over time in studies of adults and among children from high-income countries.

Conclusions After a period of major improvement, survival in SLE has plateaued since the mid-1990s. In high-income countries, 5-year survival exceeds 0.95 in both adults and children. In low/middle-income countries, 5-year and 10-year survival was lower among children than adults.

INTRODUCTION

Survival of adults with systemic lupus erythematosus (SLE) is widely recognised to have improved between the 1950s and 1990s, with 5-year survival increasing from 50% to 60% to more than 95%.[1–5] However, it is not clear if survival has continued to improve, as only one study that reported trends in survival included data after 2000.[3] Some evidence suggests that the improvement in survival may have slowed between 1980 and 1990.[6] Studies that have reported improvement in survival have largely been from high-income countries.[2 3 7 8] It is unclear if similar improvements have occurred among patients in low/middle-income countries (LMIC), where socioeconomic barriers may limit access to care. Few data are available on 10-year and 15-year survival trends.

Similarly, 5-year survival in paediatric SLE has improved from 60% to 70% in the 1950s to more than 90% in the 1980s.[9–12] These estimates are based on isolated studies, and information on recent trends are lacking. Paediatric patients experience a longer disease course and extended exposure to disease and medication complications.[13] However, few studies have provided survival estimates beyond 5 years.[14–16]

We evaluated changes in survival from the 1950s to 2016 in adults and children with SLE in both high-income countries and LMIC based on a systematic literature review. We hypothesised that survival in SLE steadily improved over time, with greater improvement in studies from high-income countries. We also examined if the principal causes of death in patients with SLE have changed over time.

METHODS

Data sources and search strategy

We conducted a systematic review of the published literature on survival in adult and paediatric patients with SLE (omitting neonatal lupus). The study protocol was developed based on Preferred Reporting Items for Systematic Review and Meta-Analysis guidelines[17] and Meta-analysis of Observational Studies in Epidemiology recommendations.[18] We searched the PubMed, Embase and Scopus databases from their inceptions to 7 June 2016, without language restrictions. The search strategy and terms were developed in collaboration with a medical informationist (online supplementary appendix A). We also reviewed the references of these studies and review articles for additional publications. The study was exempted from human subjects review by the National Institutes of Health Office of Human Subjects Research Protection.

Study selection

Two investigators independently reviewed the titles and abstracts, and when necessary, full texts, to determine eligibility for inclusion. We included prospective or retrospective cohort studies of overall survival in adult or paediatric patients with SLE. Studies were considered as paediatric if the inclusion criteria specified an age of 0–17 years. We excluded: (1) animal studies; (2) case reports, case series, reviews, meta-analyses and abstracts;

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(3) studies on unrelated topics; (4) studies of selected SLE subsets (patients with specific clinical manifestations, hospital inpatients, elderly onset patients, adults with childhood-onset SLE and male only cohorts); (5) studies based on administrative data; (6) studies with incomplete data on number of deaths or follow-up; (7) adult studies with fewer than 20 patients and paediatric studies with fewer than 10 patients; and (8) studies of the same cohort as included articles.

Data extraction

Two investigators read the full text of adult (MGT and MMW) and paediatric studies (LL and MMW) and independently performed data extraction and quality assessment. Discrepancies were resolved by consensus. We collected data on the year and country of publication, study design, inception or prevalence cohort, sample size, years of patient enrolment, patient demographic characteristics, SLE duration at study entry, proportion with nephritis or central nervous system (CNS) involvement during disease course, follow-up duration and number of deaths. We extracted data on 5-year, 10-year, and 15-year survival when provided and separately identified studies with Kaplan-Meier plots. We extracted data on causes of death and classified these as due to either SLE, infection, cardiovascular disease, malignancy or other causes.

Quality assessment

We evaluated study quality using items adapted from the Newcastle-Ottawa scale (online supplementary appendix B), as used previously.[19 20] We considered studies of inception cohorts as high quality.

Data analysis

Overall survival was the outcome of interest. Survival data were reported as either Kaplan-Meier plots, as summary survival estimates from time-to-event analyses (but without a Kaplan-Meier plot), or as per cent mortality over the observation period. We treated each format differently to arrive at pooled survival

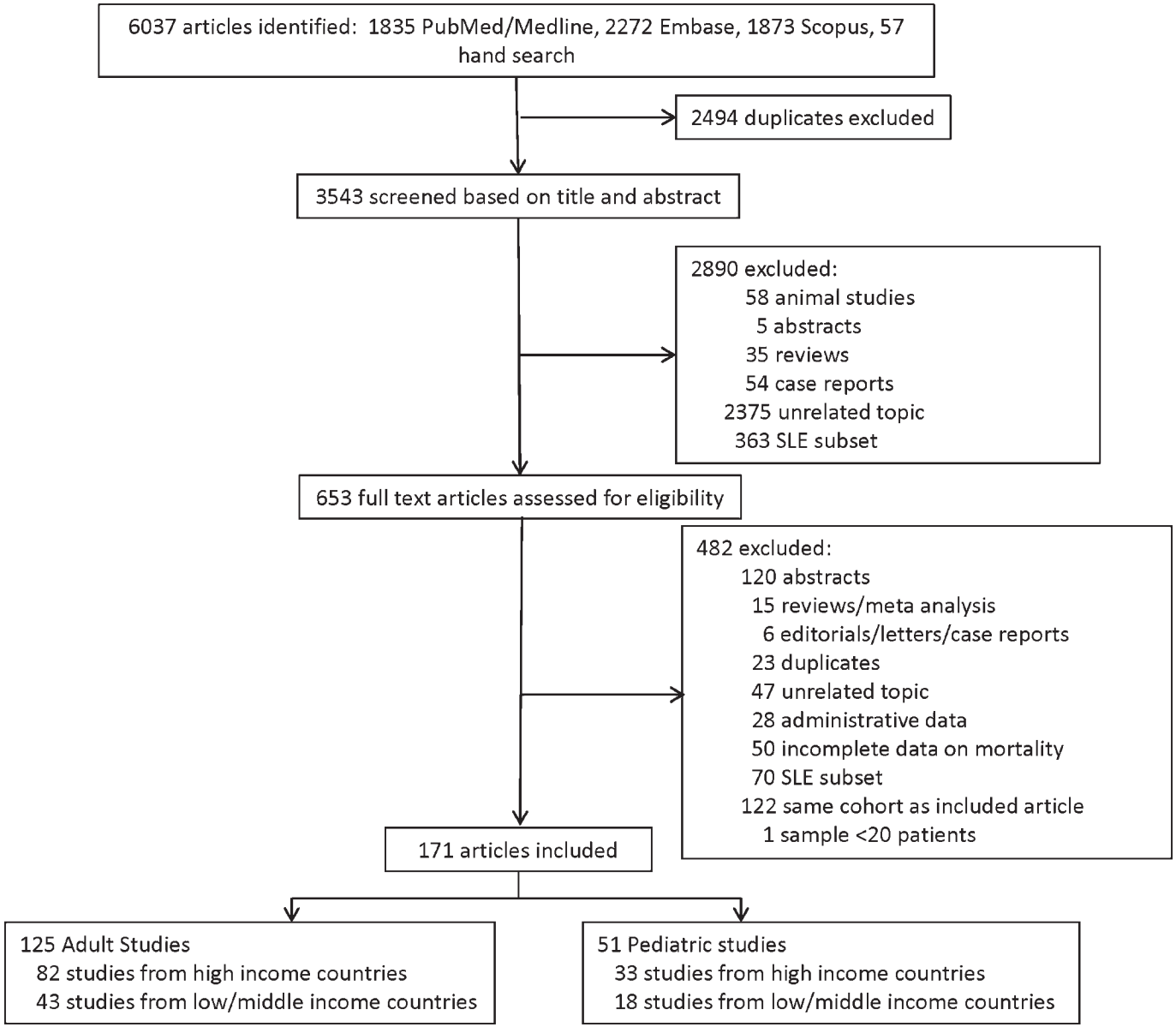

Figure 1 Flow diagram of literature search and study inclusion. Five adult studies also included data on a paediatric subset. SLE, systemic lupus erythematosus.

estimates.[19] For studies that reported Kaplan-Meier plots, we reconstructed individual patient data from the plots.[21] For studies that reported summary survival estimates, we used these to generate estimates of individual patient data. For studies that reported percent mortality, we used these percentages.

For each study, we modelled the time to death as a Weibull distribution. Each study contributed one survival estimate. We used Bayesian estimation with Markov chain Monte Carlo methods to obtain posterior distributions of the pooled Weibull estimates, which we used as the basis of 5-year, 10-year and 15-year survival estimates and corresponding 95% credible intervals. R V.3–12 package *rjags* was used for analysis.[22] For prevalence cohorts, we accounted for left truncation by adding the median duration of SLE at entry to the estimation of time to death. A full description of the methods is provided in online supplementary appendix C.

To examine trends over time, we divided calendar years from 1950 to 2016 into overlapping 5-year periods. We pooled studies that contributed data in a given five-calendar-year interval, starting from the calendar year of the midpoint of enrolment and ending with the end of follow-up. We sequentially repeated the analysis using the subset of studies represented in each 5-year calendar window. Different studies therefore entered and dropped out of the analysis across calendar years, akin to a moving average. We weighted studies by their sample size so that larger studies had greater influence on the pooled estimates.

We accounted for two main sources of heterogeneity, age and development status, by stratification. Within the adult and paediatric studies, we performed separate analyses for studies from high-income countries and LMIC, using World Bank criteria for the midpoint year of patient enrolment in each study.[23]

We performed a sensitivity analysis in which the contribution of individual studies was limited to 10 years from the midpoint of enrolment. This analysis limits the late influence of studies with long-term follow-up, when fewer patients might be under observation. This analysis may therefore be more sensitive to changes in survival over calendar years. Additionally, we performed a separate analysis of inception cohorts (ie, high-quality studies).

We examined trends in the prevalence of nephritis and CNS involvement over time using Spearman correlations, weighted by sample size. We also examined trends in causes of death across decades, based on the year of start of enrolment. We tested trends across decades in the proportion of deaths due to SLE using weighted linear regression (beta coefficient b for the indicator variable for decade). The analysis was weighted by number of deaths, so that larger studies had greater influence. Because studies of prevalence cohorts may miss deaths early in SLE, which may have different causes than late deaths, we repeated this analysis using only inception cohorts. We considered p values ≤ 0.05 as statistically significant. We used SAS program (V.9.3) for these analyses.

RESULTS

Study characteristics

We included 171 studies: 125 adult and 51 paediatric (five studies included stratified data on both adults and children) (figure 1 and online supplementary appendix D). Sixteen per cent were

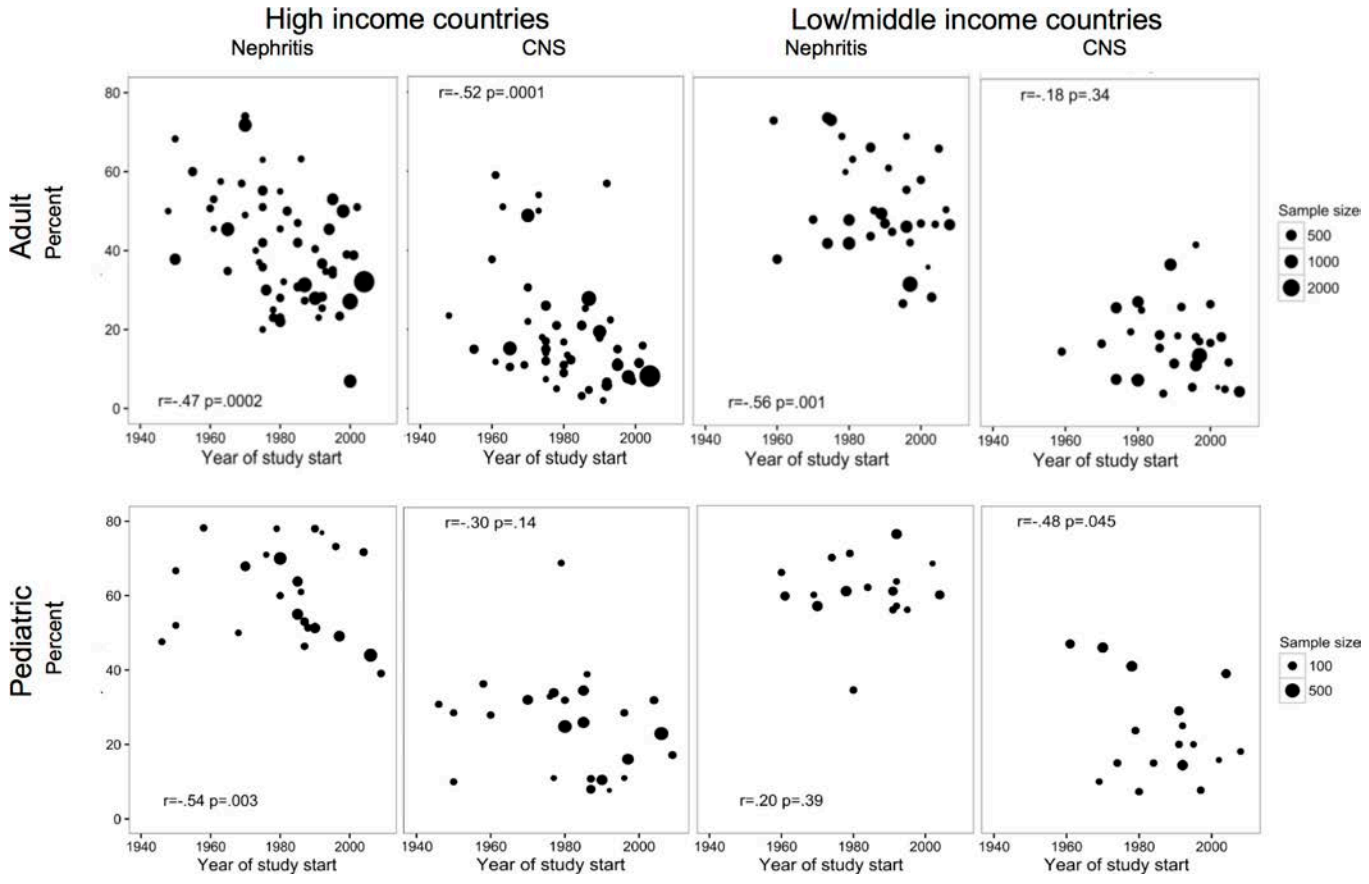

Figure 2 Prevalence of nephritis and central nervous system (CNS) involvement during the course of systemic lupus erythematosus in individual studies, by calendar year of start of enrolment. Symbol size is proportional to the study sample size.

prospective cohort studies and 84% were retrospective cohort studies.

Although our goal was to study adult and paediatric patients separately, age of inclusion was not clearly specified in all studies. Thirty-four of the 125 adult studies (27%) included only adult patients (age 18 years or older) or reported data separately for adults, while 32% did not report if they solely examined adults or also included children. We assumed most patients were adults based on the departments from which the studies originated. Forty-one per cent of the 125 studies reported that they also included children (range 1.3%–26%, median 9%) but did not provide age-stratified results. For ease of description, we termed these age-unrestricted studies as 'adult' studies, but it is important to recognise that some included paediatric patients. The adult studies comprised 46 317 patients and paediatric studies comprised 6862 patients.

The adult studies included from 21 to 3679 patients (90.2% women, mean age (at diagnosis or study entry, as reported in the primary studies) 33.8 years, mean follow-up 6.8 years). Eighty per cent of studies used American College of Rheumatology (ACR) classification criteria for the enrolment of patients, 25% examined inception cohorts, 22% examined community-based cohorts and 32% provided Kaplan-Meier plots. Only 39% of studies reported the proportion of patients lost to follow-up, which was less than 20% in 88% of these studies. The proportion of patients with nephritis decreased substantially over time in adult studies from both high-income countries and LMIC, as did the proportion of patients with CNS involvement in high-income countries, indicating a change in the nature of patients enrolled over time (figure 2).

Paediatric studies included between 13 and 1393 patients (82.3% girls, mean age at diagnosis 12.4 years, mean follow-up 5.3 years (range 1.7–13.1 years). Ninety-two per cent of studies used ACR classification criteria for the enrolment, 16% examined inception cohorts, 12% examined community-based cohorts and 51% provided Kaplan-Meier plots. Only 31% of paediatric studies reported on losses to follow-up. The proportion of patients with nephritis decreased over time in studies from high-income countries, as did the proportion with CNS involvement in studies from LMIC (figure 2).

Survival in adult studies

Eighty-two studies were from high-income countries and 43 were from LMIC (online supplementary figure 1). Among studies from high-income countries, there was a progressive increase in survival from the mid-1950s to 1990, after which survival estimates were stable (figure 3). In 2008–2016, the 5-year, 10-year and 15-year survival estimates in high-income countries were 0.95 (95% credible interval 0.94 to 0.96), 0.89 (0.88 to 0.90) and 0.82 (0.81 to 0.83), respectively.

Data from LMIC did not extend prior to 1970, but subsequent trends in survival were similar to those in high-income countries (figure 3 and online supplementary figure 2). In 2008–2016, the 5-year, 10-year and 15-year survival estimates in LMIC were 0.92 (0.91 to 0.93), 0.85 (0.84 to 0.87) and 0.79 (0.78 to 0.81), respectively.

Results were very similar in the sensitivity analysis that truncated follow-up at 10 years after the midpoint of enrolment, indicating that studies with very long follow-up did not overly influence the findings (online supplementary figure 3).

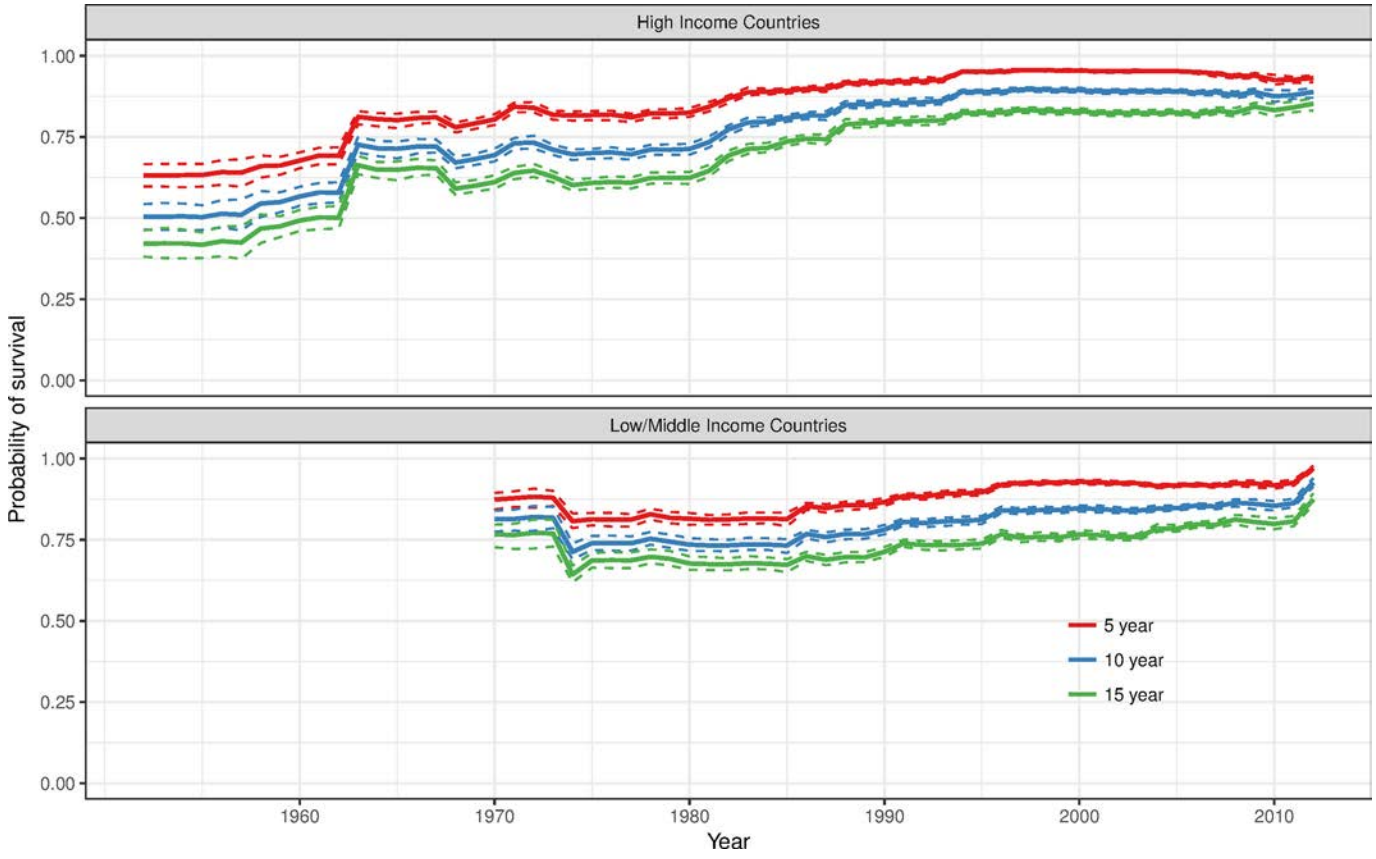

Figure 3 Estimated survival at 5 years, 10 years and 15 years in adults with systemic lupus erythematosus in high-income countries (top) and low/middle-income countries (bottom), by calendar year of observation. Dashed lines represent 95% credible limits.

Survival estimates were also very similar in the 25 inception cohort studies from high-income countries, but slightly lower in the seven inception cohort studies from LMIC (online supplementary figure 4). Only one inception cohort study, which had 5-year survival of 0.80, contributed to the LMIC estimate after 2008, accounting for the recent decrease in survival. Differences in survival estimates between high-income countries and LMIC were somewhat greater in inception cohorts, with 5-year survival of 0.94 and 0.89, 10-year survival of 0.88 and 0.81 and 15-year survival of 0.83 and 0.74, respectively, in 2008–2016.

Survival in paediatric studies

Thirty-three studies were from high-income countries and 18 were from LMIC (online supplementary figure 5). Only three studies reported 15-year survival, and therefore we did not estimate survival for this time point. Among studies from high-income countries, there was a sharp increase in survival from the 1960s to the 1970s, followed by slower improvement (figure 4). In 2008–2016, the 5-year and 10-year survival estimates from high-income countries were 0.99 (0.98 to 1.00) and 0.97 (0.96 to 0.98), respectively.

Data from LMIC did not extend prior to the 1970s. The increase in survival was pronounced between 1970 and 1990, followed by a plateau if not a slight decrease (figure 4). Between 1980 and 2000, survival persistently lagged that of high-income countries. By the end of the study period, 5-year and 10-year survival estimates from LMIC were 0.85 (0.83 to 0.88) and 0.79 (0.76 to 0.82), respectively, substantially lower than those from high-income countries.

Results were similar in the sensitivity analysis which limited the influence of studies with very long follow-up (online supplementary figure 6). There were too few paediatric inception cohort studies (n=8) for separate analysis.

Causes of death

Causes of death were reported in 87 adult studies, 22 of which examined inception cohorts (table 1). Among studies from high-income countries, the proportion of deaths attributed to SLE decreased over time in all studies and in inception cohorts (both p for trend=0.01). Similarly, the proportion of deaths due to SLE was lower in more recent years among inception cohorts from LMIC, but there was no trend among all studies from LMIC. Deaths from infections increased over time in adult inception cohorts from both high-income countries and LMIC.

Causes of death were reported in 39 paediatric studies, five of which reported on inception cohorts. There was no significant trend in cause of death in paediatric studies, possibly due to the smaller number of studies. In studies from LMIC, the frequency of deaths due to SLE demonstrated a trend to increase over time. In inception cohorts from high-income countries, SLE was the cause of more than 50% of deaths in recent studies.

DISCUSSION

Our results showed that survival in patients with SLE gradually increased from the 1950s to the mid-1990s, and then plateaued. Although it is widely recognised that survival in SLE has improved substantially over the past decades, the time course of this improvement has not been clear. Seven adult studies have

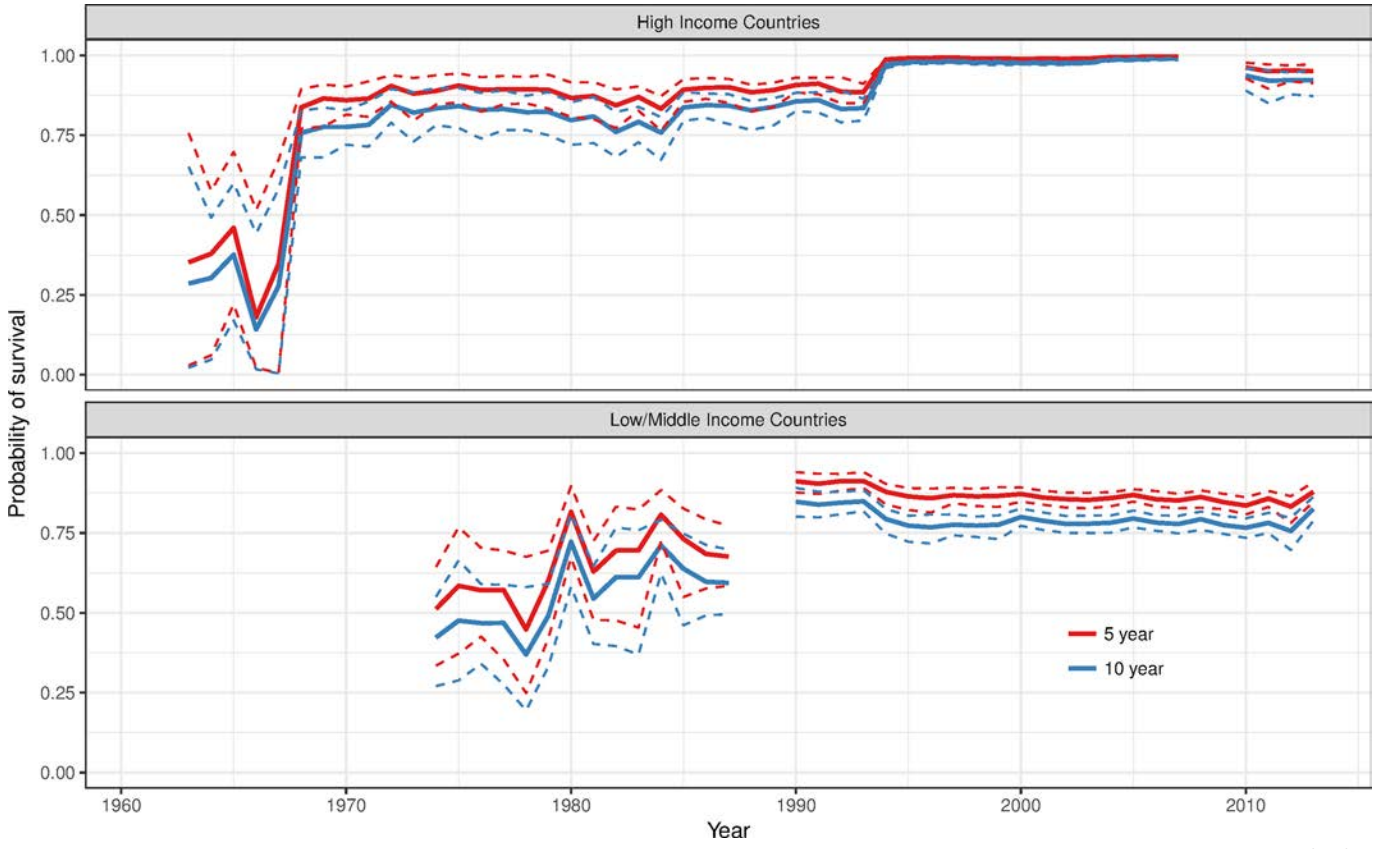

Figure 4 Estimated survival at 5 years and 10 years in children and adolescents with systemic lupus erythematosus in high-income countries (top) and low/middle-income countries (bottom), by calendar year of observation. The gaps represent periods with fewer than two studies that included Kaplan-Meier plots or summary survival estimates. Dashed lines represent 95% credible limits.

Table 1 Causes of death in patients with systemic lupus erythematosus (SLE) by year of start of enrolment and income level. Values are weighted mean percentages of death from each cause, with weights equal to the number of deaths in each study.

	All studies							Inception cohorts						
Years	Number of studies	Number of deaths	SLE %	Infection%	CVD%	Cancer%	Other%	Number of studies	Number of deaths	SLE%	Infection%	CVD%	Cancer%	Other%
	Adult high income (b=−7.1, p=0.01)							Adult high income (b=−9.6, p=0.01)						
<1980	28	1244	42.4	23.7	16.5	5.4	12.0	6	331	18.9	21.9	32.5	12.8	13.9
1980–1989	15	287	31.0	23.7	21.9	4.5	18.9	5	87	32.3	24.1	26.9	8.7	8.0
1990–1999	13	353	18.0	23.1	20.8	8.0	30.1	5	124	7.7	42.3	32.2	4.9	12.9
≥2000	2	246	12.3	15.1	11.3	7.5	53.8	0	–	–	–	–	–	–
	Adult low/middle income (b=0.51, p=0.88)							Adult low/middle income (b=−14.6, p=0.03)						
<1980	8	633	25.0	35.8	15.0	1.5	22.7	1	16	37.5	31	25	0	6.5
1980–1989	5	210	36.5	34.9	12.2	0.4	16.0	1	56	38.2	26.5	16.2	0	19.1
1990–1999	9	313	26.9	53.9	4.1	2.1	13.0	3	144	16.9	65.3	6.4	2.7	8.7
≥2000	7	90	34.3	37.5	10.6	4.2	13.4	1	6	0	50.0	0	0	50.0
	Paediatric high income (b=−1.21, p=0.78)							Paediatric high income (b=−0.16, p=0.99)						
<1980	13	173	41.4	44.9	7.9	0.6	5.1	1	3	67.0	33.0	0	0	0
1980–1994	8	61	56.3	34.9	3.4	0	5.0	1	6	33.3	66.7	0	0	0
≥1995	4	25	28.2	63.7	0	0	8.1	1	9	55.5	33.0	0	0	11.5
	Paediatric low/middle income (b=10.9, p=0.24)							Paediatric low/middle income						
<1980	2	26	26.7	42.4	19.4	0	11.4	0	–	–	–	–	–	–
1980–1994	9	96	41.8	35.7	12.6	0	9.9	2	7	28.7	28.4	28.6	0	14.3
≥1995	3	18	67.3	32.7	0	0	0	0	–	–	–	–	–	–

CVD, cardiovascular disease.

reported survival trends over different calendar periods in the same cohort. In six studies, follow-up started between 1950 and 1970 and ended in the 1980s or 1990s.[2 3 7 8 24–26] Only one study had follow-up into the 2000s.[3] Trends in survival have also been examined in studies that compared the relative risk of death in SLE to the general population.[3 27 28] For example, Urowitz *et al* found that the standardised mortality ratio decreased from 12.6 in 1970–1978 to 3.46 in 1997–2005.[3] Our results indicate that survival in adults with SLE has not continued to improve through the 2000s.

The increase in survival to the mid-1990s is likely multifactorial. Some of the improvement is undoubtedly attributable to better treatment of SLE and advances in general medical care. However, some of the improvement may also be due to inclusion of milder cases after more widespread use of antinuclear antibody testing.[29 30] Milder cases of SLE might have been underdiagnosed in earlier decades, leaving only more severely affected patients included in survival studies. Supporting this is our finding that lower proportions of adult patients had nephritis or CNS disease in more recent studies. Part of the apparent improvement in survival may therefore reflect less spectrum bias in more recent decades. Shorter delay in diagnosis may have also contributed to longer apparent survival in more recent studies (eg, lead-time bias).

Our pooled estimates are similar to the limited data from studies in the 2000s.[31–37] The plateau in survival since the mid-1990s may reflect ongoing limitations in the appropriate implementation of treatment, or in the control of comorbidities or complications such as infections. It may also represent persistent poor outcomes among patients with treatment-resistant SLE. Whether newer medications such as mycophenolate mofetil and rituximab affect survival at the population level is not clear.

Among adult studies, survival was comparable in LMIC and high-income countries. However, disparity was somewhat more apparent in inception cohort studies, particularly for 15-year survival. Differences in survival between high-income countries and LMIC were more striking in paediatric SLE studies, with a gap in 10-year survival of 0.97 and 0.79, respectively. Even more concerning is that these rates seem to plateau at this level. Barriers to healthcare access and limited availability of experienced clinicians and treatments may influence the diagnosis and management of SLE in LMIC.[38 39] Some severely affected patients may die before reaching specialists able to make a diagnosis, and therefore may not be included in survival estimates.

SLE was less frequently a cause of death in recent years among adult patients, supporting previous literature.[4 8 40] The introduction of effective immunosuppressive treatments may have reduced deaths directly due to SLE, but likely also increased complications, notably infection-related deaths.[41] The inclusion of more patients with milder SLE may have also contributed to fewer SLE-related deaths recently. In the 2000s, infections and cardiovascular disease were the main causes of death in large inception cohorts.[42–44] Our pooled results indicated no increase in the proportion of deaths due to cardiovascular disease or cancer over time among adults.

The leading cause of death in paediatric patients in LMIC continues to be SLE. High rates of lupus nephritis combined with barriers to treatment may contribute to this finding. Infections were a major cause of death among paediatric patients in high-income countries. Serious infections are common among paediatric patients.[45] Interventions to increase vaccination in children could further improve survival.[46]

Our study has several strengths. We examined both adult and paediatric studies, and separately analysed studies by income level, because pooling these may misrepresent the survival experience in each group. We also separately examined inception cohort studies.

The most important limitation is that few studies examined inception cohorts. Studies of prevalence cohorts are more likely to underestimate mortality, because they will not capture deaths early in the course of SLE. Few studies were community based, which would provide a more representative view of survival than studies from referral centres. Also, only 39% of studies reported a time-to-event curve, despite having survival as an outcome, and only 38% reported the proportion lost to follow-up. Few paediatric studies had more than 10 years of follow-up. Although we summarised data of many studies, we accounted for two main sources of variation—age and development status—by stratification. There were insufficient data to stratify further by gender or race. Some adult studies included small or unknown proportions of children, which might have affected the survival estimates.

Our results indicate that overall survival has not increased over the past 20 years in patients with SLE in high-income countries. Progress in improving survival will depend on a comprehensive understanding of the major preventable causes of death. This may be achieved most quickly by a focus on preventing infections and improving the outcomes of patients with serious infections.[41]

Correction notice This article has been corrected since it published Online First. The third author's name has been corrected to Jinxiang Hu.

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EXTENDED REPORT

Long-term outcomes and secondary prevention after acute coronary events in patients with rheumatoid arthritis

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ABSTRACT

Objectives Patients with rheumatoid arthritis (RA) are at increased risk of acute coronary syndrome (ACS) *and* suffer from poorer short-term outcomes after ACS. The aims of this study were to assess long-term outcomes in patients with RA with ACS compared with non-RA patients with ACS, and to investigate whether the use of secondary preventive drugs could explain any differences in ACS outcome.

Methods We performed a cohort study based on 1135 patients with RA and 3184 non-RA patients who all developed an incident ACS between 2007 and 2010. We assessed 1-year and overall relative risks for ACS recurrence and mortality, as well as prescriptions of standard of care secondary preventive drugs.

Results The risk of ACS recurrence, and of mortality, was increased in RA, both at 1 year after adjusting for baseline comorbidities (HR=1.30(95% CI 1.04 to 1.62) and 1.38(95% CI 1.20 to 1.59), respectively) and throughout the complete (mean 2 years) follow-up (HR=1.27(95% CI 1.06 to 1.52) and 1.50(95% CI 1.34 to 1.68), respectively). Among certain subgroups of ACS, there was a tendency of lower usage of statins, whereas there were no apparent differences in others. The increased rates of ACS recurrence and mortality remained in subgroup analyses of individuals whose prescription pattern indicated both adequate initiation and persistence to secondary preventive treatments.

Conclusions Patients with RA suffer from an increased risk of ACS recurrence and of death following ACS compared with general population, which in the present study could not readily be explained by differences in usage of secondary preventive drugs.

INTRODUCTION

Patients with rheumatoid arthritis (RA) are not only at increased risk of ischaemic heart disease (IHD)[1]; we recently reported that patients with RA and acute coronary syndrome (ACS) more often also present with sudden cardiac death or ST elevation myocardial infarction (MI), suffer from increased *short-term* mortality compared with non-RA patients with ACS, but also that the mortality differences were explained neither by underlying comorbidities nor by differences in ACS type.[2]

In addition to the impaired short-term prognosis,[2–6] smaller studies have suggested an increased risk of ACS recurrence as well as an increased longer term mortality following ACS in patients with RA.[4 7 8] In the general population, suboptimal initiation of and adherence to secondary preventive pharmacotherapies are both linked to ACS recurrence as well as to poor long-term ACS outcomes.[9] Therefore, suboptimal initiation and/or adherence to such pharmacotherapies might be one, and importantly a modifiable, explanation for a worse prognosis in patients with RA who develop ACS. The few existing studies on this topic are, however, contradictory; indicating lower usage as well as no difference in the use of secondary preventive drugs after ACS in patients with RA compared.[8 10 11]

Sparked by our recent and disturbing findings regarding the clinical presentation and short-term outcome after ACS in RA, the specific aims of this study were to assess whether the risk of (1) recurrent ACS, and (2) long-term overall mortality following ACS, or (3) the initiation of, and adherence to, evidence-based cardioprotective secondary preventive pharmacotherapies after incident ACS differ between patients with RA compared with the general population.

METHODS

Study design

We performed a cohort study based on one cohort of patients with prevalent RA and incident ACS matched to non-RA patients with incident ACS.

Study setting

Swedish residents have access to publicly funded healthcare, including specialised care at rheumatology/internal medicine clinics for patients with RA, and coronary intensive care units for patients with ACS. Drugs are subsidised; after reaching an upper annual spending limit for prescription drugs (approximately US$217 per year (February 2017)), subsequent prescriptions are free of charge. A unique personal identity number (PIN) is assigned to all Swedish residents at birth or immigration.[12]

Data sources

In this study, the PIN was used as a key to link several nationwide population-based and virtually complete registers together to define an RA cohort, a general population comparator cohort, and to collect relevant information on exposures, outcomes and covariates.

To identify individuals with RA, ACS events and comorbidities of interest the *National Patient Register* (NPR) was used. This register includes diagnoses for admissions and specialised outpatient

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care in Sweden, has full coverage for inpatient care since 1987 and covers outpatient specialised care since 2001.[13] Information on mortality was collected from the *Cause of Death Register* that, similar to NPR, holds information on deaths including cause(s) of death coded according to the International Statistical Classification of Diseases (ICD; version 10 since 1996). Information on dispensed pharmacotherapies was collected from the *Prescribed Drug Register (PDR)*, including information on all dispensed drugs from Swedish pharmacies since July 2005. Dispensed drugs are coded according to Anatomical Therapeutic Chemicals (ATC) classifications and reported with drug dose and quantity.[14] The *Total Population Register* stores demographic information and was used to identify the general population comparators. The register linkages and the study population have been described in detail elsewhere.[2]

Study population

The study population comprised patients with prevalent RA (n=1135) and all their individually matched general population comparator subjects (n=3184), non-RA patients, who developed a first ever incident ACS between 2007 and 2010.

Prevalent, that is, actively monitored, RA was defined as individuals above 18 years of age (no upper age limit) with at least two visits listing RA in the NPR, of which at least one at an internal medicine or rheumatology department. This definition has a predictive value of approximately 90%.[15] To ensure that the RA disease was subject to active monitoring, one of the visits listing RA had to occur in 2006, 2007, 2008 or 2009. For each patient with RA, up to five general population comparator subjects were matched by age, sex, area of residency and educational level.

ACS was defined as a first ever registered diagnosis of MI or unstable angina in the NPR (for RA: within 1 year after the visit defining actively monitored disease) between 2007 and 2010. None of the ACS events were identified in immediate relation to the visit listing RA that defined inclusion into the cohort. This study population has been described in detail previously.[2] The ICD codes used to detect ACS have a positive predictive value of 95%.[16] Online supplementary table 1 lists the ICD codes used.

Recurrent ACS and mortality

Recurrent ACS was defined as a new registration of ACS in the NPR 30 days or more after incident ACS date (to avoid misdiagnosis of registrations related to the incident ACS). Mortality (from any cause) was defined as 1-year mortality and as mortality during the complete follow-up period, which ended 31 December 2011.

Secondary preventive drugs

Standard of care secondary preventive pharmacotherapies were assessed as dispensed prescriptions of aspirin, P_2Y_{12} inhibitors, beta blockers, renin-angiotensin system (RAS)-blocking agents and lipid-lowering agents categorised as −7 to 90, 91–180, 181–270 or 271–365 days after the ACS event. The ATC codes used are listed in online supplementary table S1.

Statistical analyses

Baseline data were compiled and presented as frequencies and per cents for categorical variables, and means or medians with SD or IQR as appropriate for continuous variables. Pre-existing comorbidities and pharmacotherapies were defined as a diagnosis in the NPR or a dispensed drug in the prescribed drug register more than 90 days prior to the ACS to avoid potential influence from the ACS itself (see online supplementary table S1 for codes). The type of ACS, based on the registered ICD code in the NPR (transmural, subendocardial, unspecific or unstable angina), was also compiled and presented with the descriptive data.

Recurrent ACS was calculated as events per 100 person-years. All-cause mortality during follow-up was analysed using the Kaplan-Meier method. We used Cox regression with time since ACS as timescale, adjusted for age and sex to assess the relative risk (RR) (HRs) of recurrent ACS, and of death. These models were further adjusted for confounding using a propensity score (PS), in turn calculated using a multivariate model including status at start of follow-up according to demographics (age and sex), pre-existing comorbidities (stable angina pectoris, cerebrovascular lesion, venous thromboembolic disease, atrial fibrillation, heart failure, cardiomyopathy, diabetes type I/II, chronic obstructive pulmonary disease, renal failure *yes/no*) and pre-existing pharmacotherapies (insulin, oral antidiabetics, warfarin, acetylsalicylic acid, P_2Y_{12} inhibitors, nitroglycerine, diuretics, RAS-blocking agents, beta blockers, calcium antagonists, lipid-reducing agents *yes/no*). The Cox model was further adjusted with the PS in combination with ACS type.

Numbers and proportions of the study population with dispensed prescriptions of each drug, and combinations of one, two and three of the drugs under study, were calculated separately by time period following ACS (−7 to 90 days, 91–180 days, 181–270 days, 271–365 days). Only subjects alive at the end of each time period under study were included in each assessment. Logistic regression models adjusted for age and sex were used to obtain p values for differences in treatment initiation and adherence; a two-tailed p value <0.05 was considered significant.

To refine our findings, a series of sensitivity analyses were performed. Recurrent ACS, mortality and dispensed prescriptions of secondary preventive drugs were assessed and stratified by type of ACS (transmural MI, subendocardial MI, unspecific MI and unstable angina). To investigate whether any difference (between patients with RA and non-RA patients) in the use of secondary preventive pharmacotherapies could explain any increased ACS recurrence, or mortality, we performed sensitivity analyses of (1) subjects fulfilling a combination of at least three different secondary preventive drugs during the first time period after ACS for 1-year analysis, and (2) subjects fulfilling a combination of at least three drugs during at least two of (any) time periods for the complete follow-up period. To rule out that pre-existing usage of each drug affected results on filled prescriptions during the first time period, (1) the first time window was broadened to −30 (days before) to 90 and (2) in two additional sensitivity analyses, all subjects with a previous filling of any of the prescriptions under study were excluded.

All analyses were carried out with SAS software package V.9.3 (SAS Institute). This study was approved by the ethics committee in Stockholm, Sweden.

RESULTS

A total of 1135 patients with RA, and 3184 non-RA patients, with incident ACS were identified (table 1, figure 1). A total of 904 (79.6%) of the patients with RA and 2742 (86.1%) non-RA patients were alive at 90 days following ACS, and were included in the analysis of secondary preventive drugs during this time period (−7 to +90 days). At 365 days after the ACS, 803 (70.7%) patients with RA and 2536 (79.6%) non-RA patients were still

Table 1 Demographics, year and type of incident ACS, and pre-existing comorbidities and pharmacotherapies among patients with RA and general population comparators with incident ACS between 2007 and 2010

	RA n=1135	Comparators n=3184
Year of ACS		
2007	333 (29.3)	802 (25.2)
2008	283 (24.9)	832 (26.1)
2009	277 (24.4)	808 (25.4)
2010	242 (21.3)	742 (23.3)
Type of ACS*		
Transmural MI	248 (21.9)	648 (20.4)
Subendocardial MI	414 (36.5)	1158 (36.4)
Unspecific MI	363 (32.0)	906 (28.5)
Unstable angina	103 (9.1)	468 (14.7)
Reinfarction	7 (0.6)	4 (0.1)
Women	711 (62.6)	1897 (59.6)
Men	424 (37.4)	1287 (40.4)
Age, median (SD)	73.8 (±10.3)	73.6 (±9.8)
Women	74.9 (±9.7)	75.4 (±10.1)
Men	71.6 (±9.6)	71.5 (±10.3)
Educational level (years)		
<9	602 (53.0)	1739 (54.6)
10–12	390 (34.4)	1057 (33.2)
>12	124 (10.9)	344 (10.8)
RA treatment 0–6 months prior ACS		
Glucocorticoid	687 (60.5)	–
DMARD, any	648 (57.1)	–
DMARD, Mtx	491 (43.3)	–
Biological drug	105 (9.3)	–
NSAID	749 (66.0)	–
Pre-existing CVD†		
Stable angina pectoris	197 (17.4)	468 (14.7)‡
Cerebrovascular lesion	150 (13.2)	388 (12.2)
Venous thromboembolic disease	117 (10.3)	211 (6.6)§
Atrial fibrillation	114 (10.0)	286 (9.0)
Congestive heart failure	175 (15.4)	332 (10.4)§
Cardiomyopathy	10 (0.9)	33 (1.0)
Pre-existing other comorbidities†		
Diabetes, type I	98 (8.6)	283 (8.9)
Diabetes, type II	171 (15.1)	504 (15.8)
COPD	124 (10.9)	221 (6.9)§
Renal failure, chronic	28 (2.5)	53 (1.7)
Pre-existing pharmacotherapy†		
Insulin	113 (10.1)	320 (10.0)
Oral antidiabetics	108 (9.5)	410 (12.9)‡
Warfarin	118 (10.4)	241 (7.6)‡
Acetylsalicylic acid	403 (35.5)	1261 (39.6)‡
P_2Y_{12} inhibitors	51 (4.5)	98 (3.1)‡
Nitro	238 (21.0)	616 (19.4)
Diuretics	545 (48.0)	1222 (38.4)§
ACE inhibitors	459 (40.4)	1230 (38.6)
Beta blocker	557 (49.1)	1395 (43.8)‡
Calcium antagonists	298 (26.3)	871 (27.4)
Lipid reducers	280 (24.7)	932 (29.3)‡

*Based on the ICD diagnoses registered in the Swedish Patient Register (unstable angina ICD I200, transmural MI ICD I210–I213, subendocardial MI ICD I214, unspecific MI ICD I219, reinfarction ICD I22).
†All pre-existing comorbidities/dispensed pharmacotherapies are defined as a diagnosis or fulfilled prescription in national patient registry or prescribed drug registry more than 3 months prior to the ACS. ICD codes used in online supplementary file.
‡p<0.05 based on χ^2 test for dichotomous variables, t-test for normally distributed continuous variables, and Mann-Whitney U test for ordinal/non-normally distributed continuous variables.
§p<0.0001 based on χ^2 test for dichotomous variables, t-test for normally distributed continuous variables, and Mann-Whitney U test for ordinal/non-normally distributed continuous variables.
ACS, acute coronary syndrome; COPD, chronic obstructive pulmonary disease; CVD, cardiovascular disease; DMARD, disease-modifying antirheumatic drugs; ICD, International Statistical Classification of Diseases; MI, myocardial infarction; Mtx, methotrexate, NSAID, non-steroidal anti-inflammatory drugs; RA, rheumatoid arthritis.

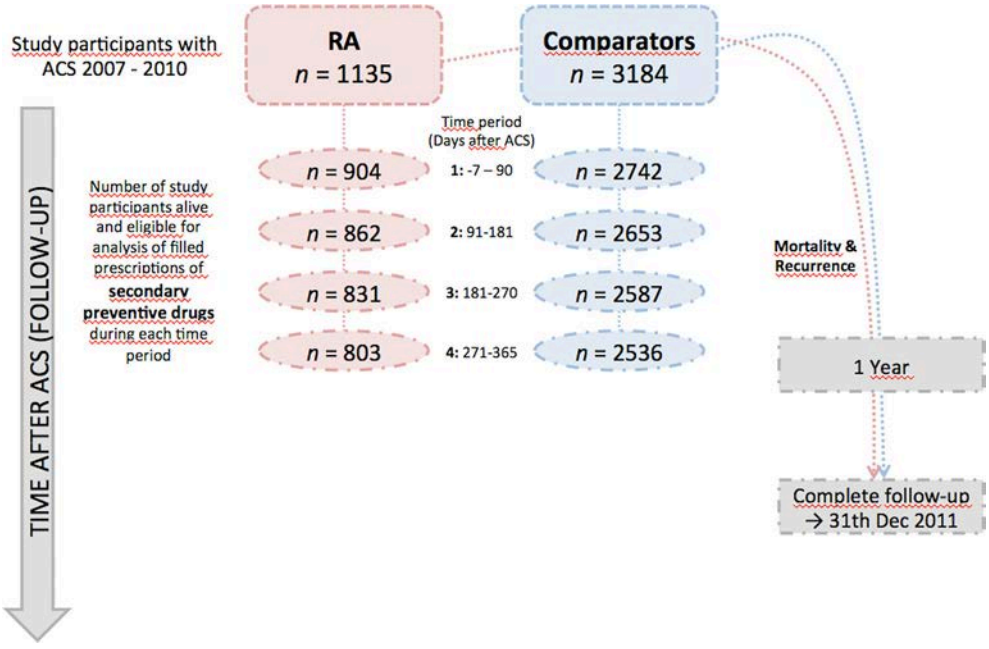

Figure 1 Flow chart study design. ACS, acute coronary syndrome; RA, rheumatoid arthritis.

alive and included in the analysis of secondary preventive drug use during the last time period under study (271–365 days).

Recurrent ACS

The rate of recurrent ACS was higher among patients with RA compared with non-RA patients, both during the first year following ACS and during the complete follow-up period (mean 2.3±1.5 years), corresponding to an approximately 30% increased recurrence risk (1-year HR 1.35 (95% CI 1.09 to 1.68); complete follow-up HR 1.34 (95% CI 1.12 to 1.60)). Further adjustment for the PS, alone and in combination with ACS type slightly decreased the HRs, which remained significantly increased (fully adjusted HR 1 year 1.28 (95% CI 1.03 to 1.60); complete follow-up 1.25 (95% CI 1.05 to 1.50)). Additional adjustment for filled prescriptions of secondary preventive drugs did not alter the HRs (figure 2, table 2).

All-cause mortality

The mortality following ACS was higher among patients with RA compared with non-RA patients with ACS (figure 3). During the

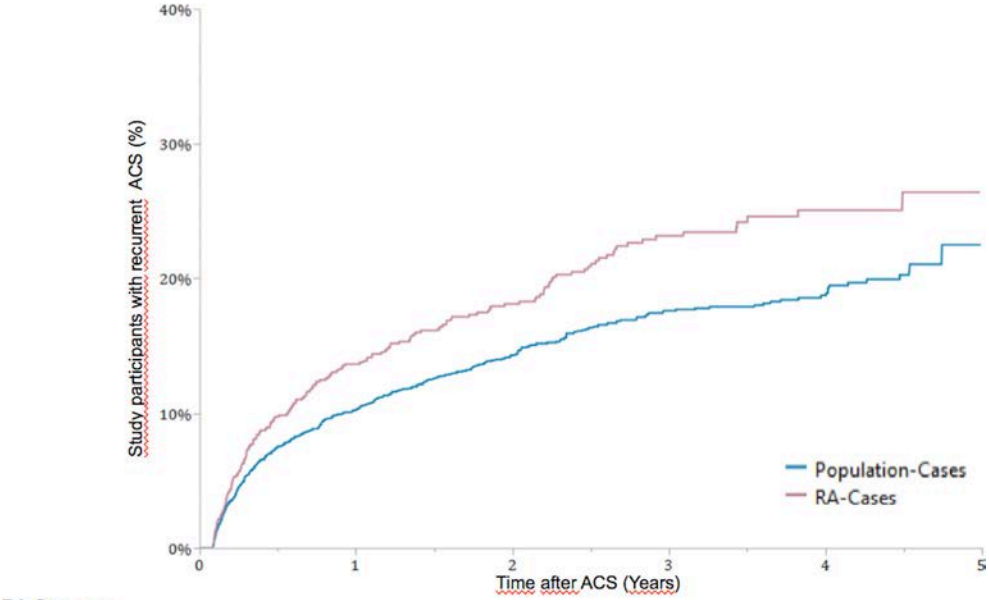

Figure 2 Recurrent ACS among RA subjects and general population comparators with incident ACS between 2007 and 2010. ACS, acute coronary syndrome; RA, rheumatoid arthritis.

Table 2 Relative risk of ACS recurrence and mortality after ACS in patients with RA and matched general population comparators. HRs with 95% CIs stepwise adjusted for potential confounders

	RA cases N events/100 person-years (95% CI)	Population cases N events/100 person-years (95% CI)	Age and sex-adjusted HR (95% CI)	PS-adjusted HR (95% CI)	PS and infarct-type-adjusted HR (95% CI)
1 year					
Mortality	58.0 (53.5 to 62.4)	35.2 (33.2 to 37.3)	1.59 (1.39 to 1.82)	1.38 (1.20 to 1.59)	1.38 (1.20 to 1.59)
Recurrence	15.2 (12.9 to 17.5)	11.2 (10.0 to 12.9)	1.35 (1.09 to 1.68)	1.30 (1.04 to 1.62)	1.28 (1.03 to 1.60)
Complete follow-up					
Mortality	21.7 (19.0 to 24.4)	12.7 (11.5 to 14.0)	1.73 (1.55 to 1.93)	1.50 (1.34 to 1.68)	1.50 (1.34 to 1.68)
Recurrence	9.1 (7.4 to 10.9)	6.7 (5.8 to 7.6)	1.34 (1.12 to 1.60)	1.27 (1.06 to 1.52)	1.25 (1.05 to 1.50)

ACS, acute coronary syndrome; PS, propensity score; RA, rheumatoid arthritis.

first year, approximately 30% of patients with RA died compared with 20% of non-RA patients, corresponding to a RR of 1.6 (HR 1.59 (95% CI 1.39 to 1.82)). During the complete follow-up period, 45% of the patients with RA with ACS versus 30% (mean 2.3±1.5 years) of the non-RA patients with ACS died, resulting in a HR of 1.73 (95% CI 1.55 to 1.93). The reported underlying cause of approximately 80% of deaths among both patients with RA and non-RA patients (79.5% vs 78.6%) was due to IHD or IHD-related complications (sudden cardiac arrest, arrhythmias, heart failure , conduction abnormalities or other complications related to IHD). These HRs remained significantly increased after adjustment for the PS alone and ACS type (fully adjusted HR 1 year 1.38 (95% CI 1.20 to 1.59); complete follow-up 1.50 (95% CI 1.34 to 1.68)) (table 2).

Secondary preventive drug use after ACS

Figure 4A–G shows the proportion of study subjects with ACS (of any type) filling prescriptions for each, and combinations of two or three drugs during each time period after the ACS.

A significantly lower (between 3% and 7% lower) proportion of patients with RA filled prescriptions of statins during all of the observed time periods (figure 4E). During some of the time periods, a significantly lower proportion of patients with RA also filled fewer prescriptions of antiplatelets and RAS-blocking agents, whereas there was no difference in beta blockers during any of the time periods (figure 4). In total, 89% of the patients with RA and 93% of the non-RA patients filled prescriptions for at least two secondary preventive drugs during the first time period following ACS (p=0.0009). By contrast, there was no appreciable difference during the following three time periods. The proportion of patients with RA that filled prescriptions for at least three secondary preventive drugs was lower than among the non-RA patients, during all time periods, for example, 75% vs 81% (p=0.0001) during the first 90 days, and 60% vs 66% (p=0.001) in the interval 271–365 days.

Sensitivity analyses

Stratifying the RR of recurrence and mortality by type of ACS resulted in a higher relative mortality risk (HR 2.4 both at 1 year and complete follow-up period) for patients with RA with unstable angina compared with non-RA patients with unstable angina. For all other ACS types, mortality HRs remained

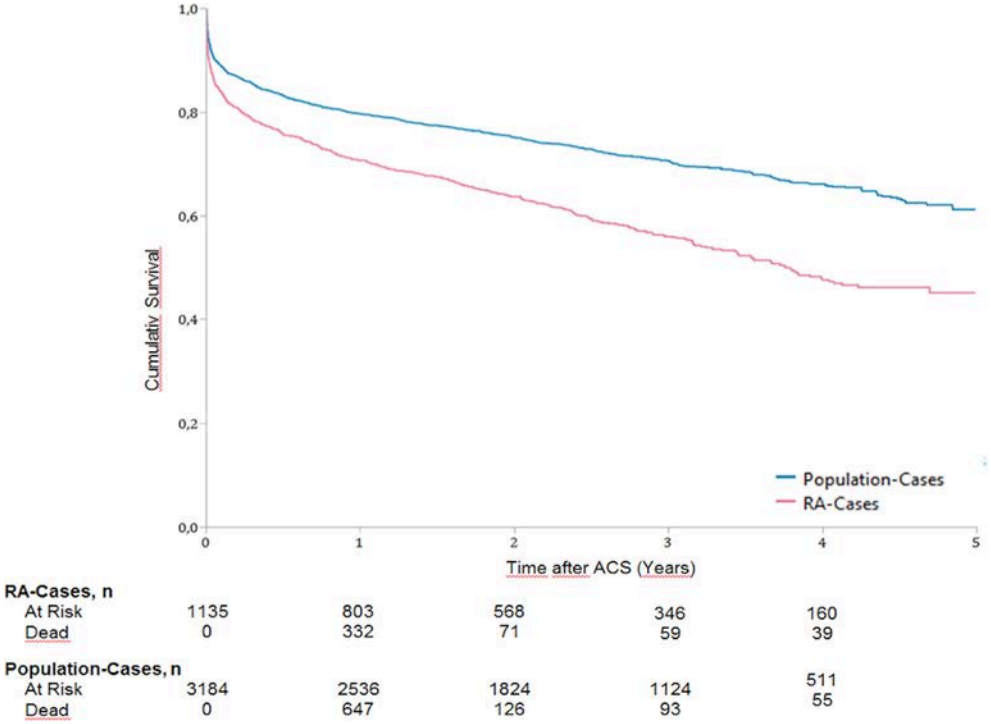

Figure 3 Overall Kaplan-Meier estimated survival in patients with RA and matched general population comparators with incident ACS between 2007 and 2010. ACS, acute coronary syndrome; RA, rheumatoid arthritis.

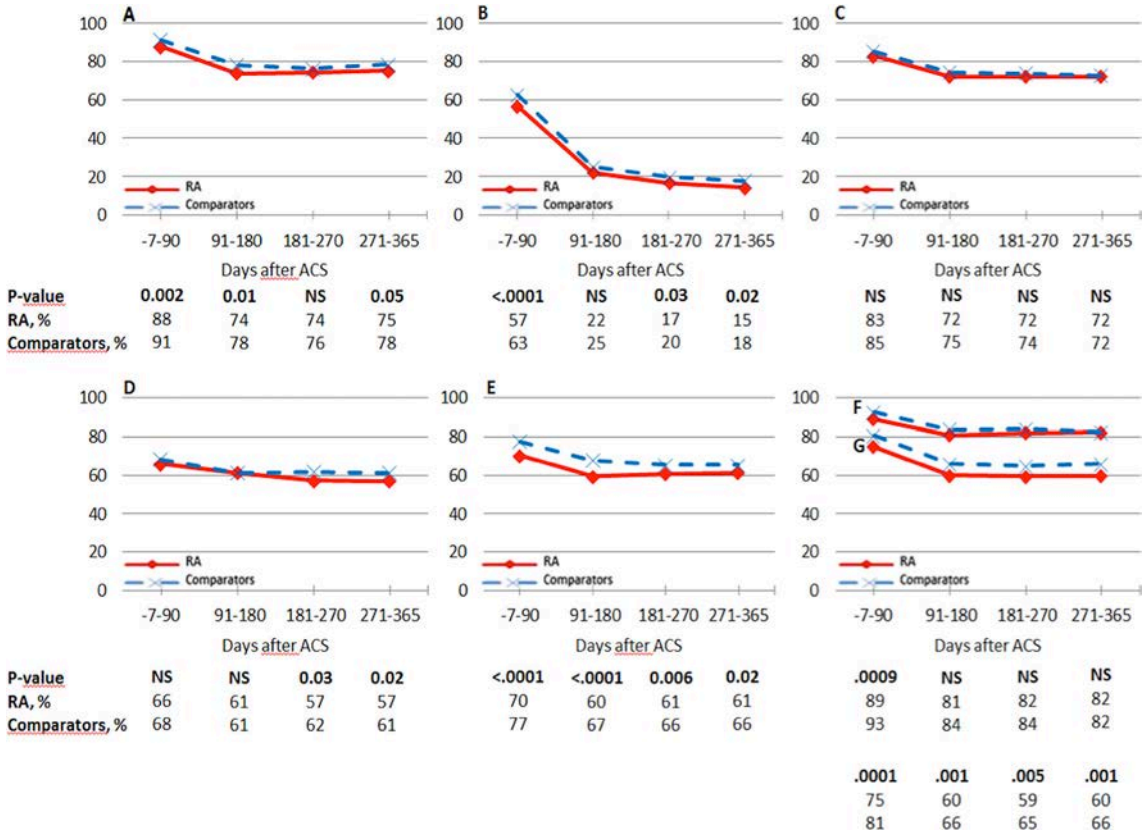

Figure 4 Proportions of dispensed prescriptions of secondary preventive drugs during four consecutive time periods following ACS: (A) Single antiplatelet. (B) Dual antiplatelets. (C) Beta blockers. (D) Renin-angiotensin system-blocking agents. (E) Statins. (F) Combination of ≥2 drugs. (G) Combination of ≥3 drugs. ACS, acute coronary syndrome; RA, rheumatoid arthritis.

similar to the HRs of the primary analysis. Recurrence HRs also remained similar, but with a poorer statistical precision especially in subgroups with fewer events, to the primary analysis (see online supplementary figures 1 and 2 and table 2). When the study population was restricted to those subjects filling prescriptions of at least three preventive drugs, the HRs for recurrent ACS, and for all-cause mortality, remained similar to the main analysis or were even more pronounced (see online supplementary table 7). Stratifying the results by sex did not reveal any major differences in relative mortality risk, whereas there was a tendency of a more pronounced increased recurrence risk among women compared with men.

When stratifying the use of secondary preventive drugs by ACS type (transmural, subendocardial, unspecific and unstable angina), most of the differences observed in the main analysis diminished or disappeared. Of the 144 statistical comparisons made (nine drugs/drug combinations during four time periods stratified by four ACS types), 26 (18%) comparisons remained statistically significant, in which 12 (8%) within the first time period, in comparison with the 17/36 (47%) significant statistical comparisons (nine drugs/drug combinations during four time periods) of the original analysis. Among study subjects with transmural MI, there were, with the exception for a lower proportion of patients with RA filling prescriptions of RAS-blocking agents time period 3 and dual antiplatelet treatment time period 4, no remaining statistically significant differences. Among other ACS subtypes, patients with RA filled significantly fewer prescriptions of statins and dual antiplatelets during several time periods (see online supplementary tables 3–6). Separate analyses among men and women did not reveal any difference in filling of prescriptions.

DISCUSSION

In this population-based and nationwide cohort study, which to our knowledge is the largest assessment of ACS recurrence, mortality and secondary preventive drug use after ACS in patients with RA, we found indications of increased risk for both recurrent ACS and for death following ACS in patients with RA. The observed risk increases could be readily explained neither by confounding from other comorbidities and/or therapies nor by ACS phenotype. Furthermore, we found that when taking ACS phenotype into account, the use of secondary preventive drugs was not consistently much lower in patients with RA than in the general population, neither when assessed separately nor when assessed in combination. The maximum differences in proportions, although statistically significant, amounted to 5% units or less. The increased risk of recurrence and death could not be readily explained by differences in usage of secondary preventive drugs among patients with RA compared with non-RA patients, although undertreatment cannot be discarded as potential explanation for the increased recurrence and mortality. Importantly, however, the impaired outcome after ACS persisted also among those individuals whose patterns of fillings of secondary preventive drugs indicated compliance with a three-drug secondary preventive regimen.

Even though the present study could not link the impaired long-term prognosis following ACS in patients with RA to poor pharmacoprevention, it is important to acknowledge that patients with RA constitute a heterogeneous group of patients frequently on several pre-existing prescribed medications and with several comorbid conditions, which both are factors that have been associated with non-adherence to secondary preventive therapies.[17]

Furthermore, adherence to secondary preventive drugs has repeatedly been pointed out to leave room from improvement[18] and is associated with adverse outcomes in terms of recurrent events *and* mortality rates in the general population[19] as well as RA.[10 20] Thus, even if similarly used in RA, drug adherence should thus be as carefully managed in patients with RA just as in the general population.

Results from previous studies on initiation and/or adherence to secondary preventive drugs in patients with RA have been contradictory. In-hospital initiation of aspirin, statins and beta blockers was reported to be lower in a cohort of 90 RA subjects with MI compared with matched controls.[10] In another study, based on an RA cohort of similar size, there was no significant difference in secondary preventive drugs received neither in-hospital nor at discharge comparing RA subjects and matched controls.[8] In a larger population-based Danish cohort study, a lower initiation of aspirin, beta blockers and statins was observed, and persisted throughout follow-up.[11] Except for potential variations related to the different geographic regions and clinical settings, there are also major differences in study designs and methodological approaches. For instance, when we stratified our results by ACS subtype (which was not the case in the previous studies), most of our observed differences disappeared. Our analyses also revealed that most of the observed differences in drug use (if any) pertained to subjects with unspecific or subendocardial MI, in contrast to virtually no difference among subjects with transmural MI. Even though these subtypes, which are based on registered ICD codes, cannot strictly be translated into ST-segment elevation MI (STEMI) versus non-ST-segment elevation MI, one may assume that a majority of subjects with transmural MI were diagnosed with STEMI and therefore underwent percutaneous coronary intervention according to existing clinical guidelines, whereas subjects with subendocardial and unspecific MI potentially suffered from more minor MIs and instead were more prone to receive conservative treatment. Invasive in-hospital treatment has been associated with increased adherence to secondary preventive drugs.[21 22] Conversely, less 'critical' events in patients with a high overall burden of disease may be associated with poorer drug adherence. Across time periods, a majority (>70%) of the lower prescription rates among patients with RA were observed in the first 6 months after ACS. It is plausible that clinical concerns regarding comorbidity, drug interactions and toxicity lead to a delay of drug initiation in this group of patients.

The increased ACS recurrence and mortality in the RA cohort in our present study corroborate findings from previous studies.[4 6 8 10 11] Importantly, we could extend these findings by demonstrating that the impaired outlook remained also after adjustment for several important cardiovascular risk factors.

Major strengths of this study include the possibility to use population-based and prospectively recorded data to identify RA, ACS, drug use as well as comorbidities of interest.[23] Additionally, the algorithms used to detect RA and ACS have high validity.[15 16] The size of the study population and the extended duration of follow-up, where virtually all outcomes of interest could be captured via linkage to other nationwide registry, are other strengths.

There are several potential limitations to take into consideration when interpreting the results from our study. First, although we could adjust for a large number of potential confounders we lacked information on certain traditional prognostic risk factors such as smoking, body mass index and actual lab values for blood lipid levels and glucose intolerance. We also lacked information on inflammatory activity which has been associated with cardiovascular disease onset and has been suggested to be associated with recurrent ischaemic episodes. Potentially, the increased inflammatory burden following ACS on top of the underlying inflammation in RA could partly explain the poorer outcomes following ACS. Second, we did not have access to information on target parameters such as blood lipid levels and blood pressure making it difficult to determine whether individual treatments were appropriate. Third, secondary prevention following ACS includes pharmacotherapies and lifestyle-related factors such as implementation or maintaining an adequate level of physical activity, dietary recommendations, stress management, and so on; we did not have access to such information. Finally, we based our definition of drug usage on (patterns of) filled prescriptions, which not necessarily equates actual drug compliance. The potential differences in disease characteristics, such as accumulated inflammatory activity, in patients with prevalent RA compared with incident RA might pose a limitation to the generalisability of the reported results to patients with new-onset RA.

In conclusion, our study suggests that by and large, the use of secondary preventive drugs in RA is not markedly lower than in ACS in general, yet patients with RA are at increased risk of recurrent ACS as well as mortality following ACS. Consequently, from an aetiological point of view, additional factors are likely to drive the impaired outlook and will be important to identify. Clinically, among patients with coronary artery disease, those with RA should be recognised as patient at elevated risk among whom preventive measures may be of particular importance.

Contributors All authors have made substantial contributions to the conception or design of the work, or the acquisition, analysis or interpretation of data.

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EXTENDED REPORT

Spectrum of lymphomas across different drug treatment groups in rheumatoid arthritis: a European registries collaborative project

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ABSTRACT

Background Lymphomas comprise a heterogeneous group of malignant diseases with highly variable prognosis. Rheumatoid arthritis (RA) is associated with a twofold increased risk of both Hodgkin's lymphoma (HL) and non-Hodgkin's lymphoma (NHL). It is unknown whether treatment with biologic disease-modifying antirheumatic drugs (bDMARDs) affect the risk of specific lymphoma subtypes.

Methods Patients never exposed to (bionaïve) or ever treated with bDMARDs from 12 European biologic registers were followed prospectively for the occurrence of first ever histologically confirmed lymphoma. Patients were considered exposed to a bDMARD after having received the first dose. Lymphomas were attributed to the most recently received bDMARD.

Results Among 124 997 patients (mean age 59 years; 73.7% female), 533 lymphomas were reported. Of these, 9.5% were HL, 83.8% B-cell NHL and 6.8% T-cell NHL. No cases of hepatosplenic T-cell lymphoma were observed. Diffuse large B-cell lymphoma (DLBCL) was the most frequent B-cell NHL subtype (55.8% of all B-cell NHLs). The subtype distributions were similar between bionaïve patients and those treated with tumour necrosis factor inhibitors (TNFi). For other bDMARDs, the numbers of cases were too small to draw any conclusions. Patients with RA developed more DLBCLs and less chronic lymphocytic leukaemia compared with the general population.

Conclusion This large collaborative analysis of European registries has successfully collated subtype information on 533 lymphomas. While the subtype distribution differs between RA and the general population, there was no evidence of any modification of the distribution of lymphoma subtypes in patients with RA treated with TNFi compared with bionaïve patients.

INTRODUCTION

Malignant lymphomas ('lymphomas') comprise a heterogeneous group of malignant diseases with presumably distinct aetiologies. Whereas the 5-year overall survival across all lymphomas is approximately 60%, there is great variation in survival depending on the lymphoma subtype, ranging from life expectancy comparable to the general population in nodular lymphocyte predominant Hodgkin's lymphoma (HL) to 5-year survival of <40% for T-cell lymphomas.[1] Furthermore, clinical characteristics and therapy approaches vary to a great extent according to subtype. The age-standardised incidence rate (IR) in Europe of approximately 25/100 000[2] makes lymphoma one of the 10 most common cancer types in the general population. There are significant gender and age-dependent differences, with men having higher IRs in most subtypes and being diagnosed at younger ages.[1]

In rheumatoid arthritis (RA) the overall incidence of lymphoma is approximately doubled compared with that in the general population.[3–9] The association between RA disease activity and lymphoma risk is considered one reason for this increased risk.[10]

Evidence that chronic immune stimulation/chronic inflammation has a pathogenic effect in lymphomagenesis comes from the publication by Baecklund *et al*.[10] This study described an 'excess' risk strongly linked to the cumulative activity of the disease, especially for diffuse large B-cell lymphoma (DLBCL), the most common type of aggressive B-cell lymphomas.[10] Moreover, an association of methotrexate (MTX) treatment with Epstein-Barr virus (EBV)-positive lymphoproliferative disorders has been described.[11] Furthermore, a possible association between the use of tumour necrosis factor inhibitors (TNFi) and a rare but prognostically unfavourable hepatosplenic subtype of T-cell lymphoma has been reported.[12]

A number of European and other rheumatology registers have reported on the overall risk of lymphoma in patients with RA treated or not with TNFi[5 13 14] and did not find a further risk increase related to the treatment. However, the influence of TNFi is a matter of debate as recent reports from Asia and French data on Crohn's disease have shown a higher lymphoma risk in TNFi-treated patients.[15–17]

The notion that RA disease activity may be a strong risk determinant suggests that the overall lymphoma risk in TNFi-treated RA compared with the general population may represent a composite wherein a decreased risk for a disease-related lymphoma subset may be replaced by an increased risk for a treatment-related subtype. However, there is no definitive evidence for any influence of

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RA treatment on subtype distribution. In contrast to estimations of overall lymphoma risk in RA, which can be accomplished in individual registers, any analysis of subtype distribution requires large data sets and hence an international collaboration of RA registers.

The main aim of this collaborative analysis was, therefore, to explore whether there might be a switch in the subtype distribution of lymphomas in RA linked to specific antirheumatic treatments; if so, the finding would support the above-mentioned 'exchange of risks.' To this end, patients with RA never exposed to bDMARDs (bionaïve) were compared with patients with RA treated with bDMARDs, mainly TNFi, with respect to lymphoma subtypes across several European RA registries. To place the RA results into context, a second rationale of the study was to analyse the size and direction of any shift in the spectrum of lymphoma subtypes in patients with RA compared with the general population.

PATIENTS AND METHODS

Participating registers

Twelve European biologic registers from nine countries participated in this collaborative project of the European League Against Rheumatism (EULAR) Registers and Observational Drug Studies (RODS) Study Group: the French biologics register 'autoimmunity and rituximab' (AIR),[18] the Swedish ARTIS linkage of the Swedish Rheumatology Quality Register (SRQ) to other nationwide registers,[13] the Czech biologics register ATTRA,[19] the Registro Español de Acontecimientos Adversos de Terapias Biológicas en Enfermedades Reumáticas (BIOBADASER),[20] the British Society for Rheumatology Biologics Register for Rheumatoid Arthritis (BSRBR-RA),[5] the Danish Rheumatologic database (DANBIO),[21] the Italian biologics register (GISEA),[22] the French biologics register 'Orencia and RA' (ORA),[18] the German biologics register 'Rheumatoid arthritis observation of biologic therapy' (RABBIT),[23] the French Research Axed on Tolerance of bIOtherapies (RATIO),[24] the French Register Tocilizumab and RA (REGATE), and the Portuguese rheumatic diseases register (Reuma.pt).[25] To participate, registers were required to have at least one lymphoma reported and consequently several other European biologic registers were not able to contribute.

Patients

Patients were required to have physician-diagnosed RA and to be prospectively followed up in one of the participating European RA registers. Patients with a history of lymphoma prior to registration were excluded. Patients diagnosed with a histology-confirmed lymphoma after study registration were included in the analysis. These patients were stratified according to their exposure status as follows: (1) bionaïve group: patients who were bionaïve at the diagnosis of the lymphoma; and (2) patients who were not bionaïve at the diagnosis of the lymphoma were stratified into four groups according to the biologic disease-modifying antirheumatic drug (bDMARD) they had received most recently prior to the development of the lymphoma: TNFi, rituximab, tocilizumab or abatacept.

Outcome

The primary endpoint was the spectrum of lymphoma subtypes. The definition of lymphoma included HL and non-Hodgkin's lymphoma (NHL), but not plasma cell neoplasias. The subtypes were defined according to the pathology reports. The WHO 2008 classification of lymphomas was used to classify the respective subtype of lymphoma.[26] Crude IRs were also calculated.

Three registries received reports of histologically confirmed lymphoma through linkage of all participants to their national cancer registry: DANBIO, ARTIS and BSRBR-RA. The remaining registers (as well as BSRBR-RA) received reports of lymphoma from the patient's rheumatologist. For BSRBR-RA, histologically confirmed lymphomas were included if reported from either record linkage or rheumatologist.

Statistical analysis

The spectrum of lymphoma subtypes was compared between RA cohorts in two steps. In the first step, the portion of HL and NHL classified into B-cell lymphoma (B-NHL) and T-cell lymphoma (T-NHL) was compared by χ^2 test and exact multinomial 95% CIs. HL, B-NHL and T-NHL with incomplete subtype information were included in this first step, whereas lymphomas not otherwise specified were excluded.

To describe the consistency of the findings, the results of analyses based on registers with at least 30 lymphomas each in the bionaïve cohort and the biologic-treated cohort are shown separately. In the second step, the subtype distributions of B-NHL were compared. In this comparison, B-NHLs with missing further subtype specification were excluded.

To compare the spectra of lymphomas observed within the RA cohorts with the spectrum of lymphoma subtypes in the general population, data from the HAEMACARE project were used.[2] HAEMACARE is a European cancer register-based project intended to improve the standardisation and availability of population-based data on haematological malignancies in Europe. It covers approximately 30% of the European population. Forty-eight cancer registers, operating in 20 countries, had incidence data for at least one of the predefined study years (2000–2002) and were hence included in the HAEMACARE analysis.[2]

To use these data for the comparison with the RA cohorts, we had to consider that the spectrum of lymphoma subtypes, especially the portion of HL versus NHL, depends on the underlying age distribution of the population being investigated. In the general population, approximately 50% of HL cases, but only 10% of NHL cases, are diagnosed in subjects aged 45 or below. In the HAEMACARE cohort, the percentage of subjects with age ≤45 years was clearly higher (55%) than that in our RA cohorts (16%). Therefore, a lower proportion of incident HL cases are expected in our cohorts. For that reason, we used direct standardisation methods and calculated the expected numbers of HL, B-NHL and T-NHL in a general population in which the age group ≤45 years has the same proportion as in our sample. These expected numbers were used to calculate percentages of the corresponding subtypes and were compared with those observed in the RA cohorts. No adjustment was made when the spectra of B-cell lymphoma were compared.

RESULTS

Baseline characteristics of more than 120 000 patients with RA included in the analysis are shown in table 1. In total, 533 lymphoma cases were identified. Since patient-years (pyrs) were not available in the RATIO and GISEA registries, we excluded the 27 lymphoma cases from RATIO and the 12 cases from GISEA in the calculation of the IR. A total of 494 lymphoma cases were reported in 584 236 pyrs in the remaining registers, corresponding to an overall crude IR of 85 per 100 000 pyrs (95% CI 77 to 92). The crude IR was similar between bionaïve and TNFi-treated patients with RA, whereas a lower incidence was reported in patients exposed to rituximab (table 1).

Table 1 Baseline characteristics and crude incidence rate of lymphomas among biologic-naïve, TNFi, rituximab, tocilizumab or abatacept-treated patients with RA

	Bionaïve	TNFi	Rituximab	Tocilizumab	Abatacept	Total
No. of patients	71 088	47 864*	9094	2029	1708*	124 997*
Follow-up time (pyrs)	322 167	242 260*	29 810	2827	3352*	584 236*
Female (%)	72.1	74.8	79.0	80.1	78.0	73.7
Age mean (mean range)	61.1 (57–62)	55.0 (50–57)	57.9 (58–58)	55.9 (55–57)	57.5 (56–58)	58.5 (50–62)
No. of lymphomas	288	230	6	6	3	533
Incidence per 100 000 pyrs (95% CI)	89 (79–100)	81 (70–94)	20 (7–44)	177 (57–413)	60 (7–216)	85 (77–92)

*Because of the type of the register these data are missing from RATIO and GISEA, 38 incident TNFi-exposed lymphoma cases (RATIO: 27, GISEA: 11) and one abatacept-exposed patient (GISEA) were for that reason excluded from the calculation of the incidence rate.
GISEA, Italian Group for the Study of Early Arthritis; pyrs, patient-years; RA, rheumatoid arthritis; RATIO, French Research Axed on Tolerance of bIOtherapies; TNFi, tumour necrosis factor inhibitor.

Spectrum of lymphoma subtypes in patients with RA

The spectrum of lymphoma subtypes was analysed in multiple steps, corresponding to progressively more detailed classifications (tables 2 and 3).

To compare possible influences of the treatment on the subtype distribution of lymphomas we compared patients with RA by treatment groups. There were no significant differences in the distribution of HL versus B-NHL versus T-NHL between bionaïve patients and TNFi-treated patients (table 2). Similar results were found in each of two biologic registers (ARTIS and BSRBR-RA) with more than 30 lymphomas in both the bionaïve and TNFi groups, as well as in the subgroup of the remaining registers (table 2). Results of the remaining registers are provided in online supplementary table S1.

B-NHL cases were further stratified by subtype (table 3). The most frequent subtype in patients with RA was DLBCL, followed by follicular lymphoma and chronic lymphocytic leukaemia (CLL). No significant difference in B-NHL subtypes was observed between bionaïve and TNFi-treated patients (table 3).

The small numbers of HL and T-NHL cases did not allow further subtype analysis. No case of hepatosplenic T-cell lymphoma was detected.

Comparison between RA and the general population

After standardisation for age, the distribution of HL versus B-NHL versus T-NHL observed in the RA group with 9.5% HL, 83.8% B-NHL and 6.8% T-NHL was similar to the values estimated from the general population data (10.1% HL, 82.6% B-NHL and 7.3% T-NHL, table 2).

Comparison within the B-NHL subtype, however, showed that DLBCL was significantly over-represented in subjects with RA compared with the general population (56% of all B-NHL in RA vs 30% in the general population; table 3); whereas CLL was significantly less frequent (16% of all B-NHL in RA vs 38% in the general population; table 3).

DISCUSSION

The main aim of this collaborative study was to compare the distribution of lymphoma subtypes between TNFi-treated and bionaïve patients with RA. Interestingly, we did not find any significant differences in these subtype distributions, neither when comparing the broader groups of HL versus B-NHL versus T-NHL nor when comparing among the B-NHL subtypes. This is reassuring as it does not indicate any bidirectional effect

Table 2 Lymphoma subtype distribution (Hodgkin's, B-cell and T-cell lymphomas) in patients with RA in treatment groups. ARTIS and BSRBR-RA, both with more than 30 lymphomas in the bionaïve and TNFi groups, are shown separately to describe the robustness of the results

		Hodgkin's			B cell			T cell			NOS
	N total	n	%	95% CI	n	%	95% CI	n	%	95% CI	N excluded
Bionaïve											
ARTIS	197	13	6.6	3.3 to 11.8	174	88.3	82.1 to 93.0	10	5.1	2.6 to 8.8	19
BSRBR	30	5	16.7	5.1 to 37.0	22	73.3	50.9 to 88.6	3	10.0	1.8 to 29.1	4
Other	31	3	9.7	1.8 to 28.6	24	77.4	55.3 to 91.2	4	12.9	3.2 to 32.5	7
Total	258	21	8.1	4.7 to 12.9	220	85.3	79.3 to 90.0	17	6.6	3.6 to 11.2	30
TNFi											
ARTIS	52	6	11.5	4.0 to 26.2	40	76.9	61.1 to 88.3	6	11.5	4.0 to 26.2	7
BSRBR	77	11	14.3	6.5 to 25.9	63	81.8	69.4 to 90.6	3	3.9	0.7 to 12.1	10
Other	73	7	9.6	3.6 to 20.4	61	83.6	71.3 to 91.8	5	6.9	2.0 to 17.0	11
Total	202	24	11.9	7.0 to 18.3	164	81.2	74.1 to 87.3	14	6.9	3.3 to 12.3	28
Rituximab	6	0	0	0 to 50.0	5	83.3	32.9 to 99.7	1	16.7	0.3 to 67.2	0
Tocilizumab	5	0	0	0 to 56.0	5	100	44.0 to 100	0	0	0 to 56.0	1
Abatacept	3	0	0	0 to 74.4	3	100	25.6 to 100	0	0	0 to 74.4	0
RA total	474	45	9.5	6.6 to 13.2	397	83.8	79.3 to 87.6	32	6.8	4.3 to 10.0	59

BSRBR-RA, British Society for Rheumatology Biologics Register for Rheumatoid Arthritis; NOS, not otherwise specified; RA, rheumatoid arthritis; TNFi, tumour necrosis factor inhibitor.

Table 3 B-cell non-Hodgkin's lymphoma subtypes

	N total	Chronic lymphocytic leukemia/ small cell lymphoma		Lymphoplasmocytic lymphoma (Waldenstrom macroglobulinaemia)		Marginal zone lymphoma		Follicular lymphoma		Mantle cell lymphoma		Diffuse large B-cell lymphoma		Burkitt lymphoma	
		n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)
Bionaïve	184	28	15.2 (9.2 to 23.2)	4	2.2 (0.4 to 6.8)	1	0.5 (0 to 4.1)	33	17.9 (11.3 to 26.6)	5	2.7 (0.6 to 7.6)	113	61.4 (51.5 to 70.8)	0	0 (0 to 3.0)
TNFi	151	26	17.2 (10.1 to 26.8)	6	4.0 (1.1 to 10.1)	10	6.6 (2.6 to 13.6)	34	22.5 (14.3 to 32.6)	0	0 (0 to 3.6)	75	49.7 (38.6 to 60.8)	0	0 (0 to 3.6)
RTX	5	1	20.0 (1.4 to 79.6)	1	20.0 (1.4 to 79.6)	0	0 (0 to 62.9)	1	20.0 (1.4 to 79.6)	0	0 (0 to 62.9)	2	40.0 (2.8 to 90.6)	0	0 (0 to 62.9)
TOC	5	2	40.0 (2.8 to 90.6)	0	0 (0 to 62.9)	0	0 (0 to 62.9)	0	0 (0 to 62.9)	0	0 (0 to 62.9)	3	60.0 (9.4 to 97.3)	0	0 (0 to 62.9)
ABA	3	0	0 (0 to 80.7)	0	0 (0 to 80.7)	0	0 (0 to 80.7)	2	66.7 (5.0 to 99.8)	0	0 (0 to 80.7)	1	33.3 (0.2 to 95.0)	0	0 (0 to 80.7)
RA total	348	57	16.4 (11.6 to 22.2)	11	3.2 (1.3 to 6.4)	11	3.2 (1.3 to 6.4)	70	20.1 (14.7 to 26.4)	5	1.4 (0.3 to 4.0)	194	55.8 (48.4 to 62.9)	0	0 (0 to 1.6)
General population	28747	11019	38.3 (37.6 to 39.1)	1859	6.5 (6.1 to 6.9)	950	3.3 (3.0 to 3.6)	4881	17.0 (16.4 to 17.6)	1012	3.5 (3.2 to 3.8)	8538	29.7 (29.0 to 30.4)	488	1.7 (1.5 to 1.9)

ABA, abatacept; RA, rheumatoid arthritis; RTX, rituximab; TNFi, tumour necrosis factor inhibitor; TOC, tocilizumab.

of treatments by reducing the risk for some subtypes while increasing the risk of other subtypes. By contrast, the spectrum of lymphoma subtypes in our RA cohort showed significant differences from the spectrum described in the general population in Europe.[2] This has been suggested in previous studies,[10 27] and it is now confirmed by our analysis which is the largest to date. It is of great clinical importance as different lymphoma subtypes show different clinical behaviour, including wide heterogeneity in both prognosis and the preferred treatment approach.

The analysis of the spectrum of lymphoma subtypes is also of importance because there are hints that certain subtypes might be associated with certain therapies, for example, very rare cases of EBV-associated lymphoproliferative disease with MTX[11] and hepatosplenic T-cell lymphomas with TNFi.[12] Hepatosplenic T-cell lymphoma is a rare subtype with a very unfavourable prognosis and poor response to currently available treatment options. It occurs more often in chronically immunocompromised patients. There has been a safety concern regarding its occurrence in patients treated with TNFi, especially in young male patients with Crohn's disease.[12] However, a very thorough analysis of all T-cell lymphoma cases reported to the Food and Drug Administration between 2003 and 2010 suggested an increased T-cell NHL risk from TNFi use in combination with thiopurines but not from TNFi alone.[28] We did not find any cases of hepatosplenic T-cell NHL in our RA patient cohorts in over 240 000 pyrs of follow-up in patients with RA exposed to TNFi, in 320 000 bionaïve pyrs or in the 36 000 pyrs in patients exposed to rituximab, abatacept or tocilizumab. Whether there were cases hidden among the group of 12 'T-cell NHL not otherwise specified,' of which five cases were in the TNFi group, remains speculative.

In a recent Swedish cohort, an increased risk of HL in patients with RA compared with the general population and compared with previously reported RA cohorts has been described.[6] There is a strong association between chronic inflammation and development of HL.[6 29] In our analysis, there was a slight numerical but not statistically significant increase in the proportion of HLs between bionaïve and TNFi-treated patients.

The development of lymphomas can occur over a prolonged period of time, with several months or years elapsing between the onset of lymphomagenesis and diagnosis. Therefore, clinical trials with their short follow-up times are not an appropriate method of studying these malignancies, whereas registers provide a unique opportunity to do so. In addition to the large sample size of 533 lymphoma cases, the largest published RA-lymphoma cohort to date, the strength of our study is the usage of clearly stated definitions for the subtypes of lymphomas. All registers used the same template to define subtypes based on the WHO 2008 classification.[30] Ideally, central pathological review of lymphoma specimens would have been preferable to standardise the lymphoma subtype classification; however, for feasibility reasons, this was not possible.

Another strength is the long follow-up time for individual patients, which is the prerequisite for analysing these safety events. Thanks to the use of unselected patients without any exclusion criteria we are confident that our results are representative of patients with RA from across Europe.

Despite the huge data set of more than 120 000 patients we were not able to analyse all different RA treatments separately for subtype distribution due to small numbers. For example, only six, six and three lymphomas occurred in patients treated with rituximab, tocilizumab and abatacept, respectively, at lymphoma diagnosis. Another limitation is the fact that the bionaïve patients are older than the bDMARD group (mean age 61

vs 55). Since age is an important factor in lymphomagenesis, the comparison between the treatment groups might be affected by this age difference.

Due to feasibility reasons, the patients were grouped into treatment groups according to the bDMARD that they have received most recently before the lymphoma diagnosis. A potential limitation is that we cannot exclude an influence of bDMARDs used prior to the last one. Furthermore, we could not analyse any potential influence of additional therapies with MTX or other conventional synthetic DMARDs.

The attribution of rare events such as lymphoma in RA to the respective RA treatment is complex. First, there is an increased lymphoma risk in patients with RA compared with the general population.[3 4 31] Second, the disease activity of RA has been identified as being of outmost importance for the development of lymphoma.[10] However, disease activity changes over time and is in itself dependent on the RA treatment. In addition, disease activity is one of the strongest factors in the treatment decision; therefore, there is a considerable confounding by indication when analysing this context. Hence, the bionaïve patients are different from the bDMARD-treated patients, since bDMARDs are used in those patients with more severe disease. It is therefore reassuring that in the bDMARD group with an even higher a priori lymphoma risk due to higher cumulative disease activity the risk is not higher than in the bionaïve patients.

We were confronted with other limitations typical for collaborative studies on register data, namely that collating data from different registers does not alter the quality of data from each register. We therefore depended on the validity of each subcohort. The impact of a possible heterogeneity in the results of the registers was partly examined in a descriptive manner by showing results of the two largest registers ARTIS and BSRBR separately. Separate results of all registers are furthermore shown in online supplementary table S1.

CONCLUSION

The evidence is growing that the risk of lymphoma in RA is more dependent on RA itself and especially the disease activity than on the RA treatment.[5 13] Furthermore, our results are reassuring as the spectrum of lymphoma subtypes seems not to be altered by TNFi.

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EXTENDED REPORT

Calprotectin as a marker of inflammation in patients with early rheumatoid arthritis

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ABSTRACT

Objectives Calprotectin is an inflammatory marker of interest in rheumatoid arthritis (RA). We evaluated whether the level of calprotectin was associated with disease activity, and if it was predictive of treatment response and radiographic progression in patients with early RA.

Methods Plasma from disease-modifying antirheumatic drug (DMARD)-naïve patients with RA fulfilling 2010 American College of Rheumatology/European League Against Rheumatism classification criteria with symptom duration <2 years was analysed for calprotectin at baseline, and after 1, 3 and 12 months. All patients received treat-to-target therapy, as part of a randomised controlled strategy trial (ARCTIC). The association between calprotectin, erythrocyte sedimentation rate (ESR) and C reactive protein (CRP) and measures of disease activity were assessed by correlations. We used likelihood ratios and logistic regression models to assess the predictive value of the baseline inflammatory markers for treatment response and radiographic damage.

Results 215 patients were included: 61% female, 82% anti-citrullinated peptide antibody positive, mean (SD) age 50.9 (13.7) years and median (25, 75 percentile) symptom duration 5.8 (2.8, 10.5) months. Calprotectin was significantly correlated with Clinical Disease Activity Index (r=0.32), ESR (r=0.50) and ultrasonography power Doppler (r=0.42) before treatment onset. After 12 months of treatment, calprotectin, but not ESR and CRP, was significantly correlated with power Doppler (r=0.27). Baseline levels of calprotectin, ESR and CRP were not predictive of treatment response, but high levels of calprotectin were associated with radiographic progression in multivariate models.

Conclusions Calprotectin was correlated with inflammation assessed by ultrasound before and during DMARD treatment, and was also associated with radiographic progression. The data support that calprotectin may be of interest as an inflammatory marker when assessing disease activity in different stages of RA.

Trial registration number NCT01205854; Post-results.

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, inflammatory disease of complex pathogenesis that can lead to joint damage and loss of function.[1] Current treatment recommendations include early initiation of conventional synthetic disease-modifying antirheumatic drugs (csDMARDs) with tight control and a defined treatment target.[1–3] Laboratory assessment of inflammatory activity relies mainly on erythrocyte sedimentation rate (ESR) and C reactive protein (CRP).

Calprotectin is a calcium-binding leucocyte protein consisting of the heterocomplex of S100A8/A9 (myeloid-related protein, MRP8/MRP14), which has gained interest as a marker of inflammation in RA.[4–10] This protein is classified as a damage-associated molecular pattern molecules, shown to be highly elevated in various immune-mediated inflammatory diseases, and is a validated marker of disease activity in inflammatory bowel diseases.[11 12] Calprotectin is mainly expressed in granulocytes and monocytes,[13] predominantly at the sites of inflammation.[14] In RA, calprotectin has also been identified in macrophages and fibroblast-like synoviocytes of the synovium.[15 16] Calprotectin can be measured in both synovial fluid and serum/plasma.[17 18] EDTA plasma is the preferred medium due to the inhibitory effect of EDTA on calprotectin release from leucocytes in vitro.[19] Previous studies have found good correlations between calprotectin concentrations in plasma and synovial fluid.[20 21] Patients with RA have higher calprotectin levels than those with osteoarthritis or spondyloarthritis.[18 20 22]

Serum and plasma levels of calprotectin are associated with levels of ESR, CRP and Disease Activity Score for 28 joints (DAS28) in established RA.[7 9] Calprotectin has been shown to be sensitive to change as well as a strong predictor of response to biologic DMARDs (bDMARDs) in patients with established RA who did not respond satisfactorily to csDMARDs,[23–25] although data are somewhat conflicting.[10] In early RA, calprotectin has been shown to decrease with csDMARD therapy,[8] and one study showed high baseline levels to predict response to methotrexate in patients with active disease (ie, DAS28>3.2).[26] Baseline calprotectin is associated with levels as well as presence of anti-citrullinated peptide antibody (ACPA) and rheumatoid factor (RF).[10 27 28] Hammer *et al* have found calprotectin levels to be associated with radiographic progression in patients with RA.[28]

In ultrasound examination of patients with RA, the presence of power Doppler signals reflects active inflammation in the synovium, and is associated with radiographic progression in early RA.[29 30]

Serum and plasma levels of calprotectin are associated with ultrasound assessments of RA disease activity,[9 23 25] and elevated levels of calprotectin may indicate sustained inflammation in patients in remission or low disease activity according to DAS28.[31]

Previous studies of calprotectin in RA have been performed mainly in smaller cohorts and/or cross-sectionally, and in patients classified according to the American College of Rheumatology (ACR) 1987 criteria. Our aim was to explore the associations of calprotectin, ESR and CRP with clinical and ultrasound measures of inflammation in patients with early RA classified according to 2010 ACR/European League Against Rheumatism (EULAR) criteria,[32] before and after aggressive treat-to-target treatment. We also assessed if calprotectin levels were predictive of radiographic progression and response to initial treatment with methotrexate.

MATERIALS AND METHODS

Patients

A total of 230 DMARD-naïve patients with indication for DMARD therapy who fulfilled the 2010 ACR/EULAR classification criteria for RA[32] were included in the ARCTIC study between September 2010 and April 2013.[33] All patients were aged 18–75 years and had symptom duration <2 years. Patients with clinical data and plasma samples available at baseline (n=215) and at 1 (n=168), 3 (n=172) and 12 months (n=164) were included in the present analyses. The study was conducted in compliance with the Declaration of Helsinki and the International Conference on Harmonisation Guidelines for Good Clinical Practice. All patients provided a written informed consent.

Study design and treatment

All patients were followed according to a tight control regimen with treatment targeting no swollen joints and DAS <1.6,[34] and in half of the patients the treatment target also included no joints with ultrasound power Doppler activity.[33] Initial treatment consisted of methotrexate monotherapy 15 mg/week escalating to 20–25 mg/week and prednisolone starting at 15 mg with tapering to stop over 7 weeks.[33] If the treatment target was not achieved, treatment was intensified following a predetermined treatment protocol, with escalation to triple therapy and then bDMARDs.[33] Swollen joints and/or joints with power Doppler activity could be injected with triamcinolone hexacetonide (up to a maximum of 80 mg per visit).

Laboratory examinations

Blood samples were collected in EDTA tubes at inclusion and after 1, 3 and 12 months, centrifuged within 30 min for 11 min, and the plasma was stored at −70°C. Calprotectin was analysed by a calprotectin ELISA alkaline phosphatase kit from CalproLab (Oslo, Norway). All samples from the individual patients were analysed on the same plate. Colour intensity was read at 405 nm wavelength by an eMax reader from Molecular Devices (Sunnyvale, California, USA) using Soft Max pro software and a Synergy H1 Hybrid Reader from BioTek (Winooski, Vermont, USA). Normal median value (25, 75 percentile) of calprotectin was provided by the manufacturer by assessment of plasma from 100 healthy blood donors and was 560 (412, 788) µg/L. ESR and CRP were analysed locally by in-house standard methodology.

Clinical and imaging assessments

Clinical joint examination was performed with 44 swollen joint count and Ritchie Articular Index for tender joints.[35] Patients and physicians reported their overall assessment of disease activity on Visual Analogue Scale, range 0–100. The composite indices DAS and Clinical Disease Activity Index (CDAI) were calculated.[34 36] CDAI was preferred as a composite measure of disease activity in most analyses as it does not include ESR or CRP, and was thus considered most suitable for comparisons between calprotectin, ESR and CRP. Clinical remission in the current analyses was defined as CDAI≤2.8.[36] Treatment response after 4 months was defined by improvement from baseline of 50%, 70% or 85% in CDAI score (ie, CDAI50, CDAI70 and CDAI85) and/or EULAR good/moderate response.[37 38]

Radiographic examinations of hands and feet were performed at baseline and 24 months, and images were scored according to the van der Heijde modified Sharp score (vdHSS).[39] Scoring was performed in chronological order by two trained readers blinded for clinical information, and the average of the two scores was used. Radiographic progression was defined as a change in vdHSS of ≥2 units over 2 years, which is above the smallest detectable change (1.94 units).

Ultrasound was performed according to a validated semiquantitative 32-joint protocol with scores 0–3 separately for grey scale synovitis and for power Doppler.[40] Half the patients underwent ultrasound examination at all visits, while the remaining patients were examined at baseline, 12 months and 24 months.[33] Examiners were thoroughly trained, and an atlas was available for reference.[40] Ultrasound remission was defined as no power Doppler activity in any examined joint.

Statistical analysis

Baseline characteristics of the patients with complete calprotectin data were compared with all patients using X^2 and t test as appropriate. Correlations were assessed using the Spearman's rank correlation coefficients due to non-normal distribution of most variables. Spearman's correlations were defined as very weak: <0.19; weak: 0.20–0.39; moderate: 0.40–0.59; strong: 0.60–0.79; and very strong: 0.8–1.0.[41] Changes in calprotectin levels according to fulfilment of remission criteria were compared between groups using Mann-Whitney U test. Levels of calprotectin, ESR and CRP at different time points were compared using the Wilcoxon signed-rank sum test. Sensitivity to change after 1, 3 and 12 months was assessed using standardised response means (SRMs, mean change divided by the SD of the change scores). Ninety-five per cent CIs for the SRMs were calculated by applying bootstrapping techniques with 5000 replications. Due to non-normal distribution, calprotectin, ESR and CRP values were log transformed prior to calculating SRMs. The thresholds introduced by Cohen for effect sizes were applied to interpret the magnitude of the SRMs: trivial: <0.20; small: ≥0.20–0.50; moderate: ≥0.50–0.80; and large: ≥0.80.[42] Likelihood ratios (LR) for CDAI70 and EULAR good/moderate response to methotrexate were calculated in quartiles of calprotectin, ESR and CRP by sensitivity/1-specificity. Calprotectin, ESR and CRP area under the curve (AUC) for measurements at baseline, and after 1, 3 and 12 months were calculated using the trapezoid rule.[43] The associations between baseline variables, including calprotectin, ESR and CRP (both in categories based on quartiles and as AUC 0–12 months), and radiographic progression after 24 months were tested in univariate and multivariate logistic regression models. The multivariate model included quartiles of calprotectin, ESR, CRP, and variables for adjustment (age, gender, RF and CDAI). A p value of <0.05 was considered statistically significant. Statistical analyses were

Table 1 Baseline characteristics

Characteristics (n=215)	
Age* (years)	50.9 (13.7)
Women (% (n))	61 (132)
Body mass index* (kg/m^2)	25.8 (4.6)
Ever smoker (% (n))	67 (144)
Time since patient reported first swollen joint† (months)	5.8 (2.8, 10.5)
Anti-citrullinated peptide antibody positive (% (n))	82 (177)
Rheumatoid factor positive (% (n))	71 (153)
Disease Activity Score*(0–10)	3.5 (1.2)
Clinical Disease Activity Index*(0–76)	23.4 (12.0)
Patient's global assessment of disease activity† (mm, 0–100)	49 (31, 70)
Investigator's global assessment of disease activity† (mm, 0–100)	36 (23, 55)
Swollen joint count†(0–44)	9 (4, 14)
Ritchie Articular Index†(0–78)	7 (4, 13)
Calprotectin† (µg/L)	1045 (567, 2235)
Erythrocyte sedimentation rate† (mm/h)	19 (11, 32)
C-reactive protein† (mg/L)	7 (3, 18)
Total van der Heijde modified Sharp score†(0–448)	4 (1.5, 8)
Erosion score (0–280)	3 (1, 4.5)
Joint Space Narrowing score (0–168)	1 (0, 3)
Ultrasound grey scale score†(0–96)	17 (10, 27)
Ultrasound power Doppler score†(0–96)	7 (3, 14)

*Mean (SD).
†Median (25,75 percentile).
h, hour; kg, kilogram; L, litre; m^2, square metre; µg, microgram; mg, milligram; mm, millimetre.

performed using Stata Statistical Software, V. 14 (StataCorp LLC, Texas, USA).

RESULTS

Patient characteristics

A total of 215 patients were included in this study: 61% female, 71% RF positive and 82% ACPA positive. The mean (SD) age was 50.9 (13.7) years and median (25,75 percentile) symptom duration was 5.8 (2.8,10.5) months. Further baseline characteristics are provided in table 1.

We found no statistically significant differences with regard to age, gender, body mass index, smoking status, DAS or CDAI for patients with complete calprotectin data compared with the full analysis set (data not shown).

Correlations between calprotectin and other markers of inflammation

Calprotectin, ESR and CRP correlated moderately to strongly with each other at baseline, and weakly to moderately after 12 months of DMARD treatment (table 2). Calprotectin was weakly to moderately correlated with CDAI and ultrasound scores before treatment onset, and the correlation coefficients were overall similar to those observed for ESR and CRP (table 2). After 12 months of treatment, calprotectin had a weak, but statistically significant correlation with grey scale and power Doppler ultrasound scores (table 2). No associations were observed between ESR/CRP and ultrasound scores at this time point (table 2).

Changes in calprotectin after initiation of DMARD treatment

Calprotectin levels decreased after 1, 3 and 12 months of treatment (figure 1), with a baseline median value of 1045 (567, 2235) µg/L and a median value after 12 months of 485 (296, 805) µg/L. ESR and CRP also decreased in the same period (online supplementary figure S1).

Sensitivity to change

SRMs for calprotectin were moderate to large, and comparable to ESR and CRP. Higher values were observed for other measures of inflammation and disease activity (figure 2), with the highest values for composite disease activity indices (CDAI and DAS).

Calprotectin and levels of disease activity

Levels of calprotectin, ESR and CRP numerically increased with categories of disease activity according to CDAI (figure 3, online supplementary figure S2). Calprotectin levels were significantly lower in patients who were in remission according to CDAI compared with patients not in remission, both at 1 and 3 months (online supplementary table S1). The same trend was found for median levels of CRP, while for ESR there was only a significant difference at 12 months (online supplementary table S1).

Calprotectin, ultrasound inflammation and CDAI remission

Patients in ultrasound remission (defined as power Doppler=0) had significantly lower median levels of calprotectin than those who were not in ultrasound remission after 1 month (519 (366, 777) vs 707 (505, 1160) µg/L; p value=0.001), 3 months (462 (349, 758) vs 605 (374, 1033) µg/L, p value=0.04), and 12 months (427 (283, 730) vs 702 (400, 1266) µg/L, p value<0.001). This association was not found for ESR and CRP (data not shown). When assessing only patients in CDAI

Table 2 Correlations between calprotectin/ESR/CRP and clinical/ultrasound measures of inflammation

	Baseline n=215			12 months n=164		
	Calprotectin	ESR	CRP	Calprotectin	ESR	CRP
Calprotectin	NA	0.50***	0.66***	NA	0.42***	0.33***
ESR	0.50***	NA	0.63***	0.42***	NA	0.25**
CRP	0.66***	0.63***	NA	0.33***	0.25**	NA
SJC44	0.31***	0.26***	0.38***	0.09	0.12	0.12
RAI	0.21**	0.16*	0.32***	0.09	0.12	0.16*
CDAI	0.32***	0.25***	0.45***	0.22**	0.18*	0.15*
US GS	0.46***	0.30***	0.42***	0.20**	0.00	0.08
US PD	0.42***	0.35***	0.36***	0.27***	0.03	0.00

Spearman's correlation coefficients
*p value<0.05; **p value<0.01; ***p value<0.001.
CDAI, Clinical Disease Activity Index; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; GS, grey scale; PD, power Doppler; RAI, Ritchie Articular Index; SJC44, swollen joint count 44; US, ultrasound.

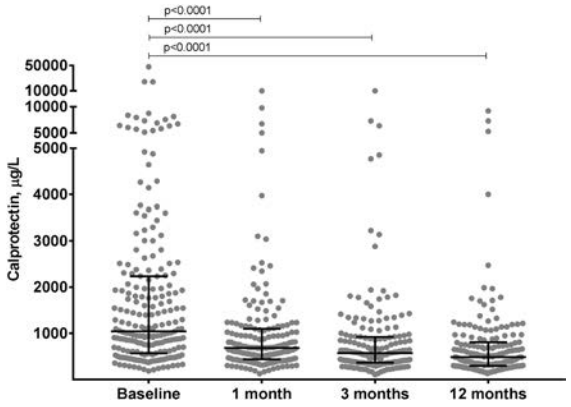

Figure 1 Calprotectin values at baseline, and after 1, 3 and 12 months of disease-modifying antirheumatic drug treatment. Centre bar indicates median value and error bars 25 and 75 percentile. p Value indicates comparison with baseline (Wilcoxon signed-rank test). L, litre; µg, microgram.

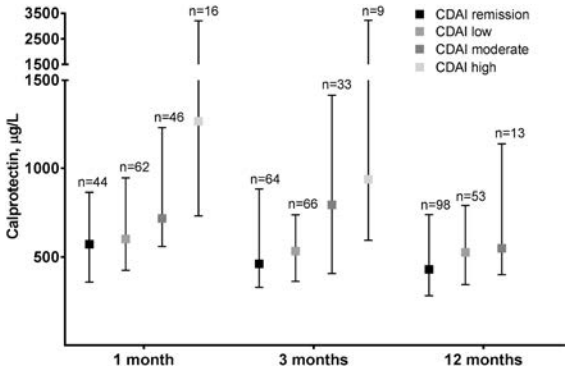

Figure 3 Calprotectin levels in patients in remission, low, moderate and high disease activity according to CDAI after 1, 3 and 12 months of disease-modifying antirheumatic drug treatment. Median values, error bars indicating 25 and 75 percentile. CDAI, Clinical Disease Activity Index; L, litre; µg, microgram.

remission, levels of calprotectin at 12 months were significantly lower for patients in both CDAI and ultrasound remission (n=82) compared with those in only CDAI remission (358 (258, 705) µg/L vs 904 (498, 1393); p value=0.001; online supplementary table S1). Thirty-five per cent (n=29) of the patients in both CDAI and ultrasound remission had calprotectin levels above the median value seen in healthy controls (>560 µg/L; data not shown). There were no statistically significant differences for ESR and CRP (online supplementary table S1).

Calprotectin as a predictor of methotrexate response

According to the treatment algorithm, medication was changed to triple therapy at 4 months if not responding to methotrexate monotherapy, thus making this the last visit to assess methotrexate response in all patients. Of the 215 patients at baseline, 194 had clinical data at 4 months. At this time point, 82% (n=159) had reached CDAI50 response, 64% (n=125) CDAI70 and 39% (n=76) CDAI85. There was no difference in baseline calprotectin when comparing patients obtaining CDAI70 at 4 months with those who did not reach the same state (online supplementary figure S3). Neither did assessing changes in calprotectin between baseline and 1 month in patients with baseline calprotectin above median (>1045 µg/L) discriminate between responders and non-responders (online supplementary figure S3). The same was found for ESR and CRP (data not shown). LRs (=sensitivity/1-specificity) for response to methotrexate at 4 months were comparable across quartiles of baseline calprotectin, ESR and CRP, both when assessing CDAI70 and EULAR good/moderate response (online supplementary table S2). Similar results were found when assessing other cut-offs for CDAI response (CDAI85 and CDAI50) and changes in levels of calprotectin, ESR and CRP after 1 month of treatment (data not shown).

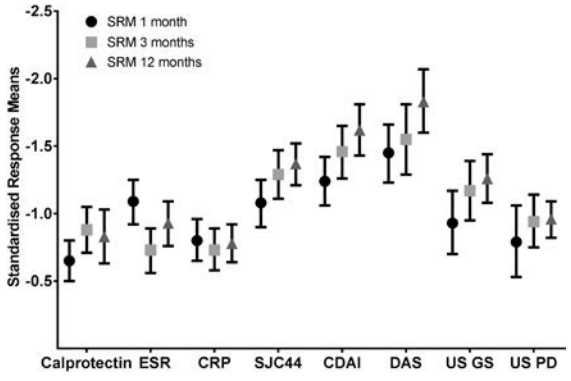

Figure 2 Standardised response means (SRMs) for measures of inflammation and disease activity after 1, 3 and 12 months of disease-modifying antirheumatic drug treatment. Mean values, error bars indicating 95% CIs. CDAI, Clinical Disease Activity Index; CRP, C reactive protein; DAS, Disease Activity Score; ESR, erythrocyte sedimentation rate; GS, grey scale; PD, power Doppler; SJC44, swollen joint count 44; US, ultrasound.

Calprotectin and radiographic damage

During the 2 years of follow-up, radiographic progression occurred in 41% of the patients with a median change in vdHSS of 1 (0.5, 3). Baseline calprotectin was correlated with change in vdHSS after 2 years (r=0.33; p value<0.0001) as was calprotectin AUC after 12 months (r=0.34; p value<0.0001). In univariate analyses of calprotectin categorised according to quartiles, the highest quartile was associated with radiographic progression, with an OR of 6.1 (95% CI 2.62 to 14.0) (table 3). Similarly, the highest quartiles of ESR and CRP predicted progression of radiographic damage in univariate models (table 3). In a multivariate model including calprotectin, ESR and CRP, in addition to age, gender, CDAI and RF status, calprotectin remained a significant predictor of radiographic damage, while no such association was found for ESR and CRP (table 3). When using time-integrated measures of calprotectin, ESR and CRP during the first 12 months, the same trend was seen in both univariate and multivariate analyses, and calprotectin, but not ESR and CRP, remained a significant predictor of radiographic damage in the multivariate model (data not shown).

DISCUSSION

In this inception cohort, calprotectin was associated with clinical measures of disease activity as well as ultrasound inflammation in treatment-naïve patients with RA. High baseline level of calprotectin was a predictor of radiographic progression in univariate and multivariate models, also when adjusting for ESR and CRP.

Median calprotectin values decreased significantly after 1 month of treatment. Previous publications have demonstrated calprotectin to be a significant predictor of response to biological treatment in patients with RA who have failed conventional

Table 3 Predictors of radiographic progression ≥1 unit/year from 0 to 24 months

	Univariate		Multivariate	
Baseline variables	**OR**	**p Value**	**OR**	**p Value**
Age	**1.04 (1.02 to 1.07)**	**<0.001**	**1.04 (1.01 to 1.06)**	**0.003**
Gender (female)	0.61 (0.35 to 1.07)	0.09	0.71 (0.37 to 1.37)	0.31
Calprotectin quartile (range)				
First quartile (186–556 µg/L)	Ref.	Ref.	Ref.	Ref.
Second quartile (567–1028 µg/L)	1.51 (0.66 to 3.46)	0.33	1.52 (0.60 to 3.87)	0.38
Third quartile (1045–2158 µg/L)	1.39 (0.61 to 3.20)	0.44	1.04 (0.39 to 2.74)	0.94
Fourth quartile (2235–48079 µg/L)	**6.06 (2.62 to 14.02)**	**<0.001**	**3.65 (1.23 to 10.87)**	**0.02**
ESR, quartile (range)				
First quartile (1–10 mm/hour)	Ref.	Ref.	Ref.	Ref.
Second quartile (11–18 mm/hour)	1.07 (0.47 to 2.43)	0.87	0.73 (0.29 to 1.84)	0.51
Third quartile (19–31 mm/hour)	1.26 (0.55 to 2.86)	0.59	0.89 (0.34 to 2.35)	0.81
Fourth quartile (32–110 mm/hour)	**3.74 (1.64 to 8.52)**	**0.002**	1.04 (0.31 to 3.50)	0.95
CRP, quartile (range)				
First quartile (0.3–2.8 mg/L)	Ref.	Ref.	Ref.	Ref.
Second quartile (3–6 mg/L)	0.69 (0.29 to 1.64)	0.41	0.42 (0.16 to 1.10)	0.08
Third quartile (7–16 mg/L)	1.29 (0.54 to 3.04)	0.57	0.62 (0.22 to 1.79)	0.38
Fourth quartile (18–117 mg/L)	**2.85 (1.20 to 6.76)**	**0.02**	0.74 (0.21 to 2.62)	0.64
CDAI	1.02 (1.00 to 1.04)	0.08	1.01 (0.98 to 1.04)	0.39
RF positivity	1.86 (0.99 to 3.48)	0.053	1.87 (0.89 to 3.91)	0.10

n=215. p Values<0.05 in bold.
CDAI, Clinical Disease Activity Index; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; Ref, reference category (lowest quartile as reference); RF, rheumatoid factor.

treatment,[23–25] and a recent study found calprotectin to predict early response to methotrexate in DMARD-naïve patients with RA with high disease activity.[26] However, in our broad RA population neither baseline calprotectin levels nor changes in calprotectin levels after 1 month were useful to differentiate between clinical responders and non-responders to methotrexate after 4 months. Based on the magnitude of the SRMs, we found calprotectin to have similar responsiveness as ESR and CRP over time, while composite and ultrasound measures of disease activity were considerably more sensitive to change than the inflammatory markers. In a previous study, calprotectin had more favourable SRMs than ESR and CRP, but comparability is limited as calculations were not based on log-transformed values and the population differed as patients had established RA with indication for biological treatment.[23]

Patients may have inflammatory activity in the joints detected by ultrasound or MRI, despite being in clinical remission.[30] Brown *et al* concluded that imaging of subclinical joint inflammation could explain the structural deterioration in patients with RA in clinical remission, while Scire *et al* found associations between absence of power Doppler and stable remission.[30 44 45] In our study, plasma calprotectin was significantly lower in patients who were in remission according to CDAI compared with those who were not in remission. Patients who were in ultrasound remission (ie, had no power Doppler activity in any examined joint) had lower median levels of calprotectin than those who were not in ultrasound remission, in line with previous findings.[31]Patients who were both in remission according to CDAI and ultrasound remission had lower levels of calprotectin at 12 months than those who were in clinical remission with persistent power Doppler activity. These observations indicate that calprotectin might contribute clinically relevant information regarding subclinical inflammation in patients with RA who are in clinical remission.

Macrophages and fibroblast-like synoviocytes are central cells in the pannus region and in the process of joint destruction.[46] Polymorphonuclear granulocytes have also been identified in this area,[47] and are abundant in the synovial fluid. As calprotectin can be released from these cells during inflammation,[13 15 16] the plasma concentration may to a certain degree reflect the local inflammatory processes inducing joint damage. Calprotectin AUC 0–12 months and baseline calprotectin were both correlated to change in vdHSS, and we found calprotectin levels, both as a continuous variable, divided into quartiles and as AUC 0–12 months, to be univariately associated with radiographic progression at 24 months. Similarly, the highest quartiles of ESR and CRP at baseline were associated with an increased risk of radiographic progression. However, in multivariate models, calprotectin was the only of the three inflammatory markers which independently predicted radiographic progression, both assessed at baseline and as AUC 0–12 months. These findings correspond well to previous results in patients followed for 10 years before the implementation of biological treatment and treat-to-target in RA care,[28] and support an association between high levels of calprotectin and radiographic progression, even with modern treatment.

Potential limitations of the study were that ESR and CRP were analysed locally at time of assessment, while calprotectin was analysed centrally after the completion of the study. Plasma had been frozen for 3 to 5 years at −70°C before the calprotectin analyses were performed, and little is known regarding deterioration of samples at −70°C, although previous studies have analysed samples that have been stored for >5 years.[4 24 28] The current study is strengthened by a relatively large sample size compared with most previous studies evaluating calprotectin in RA. This study is also to our knowledge the first exploring the performance of calprotectin relative to ESR and CRP in an inception cohort of patients with early RA fulfilling the 2010 ACR/EULAR classification criteria.[32] Another strength of the study was that all patients were DMARD and corticosteroid naïve at inclusion, and treated according to a standardised treatment protocol adhering to current treatment recommendations,[33] enabling assessment of

changes in inflammatory markers after initiation of csDMARD treatment.

In this study, high levels of calprotectin were associated with radiographic progression, also when adjusting for ESR and CRP in multivariate analyses. Calprotectin had a stronger association to ultrasound inflammation at baseline and during follow-up than both ESR and CRP, including assessments of subclinical inflammation in RA remission. However, calprotectin was not found to be a predictor of clinical treatment response to methotrexate monotherapy, and the sensitivity to change was similar to ESR and CRP. Our data suggest that calprotectin may be of interest as an inflammatory marker to assess disease activity in different stages of RA, especially at treatment onset and in patients in clinical remission. Further studies are needed to assess the clinical relevance of calprotectin as a marker of inflammation.

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Contributors All authors were involved in drafting the article or revising it critically for important intellectual content and approved the final manuscript to be submitted and agreed to be accountable for all aspects of the work. Conception and design of the study: EAH, SL, MKJ, B-TSF, ICO, HBH, DvdH and TKK. Acquisition of data: MKJ, EAH, ABA, HBH, KAB and DvDH. Analysis and interpretation of data: MKJ, EAH, SL, B-TSF, NPS, HHN, A-BA, KAB and ICO.

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Competing interests All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf (available at request from the corresponding author) and declare that NPS, HHN, HBH, A-BA, KAB, DvdH, B-TSF and SL have no competing interests.

Patient consent Obtained.

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EXTENDED REPORT

The effects of structural damage on functional disability in psoriatic arthritis

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ABSTRACT

Background Functional outcomes are central in patients with chronic inflammatory musculoskeletal diseases. In a secondary data analysis of the GO-REVEAL trial (NCT00265096), we investigated wether structural damage is linked to functional impairment in patients with psoriatic arthritis (PsA), a link that is still elusive in this disease.

Methods We analysed 363 patients enrolled in the GO-REVEAL study and obtained modified Sharp/van der Heijde Scores (mSvdHS) from X-rays performed at baseline, after 24, 52 and 104 weeks. Using longitudinal analyses, we assessed the effect of total mSvdHS (and its subscores, joint space narrowing (JSN) and erosions (ERO)) on functional status (measured by the Health Assessment Questionnaire) in all patients and in those attaining remission (n=117). Furthermore, we analysed wether structural damage reduces the responsiveness of functional limitations to treatment in a subgroup of responders who had functional impairment at baseline (n=67). Additionally, internal and external validation analyses were performed.

Results The effect of damage on function was seen in the disease activity-adjusted models using total mSvdHS (p=0.005), JSN (p=0.019) and ERO (p=0.001) as well as in the remission analyses for mSvdHS (p=0.029) and JSN (p=0.010), respectively. Functional responsiveness was limited by increasing total mSvdHS (p=0.010), JSN (p=0.002) and ERO (p=0.040). The results were validated using other functional outcomes and in an independent clinical cohort.

Conclusions In PsA, structural damage, particularly JSN, has implications for physical function. Functional outcomes have an irreversible component that is strongly related to the extent of joint destruction. This needs to be considered when targeting functional outcomes in clinical practice.

INTRODUCTION

Psoriatic arthritis (PsA) is a chronic inflammatory disease that affects the musculoskeletal system in multiple ways. Aside from overt peripheral arthritis, inflammation of entheses and the axial skeleton, is part of the disease spectrum. Particularly, the inflammatory process of the peripheral joints can lead to substantial cartilage and bony destruction, but also bony overgrowth.[1] While physical function is strongly affected by the active inflammatory process that leads to pain, swelling and stiffness ('disease activity'), it is conceivable that also the aforementioned joint damage leads to functional limitations over time. Similar to rheumatoid arthritis (RA),[2] disease activity is associated with joint damage in PsA.[3] Moreover, in RA,[4 5] there is also a well-established link between structural damage and disability, and this link is even tighter for cartilage damage than for bony damage.[6] Although peripheral joint damage is generally greater in RA than in PsA,[7] there is still evidence for some association of joint damage with disability in the latter.[3] This is indicated by larger functional impairment with increasing disease duration but not necessarily conclusive results regarding damage.[8]

Here, we investigated in detail if and to what extent joint destruction and functional status are linked in patients with PsA. The results of this study should therefore allow to estimate the functional impact of structural damage in PsA. Also, we investigated to what extent functional improvement is limited by fixed, damage-related functional components, and whether this is related to changes of cartilage or bone.

METHODS

Patients and assessments

In the present study, we performed a secondary analysis on patients who had been enrolled in the Golimumab — A Randomized Evaluation of Safety and Efficacy in Subjects with Psoriatic Arthritis Using a Human Anti-TNF Monoclonal Antibody (GO-REVEAL) study (trial registration number: NCT00265096) comparing golimumab with placebo in 405 patients with PsA.[9] The institutional review boards and ethics committees of all participating centres had approved the study and informed consent of all patients included in the GO-REVEAL trial were obtained prior to study participation. The sponsor limited the provision of patient level data to a random cut of 90% for our secondary data analyses. Among the patients, 43% had polyarticular and 57% had oligoarticular disease. We extracted modified Sharp/van der Heijde Scores (mSvdHS),[10 11] by which the structural damage was quantified in the trial at baseline and after 24, 52 and 104 weeks in all patients (n=363). The mSvdHS is based on scoring of erosions (ERO) and joint space narrowing (JSN), with a maximum score of 320 for ERO and 208 for JSN, resulting in an mSvdHS ranging from 0 to 528.[10] The smallest detectable change in the GO-REVEAL trial was 1.56 for the total score, 1.18 for ERO and 0.96 for JSN.[9] For assessment of disease activity, we calculated the Disease Activity Index for Psoriatic Arthritis (DAPSA),[12] which allows a metric quantification of disease activity at every clinical visit (DAPSA=SJC66+TJC68+Patient Global (0–10)+Patient Pain (0–10)+CRP

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(mg/dL)). Functional status was assessed using the traditional Health Assessment Questionnaire (HAQ) disability index, which has been commonly used in PsA.[3 13–16]

Influence of disease activity on physical function

As a first step, we evaluated the association of disease activity with the HAQ, in line with a similar previous analysis.[17] To this end, a longitudinal data model using generalised estimating equations (GEE) was developed. The GEE methodology provides the possibility to take multiple observations of each individual patient into account and simultaneously allows to adjust for different independent variables (e.g. disease activity, X-ray score) of each patient's observation. As HAQ, disease activity and radiographic scores may change across each patient's study visit, statistical methods as the GEE method allow to account for this aspect and provide overall effect associations, while adjusting for changes on an individual patient level.

In our analyses, HAQ was used as the outcome variable, with visit and DAPSA as independent variables. An autoregressive variance–covariance correlation matrix (AR(1)) was chosen based on the best GEE Fit Criteria.[18]

Since in a state of low disease activity (LDA), minor changes of disease activity may influence physical function more than in high disease activity states, we used a multistep approach including also quadratic and cubic terms of DAPSA in the model. While the cubic term did not show significant results, the quadratic model did and was therefore chosen for analysis of the influence of disease activity on physical function.

Influence of structural damage on physical function

For analysis of the effects of structural damage on functional disability, we used all visits of all patients that had HAQ, X-ray score and DAPSA available, that is, baseline and weeks 24, 52 and 104. Among the visits of all 363 patients, 1286 of 1322 visits (97.3%) were used, with 32 visits (2.7%) being excluded because of missing values (of HAQ, mSvdHS or DAPSA). Data were missing completely at random.

Again, we used a GEE longitudinal analysis on all patients. HAQ of each visit was used as dependent variable and mSvdHS, JSN or ERO, respectively, were used as independent variables in separate models (with total mSvdH, ERO and JSN score separately included in each model). In all GEE models, since the dependent variable (HAQ) appeared normally distributed, normal distribution with the identity link function was chosen, as well as an autoregressive correlation matrix, to account for patients' within-subject correlations over time. We adjusted the model for DAPSA scores, given the expected substantial effects of disease activity on functional scores.

The effects of disease activity on function may by far exceed the effect of structural damage on function, which might pose a problem when adjusting for this major effect statistically. We therefore also developed a model which included only the subgroup of patients who had at least one visit in DAPSA remission (n=117). We used all remission visits of these patients, in total 213, in a longitudinal model as above, with the exception that no adjustment for DAPSA was needed, given absence of active disease in the selected remission visits. Remission was defined as a DASPA of ≤4.[19]

To investigate further how damage would influence other response measures of disease activity (patient global assessment of disease activity, evaluator global assessment of disease activity, patient global assessment of pain, SJC66, TJC68), similar models as in the remission analyses were developed, using these measures as dependent variables (instead of HAQ) in separate models in the DAPSA remission cohort.

To put these results into clinical context, we also evaluated how many patients achieving DAPSA LDA (DAPSA ≤14) in each tertile of mSvdHS were able to achieve a 'normal' HAQ of <0.5 at week 52.[20] To compare the risk of HAQ normalisation between the groups, the risk ratio (RR) between the first and third tertile was calculated. Additionally, the absolute risk reduction (ARR) and number needed to treat (NNT) were calculated. Differences were compared using the χ^2 test; group differences of continuous group characteristics (disease duration, age, DAPSA at baseline, HAQ at baseline) were compared using unpaired t-tests.

Influence of structural damage on functional responsiveness

Finally, we tested the following hypothesis: if structural damage (which is presumed to be irreversible) explains parts of the functional disability in patients with PsA, then patients with a higher degree of structural damage should be expected to have a smaller functional responsiveness to therapy than those with less or no damage, leading to a floor effect of physical function, preventing further improvement beyond that point. To confirm the hypothesis that such an 'irreversible' component of disability exists in PsA, we used a longitudinal GEE model in which we assessed the effect of radiographic damage (corresponding to this putative irreversible functional component) on changes in HAQ from baseline, while adjusting for HAQ at baseline. We performed this analysis on a subgroup of patients who showed a major response of DAPSA (improvement of ≥85%) from baseline,[19] and who had a baseline HAQ≥1 (since patients with normal or near normal function at baseline would not be informative in this analysis of functional responsiveness).

Validation

To show the independence of the results from the measurement instrument used for physical function assessment, we also performed the remission and responsiveness analyses using the Physical Component Score of the 36-Item Short Form Survey Instrument (SF-36 PCS), instead of HAQ and HAQ change, as outcome variable in GO-REVEAL patients.[14 21]

Since the above analyses were based on one patient cohort, we externally validated these data using a clinical database of routine patients seen at our clinics. The use of PsA patient data was approved by the ethics committee of the Medical University of Vienna (EK Nr: 2002/2014). In total, our X-ray database includes visits of 206 PsA patients. We identified all PsA patients (n=160) who had complete cDAPSA (the clinical version of the DAPSA without C reactive protein),[19] HAQ assessment and a corresponding radiographic assessment at or within 6 months of the clinical remission visit. Fifty-five (34.4%) of these patients achieved cDAPSA remission in the course of their disease. Respective dropout numbers are provided in the online supplementary table S3. An experienced scorer (GS), blinded to the purpose of this study, scored all radiographs of all identified patients. In this cohort, the same model as described above for the overall and the DAPSA remission cohort was used.

All analyses were performed using SAS V.9.4 (SAS Institute, Cary, North Carolina, USA).

RESULTS

Patient characteristics

The baseline characteristics of the 363 patients extracted from the trial cohort are presented in table 1, together with the corresponding

characteristics of the subsets of patients for the remission analysis (n=117) and the response analysis (n=67). For the subgroup of patients who fulfilled the remission criteria, table 1 also presents the characteristics at the time of remission for both, the trial cohort (n=117) and the validation cohort (n=55). The distribution of the mSvdHS of the population is visualised as histogram in online supplementary figure S2.

Disease activity is strongly associated with physical function in a non-linear way

Longitudinal analysis showed a non-linear, significant association (p<0.001) of disease activity with physical function (visualised in online supplementary figure S1). Increases of disease activity at the low end of the disease activity scale affect physical function, and this effect attenuates with higher levels of disease activity in a non-linear way (quadratic association). Baseline characteristics of these patients were consistent with those of all patients included in the GO-REVEAL trial (data not shown).[22]

Structural damage leads to functional disability independent of disease activity

Table 2 summarises the estimates (95% CIs) and the p values for the different parameters in the GEE models for all patients (main analysis; adjusted for DAPSA), as well as in DAPSA remitters (without adjustment for disease activity). The model parameters (betas) correspond to the effects of each increment of the radiographic score on the HAQ scale. Given the large range of the mSvdHS in this cohort (0–218) and the small range of the HAQ (0–3), the HAQ increments in relation to damage are expected to be small. In the main analysis, significant effects on physical impairment were seen for the total mSvdHS (figure 1, p=0.005). The subsequent analyses of subscores showed that this was mainly related to the effects of JSN (p=0.001) and to a lesser extent to the effects of ERO (p=0.019), as visualised in figure 2A. Putting the estimate (β=0.002) of the remission model into clinical context, a patient in DAPSA remission with an mSvdHS of 10, 50, 100 or 150 would have a predicted 'residual' mean HAQ of 0.02, 0.1, 0.2 and 0.3, respectively. As the minimally clinical important difference of the HAQ in PsA lies between 0.3 and 0.35,[16 23] patients with long-standing PsA and/or substantial radiographic damage would experience a clinically meaningful irreversible change of physical function.

In the cohort achieving DAPSA remission, all radiographic changes were significantly related to HAQ scores (figure 1 and figure 2B).

Table 1 Characteristics of patient populations. (A) Baseline characteristics of the total trial population and subgroups of the trial population and the validation cohort at first visit; (B) Patient characteristics at the time of remission for the trial population and the validation cohort

	(A) At baseline					(B) At remission	
	GO-REVEAL			Validation			
	All patients	Remission*	Major response†	All patients	Remission‡	GO-REVEAL	Validation
Number of patients	363	117	67	160	55	117	55
Female (%)	153 (42.1)	39 (33.3)	30 (44.8)	75 (46.8)	15 (27.3)	39 (33.3)	15 (27.3)
Age (years)	46.9±10.8	44±11.5	43.6±11.2	52.3±12	51.8±12.1	45.1±11.6	52.4±11.7
Disease duration (years)	7.4±7.4	7.2±6.7	7.8±8.3	2.9±7.1	3.6±8.7	8.1±6.7	7.2±8.9
Swollen joints (0–66)	13.3±10.3	11.1±8.3	17.1±11.7	2.7±3.5	2.3±3.4	0.3±0.6	0.4±0.7
Tender joints (0–68)	23.1±16.5	16.7±11.4	29.3±17.6	10.1±14.5	4.2±8.9	0.5±0.8	0.2±0.4
Pain (VAS 0–100)	55.9±23.5	48.7±26.1	66±21.2	41.2±26.1	22.1±20.3	4.6±4.6	7.4±7.4
Patient global (VAS 0–100)	53.2±23.3	46.9±24.6	63.9±21	44.8±27.4	26.9±22.8	4.7±4.9	8±7.3
Evaluator global (VAS 0–100)	54.5±17.9	49.9±16.9	59.8±17.2	13.3±13.9	8.8±11.4	5±9.5	1.6±2.8
CRP (mg/dL)	1.4±1.6	1.4±1.6	2.2±1.9	0.9±0.8	0.5±0.3	0.4±0.2	0.5±0.3
HAQ (0–3)	1±0.6	0.8±0.6	1.5±0.4	0.8±0.8	0.3±0.4	0.1±0.3	0.2±0.3
SF-36 PCS (0–100)	32.5±9.8	36±10	NA	NA	NA	51.4±6.5	NA
Total mSvdHS (0–528)§	9.5 (3; 26)	9.5 (3; 26)	12 (4; 56.2)	6 (2; 14)	8 (2; 21)	8.5 (3; 23)	8 (2; 21)
ERO Score (0–320)§	5.5 (2; 15.5)	6 (2; 16.5)	9 (2; 31)	0 (0; 1)	0 (0; 3)	5 (2; 16)	0 (0; 3)
Score JSN (0–208)§	3.5 (1; 10.5)	3 (0.5; 8.5)	4.5 (1; 17.5)	5 (2; 13)	7 (2; 16)	3 (0.5; 7.5)	7 (2; 16)
DAPSA score	48.8±26.3	38.9±21.7	61.6±29.1	NA	NA	2±1.2	NA
cDAPSA score	47.4±26.1	37.4±21.1	59.4±28.5	22±19.7	11±13.4	1.7±1.2	2.2±1.4

All values are presented as mean±SD except stated otherwise.
*DAPSA ≤4 at the time of remission.
†85% DAPSA improvement from baseline and HAQ baseline ≥1.
‡cDAPSA ≤4.
§Median (first quartile; third quartile).
cDAPSA, clinical Disease Activity Index for Psoriatic Arthritis Score (TJC68+SJC66+Patient Global (0–10)+Patient Pain (0–10)); CRP, C reactive protein; DAPSA, Disease Activity Index for Psoriatic Arthritis Score (TJC68+SJC66+Patient Global (0–10)+Patient Pain (0–10)+CRP (mg/dL)); ERO, erosion; HAQ, Health assessment Questionnaire; JSN, joint space narrowing; mSvdHS, modified Sharp/van der Heijde Score; SF-36 PCS, 36-Item Short Form Survey—Physical Component Score; VAS, Visual Analogue Scale.

Table 2 Results from longitudinal analyses of the influence of structural damage on physical function (measured by the Health Assessment Questionnaire Disability Index (HAQ))

	All patients* (n=363)		Remission patients† (n=117)	
Parameter	**Estimate (95% CI)**	**p**	**Estimate (95% CI)**	**p**
Model 1 (effects of total modified Sharp/van der Heijde Score (mSvdHS))				
Intercept	0.24 (0.163 to 0.316)	<0.001	0.097 (0.025 to 0.168)	0.008
Visit	0.0002 (–0.0003 to 0.0008)	0.352	–0.0004 (–0.0011 to 0.0004)	0.312
DAPSA	0.022 (0.018 to 0.025)	<0.001	–	–
DAPSA²	–0.0001 (–0.0001 to -7.2×10^{-5})	<0.001	–	–
Total mSvdHS	**0.002 (0.001 to 0.003)**	**0.005**	**0.002 (0.0002 to 0.004)**	**0.029**
Model 2 (effects of erosion score (ERO))				
Intercept	0.246 (0.169 to 0.323)	<0.001	0.104 (0.032 to 0.176)	0.005
Visit	0.0002 (–0.0003 to 0.0008)	0.369	–0.0004 (–0.0012 to 0.0004)	0.297
DAPSA	0.022 (0.018 to 0.025)	<0.001	–	–
DAPSA²	–0.0001 (–0.0001 to -7.2×10^{-5})	<0.001	–	–
ERO score	**0.003 (0 to 0.005)**	**0.019**	**0.003 (0 to 0.005)**	**0.058**
Model 3 (effects of joint space narrowing score (JSN))				
Intercept	0.238 (0.163 to 0.314)	<0.001	0.092 (0.023 to 0.161)	0.009
Visit	0.0003 (–0.0003 to 0.0008)	0.348	–0.0004 (–0.0011 to 0.0004)	0.325
DAPSA	0.022 (0.018 to 0.025)	<0.001	–	–
DAPSA²	–0.0001 (–0.0001 to -7.3×10^{-5})	<0.001	–	–
JSN score	**0.005 (0.002 to 0.007)**	**0.001**	**0.005 (0.001 to 0.009)**	**0.010**

Estimates are presented as estimate of HAQ (95% lower CI to 95% upper CI).
*All visits of patients with available radiographic scoring.
†Visits in DAPSA remission (DAPSA ≤4) of patients with available radiographic scoring.
DAPSA, Disease Activity Index for Psoriatic Arthritis.

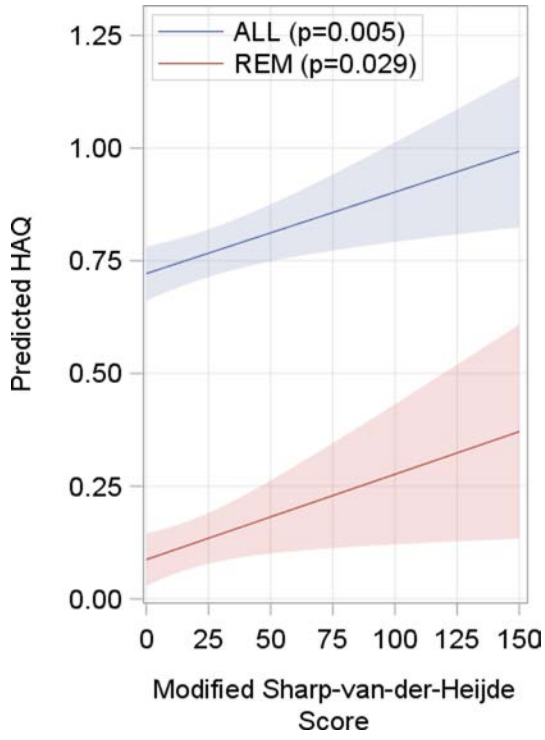

Figure 1 Predicted association of structural damage on physical function in patients with psoriatic arthritis, estimated for week 24. Blue curve: model in all patients (ALL, n=363), adjusted for Disease Activity Index for Psoriatic Arthritis (DAPSA) and estimated for for the mean DAPSA over all visits (DAPSA=25); red curve: model in all remission patients (REM, DAPSA≤4, n=117) without additional disease activity adjustment. Shaded areas represent the 95% CIs. HAQ, Health Assessment Questionnaire.

Additionally, we investigated how other core disease activity variables are affected by damage and found no significant association besides the HAQ (see online supplementary table S4).

NNT to prevent irreversible functional impairment

At week 52, 44 of 60 patients (73.3%) achieving DAPSA LDA (DAPSA≤14) in the first (lowest) tertile of the mSvdHS normalised their HAQ (HAQ<0.5), while 29 of 54 (53.7%) of the third tertile achieved a normal HAQ. Comparing the first and third tertile, the RR of achieving a normal HAQ is 0.58 (95% CI 0.35 to 0.96, p=0.029). Thus, overall, the potential of achieving a normal HAQ is highly reduced in patients in the highest damage tertile. Further, patients achieving LDA in the first mSvdHS tertile have an ARR of 0.196 in HAQ normalisation. In a classical invention study, this risk reduction would correspond to an NNT of 5 (95% CI 2.7 to 42.4).

The mean disease duration was different between the first and third tertile achieving LDA at week 52 (5.15±5 and 11.3±9.1; p<0.001), as well as the mean age (40.5±8.7 and 49.4±11.5; p<0.001). While there were no differences in mean DAPSA at baseline, the mean HAQ scores at baseline were significantly lower in the first tertile, compared with the third (0.81±0.60 and 1.11±0.69, p=0.015).

Functional responsiveness is impaired in patients with structural damage

In the analysis of DAPSA major responders, the change of HAQ scores decreased significantly with increasing levels of overall structural damage (total mSvdHS; p=0.010 and p=0.013 for absolute or relative HAQ change, respectively) (table 3, figure 3). This was driven mainly by JSN and less by ERO (figure 2C,D).

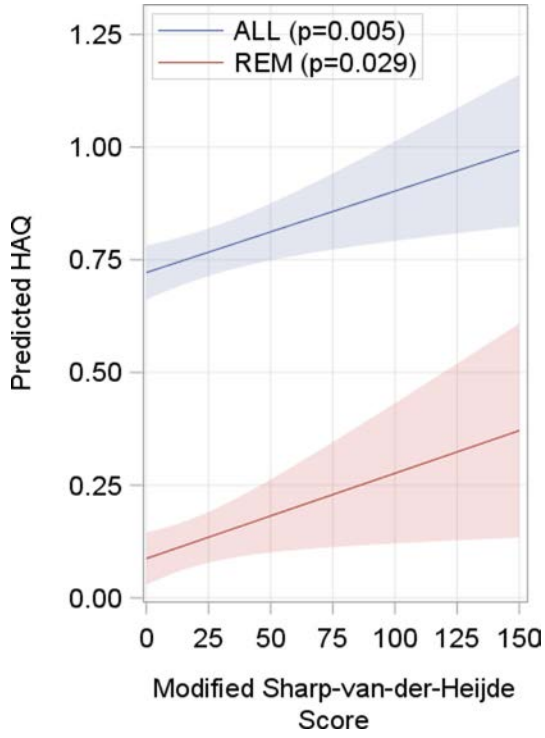

Figure 2 Predicted association of joint space narrowing (JSN) and erosion scores on physical function and functional responsiveness in patients with psoriatic arthritis, estimated for week 24. Red curves: JSN; blue curves: ERO score; (A) Predicted Health Assessment Questionnaire (HAQ) in all patients, adjusted for Disease Activity Index for Psoriatic Arthritis (DAPSA) and estimated for the mean DAPSA over all visits (DAPSA=25, n=363); (B) Predicted HAQ in all DAPSA remission patients (DAPSA ≤4) without adjustment for disease activity (n=117); (C) Predicted absolute HAQ change in patients with a baseline HAQ of ≥1 and a DAPSA major response (≥85% DAPSA improvement from baseline) (n=67), adjusted for baseline HAQ. (D) Predicted relative HAQ change in patients with a baseline HAQ of ≥1 and a DAPSA major response (≥85% DAPSA improvement from baseline) (n=67), adjusted for baseline HAQ. Shaded areas represent the 95% CIs.

Validation analyses using a different cohort and a different functional measure

The analyses including all patients and the remission analyses were validated in the clinical practice cohort, in which the significant association of HAQ with mSvdHS ($p<0.001$), JSN ($p<0.001$) and ERO ($p<0.001$) was confirmed. Additionally, we validated the remission as well as the responsiveness analyses using the SF-36 PCS instead of HAQ as outcome variable in GO-REVEAL patients (data provided as online supplementary material).

DISCUSSION

PsA is associated with significant disability. A major factor in this respect is disease activity, since especially pain, swelling and stiffness impair physical function.[17 24] In the present study, we show that disability increases with increasing PsA disease activity, as assessed by the DAPSA. Moreover, in line with similar reports,[3 8] we also observed a significant association of disability with joint damage, since HAQ scores increased with higher mSvdHS. However, here, we provide a numerical estimate for the irreversible disability associated with joint damage. Importantly, as joint damage in PsA relates to both, bony as well as cartilage changes, like in RA,[2 4–6] JSN as a surrogate of cartilage damage was more strongly associated with functional impairment than ERO. Therefore, also for PsA, a focus on preserving joint integrity can be called on, with a specific consideration of JSN in radiographic assessment. Other core set disease activity characteristics, including joint counts, patient global assessment, evaluator global assessment and pain, do not show associations with higher degrees of damage.

While the estimates of the models are small, they clearly cross the reported threshold of clinical meaningfulness of the HAQ if early, established and late PsA are considered. The association of disability with joint damage was particularly prominent when we focused on patients who were in clinical remission and whose physical function was, therefore, not affected by disease activity. As joint damage is presumed to be irreversible, so would also be the residual disability caused by joint damage. Greater amounts of damage, therefore, preclude patients with PsA to normalise physical function, even if their disease activity is optimally controlled. Thus, prevention of joint destruction from occurring and, especially, progressing constitutes a very important principle for the treatment of PsA.

With all these data at hand, the claim can be made that structural changes in PsA are not mere epiphenomena of the disease, but clearly relate to physical functioning and overall health status of these patients. On the other hand, however, the main result of our study also reveals that the responsiveness of the HAQ decreases with higher structural damage in patients achieving major treatment response. Physical function is a major outcome in patients with chronic musculoskeletal disease, such as PsA. For that reason, functional assessment is often included in composite disease activity and outcomes scores of PsA.[13] Since, as the present data reveal, impairment of physical function may be partly irreversible and thus will not normalise in the presence of significant joint damage, the inclusion of functional scores such as the HAQ in composite scores that measure the disease process may have to be revisited. Indeed, we have observed that similar effects as on the HAQ are seen when assessing the physical component subscale of the SF-36, which supports the fact that the concept is independent of the functional instrument used.

Our findings were initially derived from a clinical trial cohort. Patients in clinical trials may only partly reflect those seen in clinical practice. However, we were able to validate the initial observations in a cohort of patients from routine clinical care. Thus, the data obtained are pertinent for both, patients included in clinical trials as well as those seen in practice.

While our study reveals novel evidence regarding joint damage-induced irreversible disability in PsA, it may not provide the full spectrum of the complex interplay between disability and disease-related factors. Our study has several limitations: (1) We did not address comorbidities and psychological factors in the context of disability. Indeed, comorbidities have been shown to significantly impact irreversible disability in RA,[25] and this is also likely the case in PsA; however, we did not have data on comorbidities available in the cohorts studied. Also, skin involvement does not seem to affect physical function in PsA,[22] even if a PsA modified version of the HAQ is used.[26] (2) Non-pharmaceutical treatment, including physiotherapy, may also contribute to the improvement of physical function, even in patients with advanced radiographic damage. (3) We are mainly addressing physical function of PsA patients with oligoarticular or polyarticular peripheral joint disease, which is predominant in PsA,[27] but the mSvdHS does not take axial skeleton involvement into account and axial changes may also contribute to disability. Therefore, in patients who have only one or very

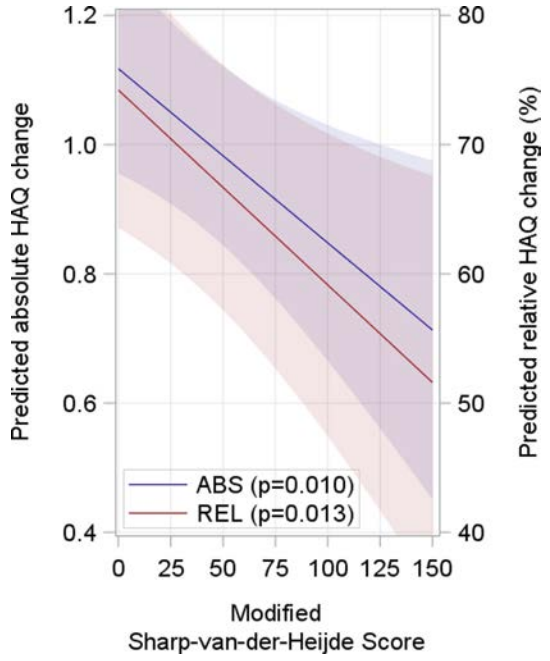

Figure 3 Predicted association of radiographic damage and functional responsiveness in patients with psoriatic arthritis. Analysis of patients with a baseline Health Assessment Questionnaire (HAQ) of ≥1 and a Disease Activity Index for Psoriatic Arthritis (DAPSA) major response (≥85% DAPSA improvement from baseline) (n=67), adjusted for baseline HAQ. Absolute (ABS) and relative (REL) HAQ changes at week 24 are shown for different levels of radiographic scores, and are estimated for patients with a baseline HAQ=1.5. Shaded areas represent the 95% CIs.

few peripheral joints involved or predominantly axial disease, irreversible disability may be underestimated by using mSvdHS only. (4) Furthermore, bony proliferation is not included in the mSvdHS and may also contribute to loss of physical function. (5) Additionally, secondary osteoarthritis has not been taken into account. (6) Finally, most patients in the GO-REVEAL study had low degrees of structural damage (visualised in online supplementary figure S2), but we could still observe significant implications on functional outcomes. Nevertheless, extrapolation to values beyond the observed data may not be legitimate.

The data presented reveal that damage in PsA is associated with irreversible disability as in RA and that the major culprit in this respect is cartilage destruction. This implies that prevention of joint damage and especially preservation of cartilage structure is of particular importance and, therefore, would support the claim to diagnose and treat PsA rapidly and effectively, as well as the currently accepted treatment targets of remission of disease activity. Remission will best prevent joint damage progression,[28] and thus will also lead to best possible functional outcomes in PsA over time.

In conclusion, our results reveal that responsiveness of functional limitations decreases with increasing joint damage. They further suggest that—similar to what has been shown in RA—JSN is functionally more important than ERO. Both achievable HAQ levels and HAQ responses are negatively impacted by a high degree of structural damage. Consideration of these components is clinically and therapeutically relevant, as the HAQ component related to inflammation is expected to be reversible, while the component related to destructive changes is not.

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Contributors Study design: AK, JSS, DA. Data acquisition: DB. Data analysis: AK, JSS, DA. Manuscript writing: AK, DB, JSS, DA.

Competing interests None declared.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement Data were partly provided by Janssen, who did not provide any funding for the study.

Table 3 Impaired functional responsiveness in patients achieving major response of the Disease Activity Index for Psoriatic Arthritis (DAPSA; 85% improvement from baseline). Results of longitudinal analyses for absolute and relative change in physical function (measured by the Health Assessment Questionnaire Disability Index (HAQ))

	Absolute HAQ change* (n=67)		Relative HAQ change† (n=67)	
Parameter	**Estimate**	**p**	**Estimate**	**p**
Model 1 (effects of total modified Sharp/van der Heijde Score (mSvdHS))				
Intercept	−0.026 (−0.447 to 0.396)	0.905	0.759 (0.492 to 1.027)	<0.001
Baseline HAQ	0.747 (0.489 to 1.005)	<0.001	−0.023 (−0.168 to 0.122)	0.758
Visit	0.001 (−0.001 to 0.003)	0.338	0.001 (−0.001 to 0.002)	0.279
Total mSvdHS	**−0.003 (−0.005 to −0.001)**	**0.010**	**−0.002 (−0.003 to −0.0003)**	**0.013**
Model 2 (effects of erosion score (ERO))				
Intercept	−0.017 (−0.447 to 0.413)	0.937	0.764 (0.493 to 1.036)	<0.001
Baseline HAQ	0.73 (0.466 to 0.994)	<0.001	−0.034 (−0.181 to 0.114)	0.655
Visit	0.001 (−0.001 to 0.003)	0.308	0.001 (−0.001 to 0.002)	0.258
ERO score	**−0.004 (−0.008 to −0.0002)**	**0.040**	**−0.002 (−0.004 to 0.0001)**	**0.062**
Model 3 (effects of joint space narrowing score (JSN))				
Intercept	−0.038 (−0.449 to 0.372)	0.854	0.752 (0.49 to 1.014)	<0.001
Baseline HAQ	0.765 (0.512 to 1.019)	<0.001	−0.011 (−0.154 to 0.132)	0.882
Visit	0.001 (−0.001 to 0.003)	0.3A85	0.001 (−0.001 to 0.002)	0.314
JSN score	**−0.007 (−0.011 to −0.003)**	**0.002**	**−0.004 (−0.007 to −0.002)**	**0.002**

*Absolute HAQ change was defined as HAQ at baseline—HAQ at visit.
†Relative HAQ change was defined as (HAQ at baseline—HAQ at visit)/HAQ at baseline.

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EXTENDED REPORT

Genome-wide association and functional studies identify a role for matrix Gla protein in osteoarthritis of the hand

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ABSTRACT

Objective Osteoarthritis (OA) is the most common form of arthritis and the leading cause of disability in the elderly. Of all the joints, genetic predisposition is strongest for OA of the hand; however, only few genetic risk loci for hand OA have been identified. Our aim was to identify novel genes associated with hand OA and examine the underlying mechanism.

Methods We performed a genome-wide association study of a quantitative measure of hand OA in 12 784 individuals (discovery: 8743, replication: 4011). Genome-wide significant signals were followed up by analysing gene and allele-specific expression in a RNA sequencing dataset (n=96) of human articular cartilage.

Results We found two significantly associated loci in the discovery set: at chr12 ($p=3.5 \times 10^{-10}$) near the matrix Gla protein (MGP) gene and at chr12 ($p=6.1\times10^{-9}$) near the CCDC91 gene. The DNA variant near the MGP gene was validated in three additional studies, which resulted in a highly significant association between the MGP variant and hand OA (rs4764133, $Beta_{meta}=0.83$, $P_{meta}=1.8*10^{-15}$). This variant is high linkage disequilibrium with a coding variant in *MGP*, a vitamin K-dependent inhibitor of cartilage calcification. Using RNA sequencing data from human primary cartilage tissue (n=96), we observed that the MGP RNA expression of the hand OA risk allele was significantly lowercompared with the MGP RNA expression of the reference allele (40.7%, $p<5*10^{-16}$).

Conclusions Our results indicate that the association between the MGP variant and increased risk for hand OA is caused by a lower expression of *MGP*, which may increase the burden of hand OA by decreased inhibition of cartilage calcification.

INTRODUCTION

Osteoarthritis (OA) is the most frequent joint disorder worldwide. An estimated 22% of the adult population has a joint affected by OA and this incidence increases to 49% in individuals over 65 years of age.[1] All synovial joints can be affected by OA, with hand OA as one of the most common forms of OA. Hand OA is characterised by osteophyte formation, bony enlargements of finger joints and cartilage degradation in the joints. One of the factors contributing to cartilage degradation is the increase of calcified cartilage in the joint.[2 3] In addition, hand OA is related to the occurrence of OA at other sites, most notably with knee OA.[4 5] Patients affected by hand OA suffer from pain and disability, impacting their quality of life. OA is a leading cause of chronic disability,[6] yet currently no effective therapeutic treatments against OA are known. It is therefore imperative to dissect the underlying mechanism of disease aetiology as this may enhance effective and targeted drug development.

OA has a strong genetic component. Depending on the joint affected, the heritability of OA is estimated in the range of 40%–60%,[7 8] with hand OA having the largest heritability, that is, ~60%.[9 10] Therefore, in recent years, several large-scale genetic studies have been performed to identify the underlying genes and pathways leading to OA. Multiple significantly associated loci for OA of the hip and knee have been identified through genome-wide association studies (GWAS).[11–18] However, thus far, only one report has described a robust association with OA of the hand.[19] In this previous report, common variants in the *ALDH1A2* and rare variants in chromosome 1p31 were genome-wide significantly associated with hand OA using a discovery cohort of 837 cases and 77 325 controls.

In this study, we aimed to identify novel genes and pathways involved in the aetiology of OA of the hand by performing a large-scale genome-wide association study (GWAS). We used a semiquantitative measure for OA of the hand in order to increase statistical power. We gathered a large sample size of 12 754 individuals for analysis, by combining data from three studies in the discovery phase and an additional three cohorts for replication. Next, we conducted functional follow-up of our top finding to investigate the underlying mechanism.

METHODS

Discovery GWAS, replication and meta-analysis

For a detailed description on the GWAS methods, participating studies, quality control procedures for genotyping and imputation, see online supplementary text S1 and table S1.

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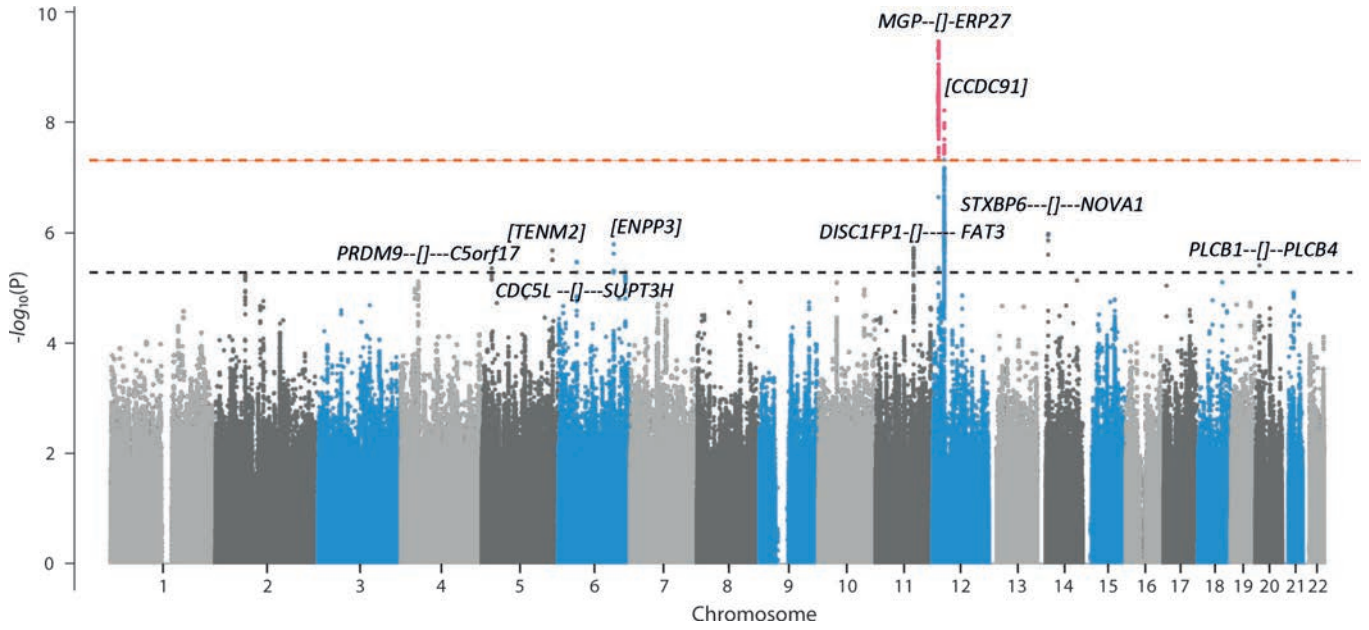

Figure 1 GWAS results for association with the KL sum score in the discovery phase. Manhattan plot for association with the KL sum score, adjusted for age and sex, in the discovery cohorts of RSI, RSII and RSIII. The –log10 p values, for each of the ~11 million SNPs analysed (remaining after EASYQC quality control) as part of the genome-wide association with the KL sum score, plotted against their position per chromosome. The red dotted horizontal line corresponds to the genome-wide significant threshold ($p=5\times10^{-8}$). The dotted grey line corresponds to the selection for replication threshold ($p=5\times10^{-6}$). SNP location represented by [], if the SNP is localised intergenic the dashes denotes the distance, -≤10 kb, --≤100 kb, ---≤1000 kb, ----≤1 Mkb, -----≥1 Mkb. GWAS, genome-wide association studies; KL, Kellgren and Lawrence score.

Detailed phenotype description of Kellgren and Lawrence sum score

We have used a semi-quantitative bilateral measure of OA of the hand based on the radiographic Kellgren and Lawrence (KL) score.[20] Using radiographs of both hands, the KL score was determined for each joint in the hand. Using these KL scores we defined the KL sum score: the total KL score, the sum, of the following hand joints for both hands (left and right): all distal interphalangeal joints, all proximial interphalangeal joints, all metacarpophalangeal (MCP) joints, the interphalangeal joint and the first carpometacarpal joint, which gives the sum of 15 joints on each hand, and in total 30 joints for both hands together, resulting in a minimum score of 0 and a maximum score of 120. In the Leiden Studies (LS) cohort, no KL scoring was done of the MCP joints, resulting in a KL sum score of maximum 88. Individuals lacking KL grading for both hands or one hand and individuals with missing age or gender information were excluded from all analyses in all cohorts. As the KL sum score has a skewed distribution, the top finding of the meta-analysis was repeated in the discovery cohorts using a Poisson regression.

Visualisation of the associated loci and the regulatory landscape

For the top GWAS-associated single nucleotide polymorphism (SNP), the linkage disequilibrium (LD) region (r^2 >0.8) was determined using the 1000G Phase-1 population using the HaploReg V3 tool.[21] Using the ROADMAP-generated reference epigenomes, we determined if any of the variants in high LD were located in potential gene regulatory regions in primary osteoblasts (generated by ENCODE) and bone marrow-derived chondrocytes (ROADMAP).[22 23] The 18-state chromatin reference epigenomes were downloaded from the ROADMAP epigenomes data portal.[23] SNPs and regulatory annotations were visualised using the UCSC Genome Browser on GRCh37/hg19.[24] For each variant, it was also determined if the alternative allele would disrupt a protein binding motif; this was done using the HaploReg V3 tool.[21]

RNA sequencing data

Post-RNA isolation (Qiagen RNeasy Mini Kit, RIN >7) of 40 knee (15 paired preserved (P) and OA lesioned (OAL), 7 P only and 3 OAL only) and 28 hip (six paired P and OAL, 14 P only and 2 OAL only) cartilage samples (online supplementary table S2), paired-end 2×100 bp RNA library sequencing (Illumina TruSeq RNA-Library Prep Kit, Illumina HiSeq2000) resulted in an average of 10 million fragments per sample. Reads were aligned using GSNAP against GRCh37/hg19, in which SNPs from the Genome of the Netherlands consortium with a minor allele frequency (MAF) >1% were masked to prevent alignment bias. Number of fragments per gene were used to assess quantile-adjusted conditional maximum likelihood (edgeR, R-package). Subsequently, differential gene expression analysis was performed pairwise between P and OAL samples for which we had RNA of both (n=21). Allele-specific expression (ASE) was assessed using SNVMix2[25] with default settings(min coverage=25, 10 reads per allele). The extent of ASE was defined as the fraction of risk allele among all counts at the respective location. Meta-analysis was done only across P samples or OAL when no P counterpart sample was present. p Values were calculated using canonical binominal test (metagen R-package).

TaqMan assay

Conventional TaqMan genotyping was performed on both genomic DNA (gDNA), articular cartilage and subchondral bone cDNA. An allele-specific custom TaqMan assay for rs1800801 (Thermo Fisher Scientific) was used to quantify the allele ratio in cDNA samples and were normalised against the gDNA ratio, which was used as an 1:1 allele ratio reference. Each sample

has been measured in four (cartilage) or eight (subchondral bone) times, while calculations and statistics were performed as described previously.[19 26] Cartilage samples that yielded fewer than four measurements (n=2) were discarded prior to further analyses. All subchondral bone samples were assessed by eight technical replicates.

RESULTS

GWAS of KL sum score

We conducted a GWAS of a semiquantitative measure of hand OA, a bilateral summed score of KL scores,[20] which grades radiographic OA severity, across all hand joints (KL sum score, range of 0–120). The discovery set consisted of three Rotterdam Study cohorts (RSI, RSII and RSIII) and included 8743 participants with KL sum scores. Replication was done in another 4011 individuals from three different cohorts; LS, Framingham Heart Study and Twins UK (TUK). General characteristics of the discovery cohorts and replication cohorts can be found in online supplementary table S3 and text S1.

The discovery analysis yielded two novel independent genome-wide significant loci ($p \leq 5*10^{-8}$) on chromosome 12, an intergenic region between *matrix Gla protein (MGP)* and *ERP27* and an intronic region in *CCDC91*. We also identified seven other novel loci with suggestive significance ($p<5*10^{-6}$) (figure 1). In total, nine loci were selected for replication in 4011 individuals from three different cohorts (LS, FHS and TUK). Using a Bonferroni corrected p value $<5.56*10^{-3}$, we significantly replicated one of nine loci, rs4764133 ($Beta_{meta}=0.83$, $SE_{meta}=0.10$, p $value_{replication}=3.4*10^{-07}$, p-$value_{meta}=1.8*10^{-15}$) with the same direction of effect as identified in the discovery analysis (table 1 and online supplementary figure S1 Forrest plot). This locus maintained genome-wide significance and another locus near *ENPP3* reached near genome-wide significance (chr6: 132063842:D, $Beta_{meta}=0.58$, $SE_{meta}=0.11$, p $value_{meta}=3.8*10^{-7}$) in the combined discovery and replication joint meta-analysis (table 1). Since the KL sum score has a skewed distribution, the top hit was also reanalysed in the discovery set using a Poisson regression (rs4764133, $Beta_{poisson}=0.12$, $SE_{poisson}=0.02$, p $value_{poisson}=1.98*10^{-11}$).

Our top replicated and genome-wide significant finding, rs4764133 [T] ($P_{meta}=1.80*10^{-15}$, Beta=0.83, MAF=0.39) is located in a non-coding intergenic region between *MGP* and *endoplasmic reticulum protein 27* (*ERP27*). However, variants in high LD with rs4764133 ($r^2 \geq 0.8$) span a ~80 Kb region encompassing multiple genes, including *MGP* and an open-reading frame *C12orf60* (figure 2A). Moreover, several of these variants are located in an mRNA transcript, including a non-synonymous variant in *MGP,* and variants in 3′ and 5′UTR of *MGP* and *C12orf60* (table 2, figure 2B). The non-synonymous variant in *MGP*, rs4236, is predicted to be non-damaging (STIFT=1, tolerated; polyPhen=0, benign) causing a threonine to alanine amino acid substitution. Two variants are located in predicted active promoter region of *MGP* (rs1800801) and *C12orf60* (rs9668569) in chondrogenic cells and primary osteoblasts (table 2).

Next, we investigated the association of rs4764133 with bilateral severe hand OA and bilateral finger OA using the discovery set (RSI, RSII and RSIII). We found a strong association with finger OA (p value=$3.09*10^{-08}$, OR=1.25) and nominal significant association with severe hand OA (p value=$2.80*10^{-2}$, OR=1.36), which has a low frequency in the population (online supplementary table S4). To see if rs4764133 also confers risk for other forms of OA, that is, OA of the hip and

Table 1 Results GWAS quantitative bilateral phenotype of osteoarthritis of the hand (KL sum score), discovery, replication and meta-analysis

SNP	Chr	Position (hg19)	Effect allele	Other allele	EAF	Discovery*			Replication†			Combined			Genomic location‡
						Beta	SE	p Value	Beta	SE	p Value	Beta	SE	p Value	
rs1494593	5	23574856	T	C	0.88	−0.83	0.18	4.46E-06	−0.15	0.32	6.31E-01	−0.67	0.16	2.28E-05	PRDM9--[]---C5orf17
rs114370021	5	167398535	A	G	0.27	−0.84	0.18	2.12E-06	0.48	0.32	1.28E-01	−0.53	0.16	6.96E-04	[TENM2]
rs7770034	6	44447004	A	G	0.48	−0.54	0.12	3.42E-06	−0.36	0.21	8.71E-02	−0.50	0.10	1.01E-06	CDC5L --[]--SUPT3H
6:132063842:D§	6	132063842	D	I	0.27	0.64	0.13	1.28E-06	0.41	0.23	7.82E-02	0.58	0.11	3.79E-07	[ENPP3]
11:90657297:D	11	90657297	D	I	0.11	0.93	0.19	1.35E-06	0.33	0.33	3.11E-01	0.78	0.17	2.91E-06	DISC1FP1-[]---FAT3
rs4764133	12	15064363	T	C	0.39	0.75	0.12	3.45E-10	1.11	0.22	3.34E-07	0.83	0.10	1.80E-15	MGP--[]- ERP27
rs7139060	12	28693144	A	G	0.67	−0.73	0.12	6.12E-09	0.11	0.22	6.16E-01	−0.52	0.11	1.47E-06	[CCDC91]
rs1950427	14	25955502	T	C	0.12	0.86	0.18	1.09E-06	−0.28	0.30	3.56E-01	0.57	0.15	1.80E-04	STXBP6---[]---NOVA1
rs6108226	20	8960884	T	C	0.77	0.70	0.15	3.94E-06	0.20	0.27	4.43E-01	0.58	0.13	1.14E-05	PLCB1--[]--PLCB4

*Discovery: RSI, RSII and RSIII, n=8743.
†Replication: LS, TwinsUK and FHS, n=4011.
‡SNP location represented by [], if the SNP is localised intergenic the dashes denotes the distance, -≤10 kb, --≤100 kb, ---≤1000 kb, ----≤1 Mkb, -----≥1Mkb.
§For TwinsUK, a proxy SNP was used: rs3850251 $r^2=1$ D′=1 (as calculated in the RSI, RSII and RSIII cohorts).
EAF, effect allele frequency; FHS, Framingham Heart Study; GWAS, genome-wide association studies; RS, Rotterdam study cohorts.

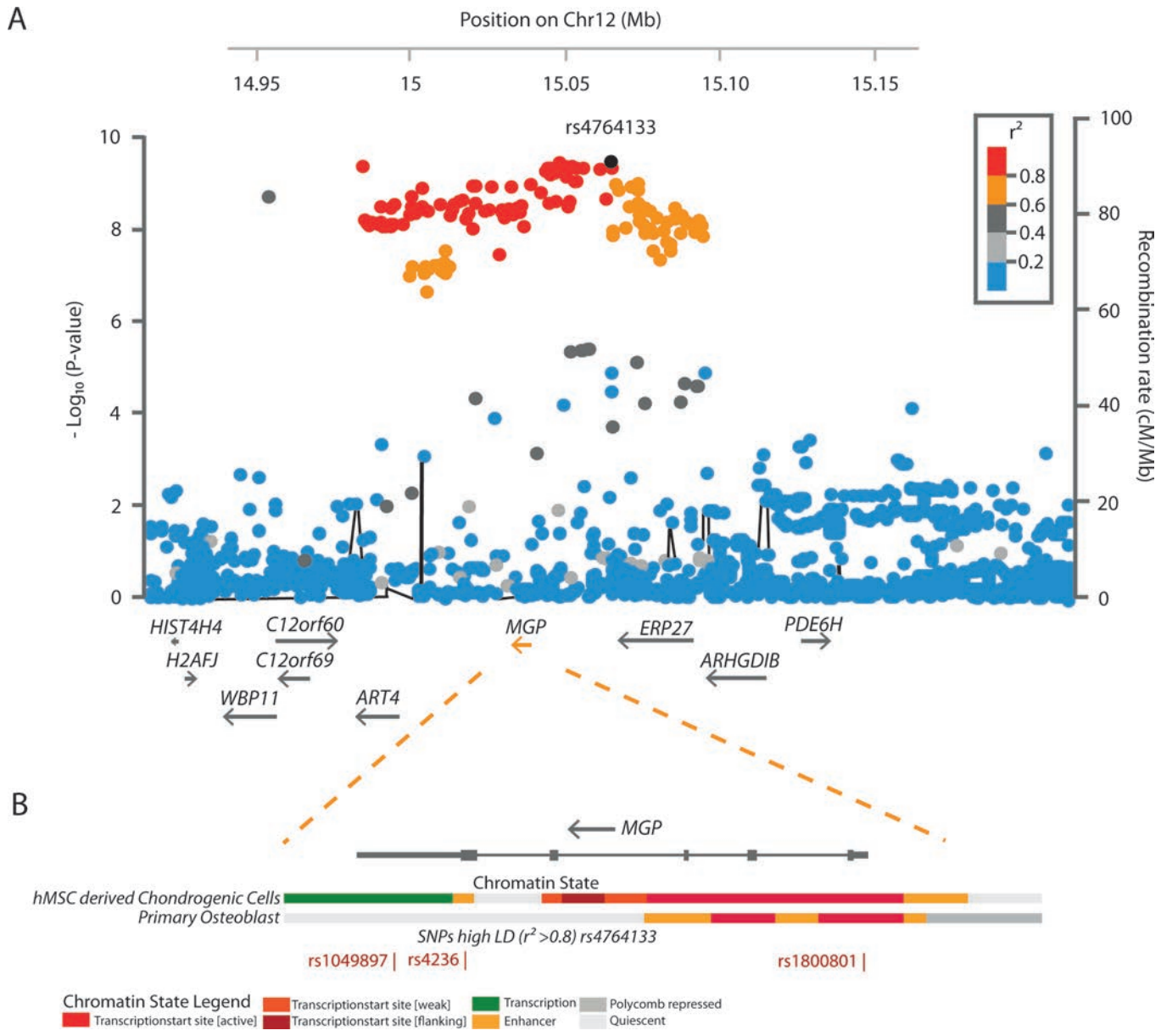

Figure 2 Locus zoom plot for rs4764133, 150 kb upstream and downstream of rs4764133 has been taken as plotted region (A). Zoom in on *MGP* and three SNPs in high LD with top SNP that are located in the MGP mRNA transcript (B). Also represented is ROADMAP chromatin 18-state data of two tissue types: human mesenchymal stem cell (hMSC)-derived cultured chondrogenic cells and primary osteoblasts. In both these cell types, the chromatin contains active marks surrounding the *MGP* promoter. LD, linkage disequilibrium; MGP, matrix Gla protein.

knee, we used the GWAS summary data of the treat OA consortium[27] and the recently published minimal joint space width of the hip (mJSW) meta-analysis.[18] No association was found between rs4764133 and hip or knee OA (online supplementary table S4). However, we did find a nominal significant association between rs1049897 and cartilage thickness in the hip joint(mJSW) (r^2=0.98 with rs4764133) (p value=$1.28*10^{-2}$, Beta=−0.398).

Gene expression analyses

In order to identify potential causal genes located in the LD block surrounding rs4764133, we assessed gene expression of *MGP*, *ERP27*, *ART4*, *SMOC3 (C12orf69)* and *C12orf60* in articular cartilage, the primary OA affected tissue. RNA sequencing was obtained on articular cartilage from patients with primary OA who had total joint replacement surgeries of either the knee (n=25) or hip (n=22) joint. Expression levels of *ERP27*, *C12orf60*, *ART4* and *SMOC3* were substantially lower than *MGP* expression levels in articular cartilage (online supplementary figure S2A). Nonetheless, neither *MGP*, *ERP27*, *ART4,SMOC3* nor *C12orf60* showed significant difference in gene expression between paired P and OAL articular cartilage. However, while these genes are not differentially expressed in OA affected cartilage, it is possible that the identified GWAS SNPs affect gene transcription. When we analysed the relationship between the top SNP and expression analysis in a classical expression quantitative trait loci (eQTL) analysis, we did not to detect significant correlations between rs1049897, rs4236 or rs1800801 and absolute *MGP*, *ERP27*, *ART4*, *SMOC3* or *C12orf60* expression levels (online supplementary figure S2B). However, we did observe several variants in high LD located in the mRNA transcript of *MGP* and *C12orf60*, allowing us to assess allele specific expression (ASE) for these genes. We were unable to study ASE for *ART4*, *SMOC3* and *ERP27*, since no

Table 2 rs4764133 LD block (r^2>0.8) annotation of potential functional elements in osteoblasts and chondrogenic cells, X marks no potential functional annotation, that is, enhancer region, promoter region or altered protein binding motifs

				Regulatory chromatin marks†		Altered protein binding motifs (HaploReg v3)
SNP	p Value discovery	r^2	Annotation*	Chondrogenic cells	Osteoblasts	
rs1049897	3.48E-09	0.88	MGP 3′-UTR	Transcription	X	X
rs4236	4.16E-09	0.86	MGP non-synonymous	Enhancer region	X	HNF4, PLAG1
rs1800801	1.12E-09	0.95	MGP 5′UTR	Promoter region	Promoter region	Zfp410
rs7310951	4.04E-09	0.86	C12orf60	Enhancer region	X	DMRT7, Gfi1, Pax-5
rs12320004	4.04E-09	0.86	C12orf60	Enhancer region	X	BHLHE40, P300, HEN1, LBP-1, RAD21,TATA, Zfx
rs10772814	3.76E-09	0.88	C12orf60	Enhancer region	X	HNF4
rs10492151	1.21E-09	0.95	C12orf60	Enhancer region	X	AIRE, Hoxa13
rs725445	3.58E-08	0.82	C12orf60	Enhancer region	X	Hand1
rs725444	3.92E-09	0.87	C12orf60	Enhancer region	X	Foxf1, Foxi1, Foxo, Foxq1, Mef2
rs4764131	6.31E-10	0.97	C12orf60	Enhancer region	Enhancer region	Myc
rs9668569	5.91E-10	0.97	C12orf60	Promoter region	Promoter region	X
rs2430687	2.44E-09	0.89	C12orf60	Enhancer region	Enhancer region	BHLHE40
rs12311463	6.91E-10	0.97	C12orf60	Enhancer region	Enhancer region	Pou1f1, Pou2f2, TATA
rs67482087	4.61E-10	0.95	C12orf60	Enhancer region	Enhancer region	Foxp1, Irx, Pou1f1, Pou2f2, Pou3f3, TATA
rs67436073	6.76E-10	0.97	C12orf60	Enhancer region	Enhancer region	Foxj2, Foxk1, Foxo, GATA, Mef2, Pou2f2, Pou3f2, Pou6f1, TATA, Zfp
rs11276	8.05E-09	0.96	C12orf60 non-synonymous	X	X	SPIB,NF-AT
rs3088189	9.46E-09	0.96	C12orf60 synonymous	X	X	SPIB
rs1861698	3.56E-09	0.96	C12orf60 synonymous	X	X	Bbx, Pou1f1, TATA

*Gene annotation based on the hg19 release of the UCSC Genome Browser.
†Regulatory chromatin marks taken from the ROADMAP Epigenomes project chromatin state learning core 18-state model.
LD, linkage disequilibrium.

SNP in high LD with rs4764133 is present in the coding region. In ASE, the influence of exonic alleles on gene expression *in -cis* is measured within heterozygote subjects, circumventing strong effects from environmental or trans-acting influences. This property results in ASE analysis to be a more statistically powerful approach, when compared with classical eQTL analysis.[28] Subsequently, we found that the OA risk alleles for three coding variants in high LD with the lead variant, rs4236 (figure 3SA, 39.6% C allele, $p<5*10^{-16}$), rs1049897 (online supplementary figure S3B, 44.4% A allele, $p<5*10^{-10}$) and rs1800801 (figure 3A, 40.7% T allele, $p<5*10^{-16}$), were significantly correlated with lower expression of *MGP*, marking imbalanced expression among heterozygotes, independent of the disease status of the articular cartilage. No ASE was observed between SNPs rs11276, rs3088189 and rs1861698 (residing in *C12orf60* and in high LD with the lead SNP, $r^2>0.8$, table 2). Technical and biological replication was performed using a custom allele-specific TaqMan assay for rs1800801 in eight additional heterozygous individuals for which we isolated RNA from P cartilage (n=2), OAL (n=2) or both (n=4) from patients with primary knee OA and confirmed the observed imbalance in preserved articular cartilage (figure 3B, relative allelic difference=0.92, $p<1*10^{-6}$), as well as in eight knee subchondral bone samples (figure 3C, relative allelic difference=0.78, $p<1*10^{-4}$).

DISCUSSION

Here, we show for the first time, that there is a robust genome-wide significant association between rs4764133, located near *MGP*, and hand OA. Furthermore, we performed functional validation showing that *MGP* coding variants in LD with rs4764133 are associated with ASE of *MGP*, which may increase risk of hand OA by lowering inhibition of articular cartilage calcification, since MGP is an essential inhibitor of cartilage calcification.[29 30] These findings suggest that MGP could be considered a prioritised drug target for hand OA, since genetically supported drug targets double the success rate of therapeutics in clinical development.[31]

MGP is an essential inhibitor of cartilage calcification, and genetic deficiencies of *MGP* in humans and mice have been linked to abnormal mineralisation of soft tissues, including cartilaginous tissue.[29 32] Furthermore, *MGP* has been previously implicated in relation to OA. A small candidate study reported marginally significant association between hand OA and genetic variants in *MGP* (rs1800802 and rs4236).[33] This is consistent with our findings that the minor allele for rs4764133 and related coding variants in high LD (r2>0.8), rs1800802 and rs4236, increase the risk of hand OA and that we found high expression of *MGP* in both P and OAL articular cartilage. In contrast, another study showed that an MGP protein complex is excreted by healthy articular chondrocytes, but not by OA-affected chondrocytes,[34] although we only assessed *MGP* expression and not MGP protein complex excretion.

Although the loci with ASE are known to be enriched for eQTLs,[35] we were unable to detect an association between the MGP genotype and *MGP* RNA expression levels in cartilage. This could have been due to our modest sample size (knee joint, n=25 and or hip joint, n=22) in combination with large heterogeneity of the tissue. Notably, the available cartilage

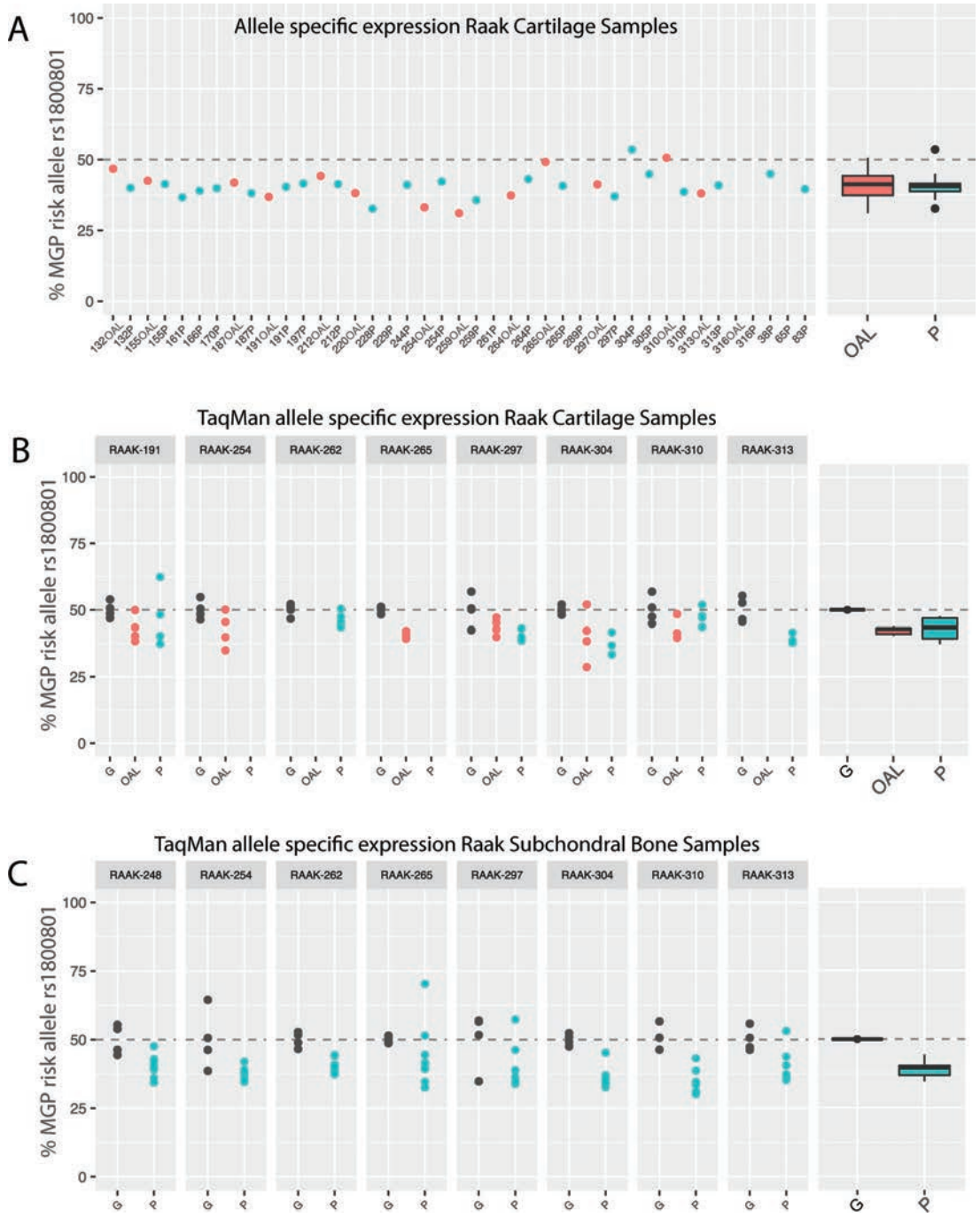

Figure 3 Allelic imbalanced expression of *MGP* marked by the alleles among heterozygotes of rs1800801 (A), in the assessed cartilage RNA sequencing dataset. Validation of selected rs1800801 using a custom TaqMan assay confirmed the imbalance (B). Allelic imbalance was also assessed with a custom TaqMan assay in subchondral bone samples (C). Preserved (P) and OA lesioned (OAL) samples are shown respectively in blue and red, and genomic DNA (TaqMan control) in black (G). For ASE results for rs4236 and rs1049879, see online supplementary figure S3 and for information on the samples, see online supplementary table S2. ASE, allele-specific expression; MGP, matrix Gla protein; OA, osteoarthritis.

samples originated from different joint sites (knee and hip) and different disease stage (preserved versus affected) and had large age range of the individuals. Also, it is known that ASE is a more powerful technique than classical eQTL analysis to identify functional SNPs influencing expression of genes.[28] While the extent of imbalance could be considered relatively modest, an increasing number of OA associated SNP alleles appear to mark ASE by comparable amount.[19 36–38] From a more biological perspective, one could consider a prolonged, although slight, deviation from homeostasis due to modest ASE of cartilage relevant genes to be of substantial influence over time. This latter hypothesis could contain the molecular basis for increased risk towards developing OA among the ageing population. Additionally, we observed that the rs1800801 alleles also affected expression of MGP in subchondral bone samples. This could imply that, in parallel to an effect in cartilage, the presumed disturbed cartilage homeostasis is further affected by the underlying bone, further enabling the view that OA is a pathology of the entire joint.

Our findings may give an explanation for the known vitamin K association with OA: MGP-mediated calcification inhibition is dependent on γ-carboxylation by vitamin K.[39] It

has been shown that low vitamin K intake is correlated with OA.[40] Thus, vitamin K intake may be a potential therapeutic treatment in OA. Recently, a first randomised control trial testing the effects of vitamin K on OA was published, which reported no overall effect of vitamin K on hand OA.[41] Despite the low power of the trial, there was a significant beneficial effect on joint space narrowing (cartilage degradation) among those individuals that were vitamin K deficient at the start of the trial.[41] Thus, an adequately powered study of vitamin K may be justified based on the found MGP association. Furthermore, genetic predisposition for hand OA was not taken into account in the trial. Perhaps, genetic predisposition for hand OA (*MGP*-risk variants) in combination with insufficient vitamin K intake might potentiate cartilage calcification and subsequent risk for developing hand OA. Therefore, future OA trails, therapeutic and preventive treatments might benefit from taking a personalised medicine approach since genetically supported drug targets double the success rate of therapeutics in clinical development.[31]

Styrkarsdottir *et al*[19] reported on common genetic variants that associate with severe hand OA, among the replication cohorts were the Leiden and Rotterdam cohorts.[19] Although we observe suggestive signals at the reported locus (*ALDH1A2* gene, 1p31), the respective variants did not meet the genome-wide significance threshold in our analyses (online supplementary table S5). This difference is likely caused by the markedly different phenotypes that were used for either analyses. Where Styrkarsdottir *et al* studied a dichotomous severe hand OA phenotype, our phenotype was semiquantitatively phenotype.

To conclude, we here present coding variants in *MGP* that are associated with radiographic hand OA, and the hand OA risk allele marks lower expression of *MGP* in articular cartilage. Our findings suggest that *MGP* might play an important role in hand OA pathogenesis through pathways related to articular cartilage calcification and vitamin K. Better understanding of MGP gene and protein regulation and its relation to vitamin K intake and OA may reveal novel therapeutic drug targets for hand OA.

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Contributors WdH and CGB contributed equally to this work. DJH, MSY, YFMR and SM performed replication analysis for this work, and LB provided analysis help. LAC and FR provided data. MK provided phenotypic contribution to the GARP study. MP provided data and analyses. TDS contributed data for replication. AH contributed data of the RS cohorts. JD, and PES contributed to genotyping data and analyses of LLS cohort. RGHHN provided contribution to the RAAK study. AGU contributed genotype data of RS cohorts. DTF and AMV contributed replication data for this work. IM and JJBvM jointly supervised this work.

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Competing interests None declared.

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

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EXTENDED REPORT

Optimal methotrexate dose is associated with better clinical outcomes than non-optimal dose in daily practice: results from the ESPOIR early arthritis cohort

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ABSTRACT

Background Although methotrexate (MTX) is the consensual first-line disease-modifying antirheumatic drug (DMARD) for rheumatoid arthritis (RA), substantial heterogeneity remains with its prescription and dosage, which are often not optimal.

Objective To evaluate the symptomatic and structural impact of optimal MTX dose in patients with early RA in daily clinical practice over 2 years.

Methods Patients included in the early arthritis ESPOIR cohort who fulfilled the ACR-EULAR (American College of Rheumatology/European League against Rheumatism) criteria for RA and received MTX as a first DMARD were assessed. Optimal MTX dose was defined as ≥10 mg/week during the first 3 months, with escalation to ≥20 mg/week or 0.3 mg/kg/week at 6 months without Disease Activity Score in 28 joints remission. Symptomatic and structural efficacy with and without optimal MTX dose was assessed by generalised logistic regression with adjustment for appropriate variables.

Results Within the first year of follow-up, 314 patients (53%) with RA received MTX as a first DMARD (mean dose 12.2±3.8 mg/week). Only 26.4% (n=76) had optimal MTX dose. After adjustment, optimal versus non-optimal MTX dose was more efficient in achieving ACR-EULAR remission at 1 year (OR 4.28 (95% CI 1.86 to 9.86)) and normal functioning (Health Assessment Questionnaire ≤0.5; OR at 1 year 4.36 (95% CI 2.03 to 9.39)), with no effect on radiological progression. Results were similar during the second year.

Conclusion Optimal MTX dose is more efficacious than non-optimal dose for remission and function in early arthritis in daily practice, with no impact on radiological progression over 2 years.

INTRODUCTION

Even in the current era of biological or targeted therapies, methotrexate (MTX) remains the initial recommended disease-modifying antirheumatic drug (DMARD) and is widely prescribed for patients with rheumatoid arthritis (RA). Recent national and international recommendations support the use of MTX as the first-line DMARD for RA because of its substantial effectiveness, acceptable safety and low cost.[1–3] However, despite more than two decades of experience with the drug, considerable heterogeneity exists in rheumatologists' prescription behaviours, including the dosage and route of administration. In controlled studies of first-line biological therapy for RA, more than one-third of patients achieved clinical remission with MTX alone, but another one-third had no treatment response.[4–7] The absence of response may indicate a primary lack of efficacy or suboptimal MTX use. However, because randomised controlled studies may not reflect current clinical practice, the results should be interpreted with caution.

Starting MTX at least 10 mg/week orally, escalating with 5 mg/month to 25–30 mg/week, or the highest tolerable dose, with a subsequent switch to subcutaneous administration in case of inadequate response, seems to be the optimal evidence-based dosing and route recommendation for MTX for RA.[3 8] However, evidence is scarce concerning the impact of optimal MTX dose on symptoms and structural damage in early RA in daily practice.

We aimed to describe the optimisation of MTX in a large cohort of patients with early RA and to evaluate its symptomatic and structural impact over 2 years in a real-life setting.

PATIENTS AND METHODS

Patients

Between December 2002 and March 2005, up to 813 patients with early arthritis from 14 French regional centres were included in the ESPOIR cohort.[9] Inclusion criteria were ages 18–70 years, more than two swollen joints for >6 weeks and <6 months, suspected or confirmed diagnosis of RA and no previous intake of DMARDs or glucocorticoids (except if <2 weeks). Patients were excluded if the referring physician judged that they had other clearly defined inflammatory rheumatic diseases. Each centre acted as an observational centre and did not interfere with patient treatment, except if managing care of a patient. In this study, we excluded patients who were included in randomised controlled trials and patients not fulfilling the ACR-EULAR (American College of Rheumatology/European League against Rheumatism) criteria for RA at baseline. We considered only patients fulfilling the ACR-EULAR criteria for RA at baseline and receiving MTX as a first DMARD within the first year of follow-up (figure 1).

Patients were followed up every 6 months during the first 2 years. At baseline and at each visit, data for a set of clinical and biological variables were recorded, including the Disease Activity Score in 28 joints (DAS28),[10] Simplified Disease Activity Index (SDAI)[11] and Health

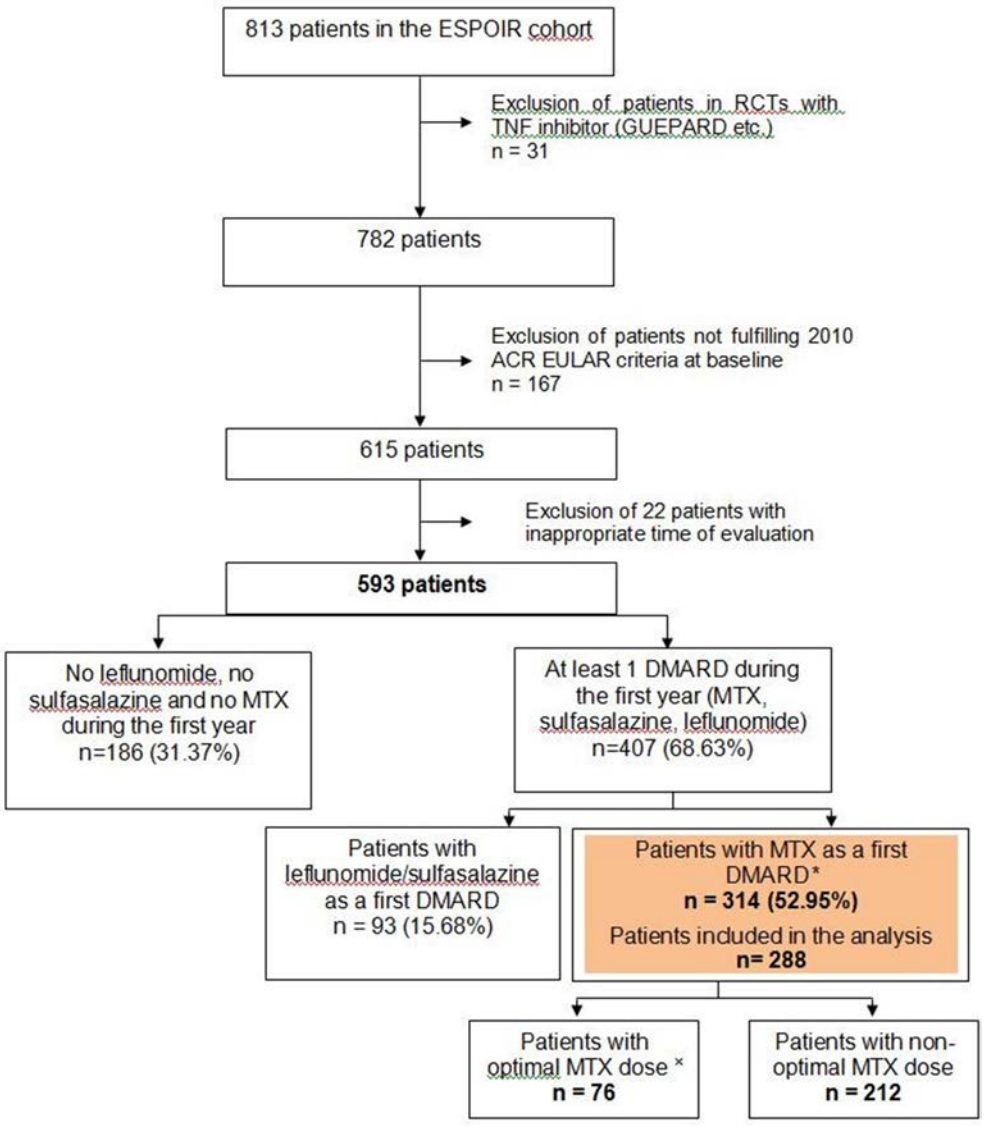

Figure 1 Flow of patients in the study. *Two patients started MTX in combination with leflunomide (n=1) and sulfasalazine (n=1). ×Introduction during the first 3 months after inclusion in the ESPOIR cohort, initially received at least by 10 mg/week and achieving at least 20 mg/week or 0.3 mg/kg/week at 6 months with DAS28 >2.6 noted at the 6-month visit in the ESPOIR cohort (or any dose noted at month 6 with DAS28 <2.6). ACR, American College of Rheumatology; DAS28, Disease Activity Score in 28 joints; DMARD, disease-modifying antirheumatic drug; EULAR, European League Against Rheumatism; MTX, methotrexate; RCT, randomised controlled trial; TNF, tumour necrosis factor.

Assessment Questionnaire (HAQ).[12] All comorbidities and toxic effects were not systematically reported in the ESPOIR cohort. Data were collected on the alanine transaminase (ALT) and aspartate transaminase (AST), gamma-glutamyl-transpeptidase at M0 and M6, blood creatinine level at M0, severe gastrointestinal events (haemorrhage, perforation, ulcer) at M0 and M6, and bronchitis and chronic obstructive pulmonary disease at M0 and M6. However, all the patients were monitored by their treating rheumatologist and have been investigated more frequently in agreement with the recommendations.

Baseline and 1 and 2-year hand and foot radiographs were read by one experienced reader in their known chronological order, with blinding to patient identity, characteristics and treatment. Structural damage quantification involved the modified Sharp/van der Heijde score (mSHS).[13]

Optimal MTX dose was defined as fulfilling the following three criteria: (1) MTX introduction during the first 3 months after inclusion in the ESPOIR cohort; (2) initial dosage ≥10 mg/week; and (3) achieving ≥20 mg/week or 0.3 mg/kg/week MTX with DAS28 >2.6 at 6 months (or any dose with DAS28 <2.6 at 6 months).

The benefits of optimal MTX dose at 1 and 2 years of follow-up were evaluated as the proportion of patients (1) in remission according to the ACR-EULAR Boolean,[14] SDAI[11] and DAS28[10] definitions; (2) with normal functioning (ie, HAQ≤0.5); and (3) with no rapid radiographic progression, defined as ΔSHS score <5 per year.[15]

The reproducibility of the radiographic assessment was assessed in the ESPOIR cohort: intraclass correlation coefficients were >0.99 for both status and change scores. The smallest detectable change was calculated as 1.0 SHS unit.[16]

The protocol of the ESPOIR cohort study was approved by the Ethics Committee of Montpellier, France (no. 020307). All patients gave their signed informed consent before inclusion.

Statistical analysis

Data are described with descriptive statistics (mean (SD), median (IQR), minimum, maximum) and distribution of MTX doses. The route of administration was described. We compared baseline characteristics of patients by optimal and non-optimal MTX dose. Qualitative variables were compared by χ^2 test or Fisher's exact test as appropriate and quantitative variables by one-way analysis of variance or Mann–Whitney U test as appropriate.

Multivariate logistic regression was used to evaluate the symptomatic and structural efficacy of optimal MTX dose after adjustment, estimating ORs and 95% CIs. Variables potentially associated with optimal MTX dose were first analysed by bivariate analysis, then variables significant at $p \leq 0.20$ were included in the multivariate logistic regression model. Centre (hospital) and other variables known to be clinically relevant or previously used in the literature were included in the model.[17 18]

To assess the robustness of the main conclusions, sensitivity analyses were performed by mSHS score at baseline instead of presence of erosion, or DAS28 at baseline instead of swollen joint count (SJC).

All analyses involved use of SAS V.9.3 (SAS Institute). $p<0.05$ was considered statistically significant.

RESULTS

Characteristics of the population

Within the first year of follow-up, 314 of 593 patients (53%) with RA received MTX as a first DMARD (figure 1). Optimal MTX dose could be analysed in 288 patients: table 1 shows their demographic and baseline clinical characteristics. In 26 patients, optimal MTX dose could not be determined because of lack of data or inappropriate time point of evaluation, with no difference from patients included in the study (shown in online supplementary table A).

Optimal MTX dose

The mean dose of MTX at initiation was 12.2±3.8 mg/week (median 12.5, IQR 10.0–15.0, range 2.5–25 mg/week). Figure 2A shows the distribution of MTX doses at initiation. The mean dose of MTX during the first 6 months was 12.6±3.8 mg/week (median 12.5, IQR 10.0–15.0, range 2.5–25 mg/week). Figure 2B shows the distribution of the MTX doses at 6-month follow-up. The route of administration was mainly oral (96.8% of patients during the first year). Overall, 17.2% of patients had their MTX dose escalated during the first 6 months. During the first year, only 65% of the patients received folic acid supplementation (mean dose 13.0±4.8 mg/week).

Among the 288 patients, 263 (91.3%) received at least 3 months of MTX during the first 6 months and 79 (27.4%) initially received at least 10 mg/week, with escalation to ≥20 mg/week or 0.3 mg/kg/week at 6 months with DAS28 >2.6. In total, 76 patients (26.4%) fulfilled all criteria for optimal MTX dose and therefore were considered to have optimal dose. Optimal MTX dose was initiated in young patients with high C-reactive protein (CRP) level (table 1). At 6 months, more patients received folic acid supplementation with optimal than non-optimal MTX dose: 63.2% vs 49.1% (p=0.0346).

During the first year, the proportion of synthetic DMARD (sDMARD) combinations was greater with optimal than non-optimal MTX dose: 25% (n=19) vs 10.4% (n=22) (p=0.0035). These combinations were varied (including only one triple therapy MTX+salazopyrine+hydroxychloroquine with optimal MTX dose) (shown in online supplementary table B).

Table 1 Characteristics of patients in ESPOIR cohort included in this study of MTX use at baseline (n=288)

Characteristic	Optimal MTX dose* (n=76)	Non-optimal MTX dose (n=212)	p
Age (years)	44.5±12.6	50.3±11.3	**0.0008**
Female sex, n (%)	56 (73.7%)	163 (76.9%)	0.57
Symptom duration (months)†	8.8±9.7	7.7±9.2	0.20
Smoking	31 (40.8%)	104 (49.1%)	0.22
DAS28	5.3±1.3	5.5±1.2	0.057
SJC	7.5±5.7	8.8±5.7	**0.04**
TJC	7.8±6.7	10.5±7.4	**0.0025**
HAQ	1.1±0.7	1.06±0.7	0.63
C-reactive protein level‡	28.2±46.8	21.0±29.5	**0.02**
Rheumatoid factor positivity, n (%)‡	49 (64.5%)	131 (61.8%)	0.68
Anti-CCP2 antibodies positivity, n (%)‡	48 (63.2%)	116 (54.7%)	0.20
Erosions present	24 (33.3%)	92 (45.1%)	0.82
SHS score	5.9±8.3	5.7±7.3	0.75
1987 ACR criteria	57 (75%)	190 (89.6%)	**0.0017**
Use of corticosteroids in the first year§	10 (13.5%)	34 (16.2%)	0.58
Use of corticosteroids during the second year¶	4 (5.71%)	33 (17.01%)	**0.0255**
Combination with synthetic DMARDs	19 (25%)	22 (10.4%)	**0.0035**
Use of biological DMARDs			
During the first year	9 (11.8%)	16 (7.6%)	0.25
During the first 2 years	11 (14.5%)	30 (14.2%)	0.95
MTX dose			
At initiation (mg/week)	14.90±4.48	11.18±3.12	**<0.0001**
At 6 months (mg/week)	15.09±4.42	11.68±3.13	**<0.0001**
Escalation between 6 and 12 months	11 (14.47%)	52 (24.53%)	0.069
Folic acid supplementation at 6 months	48 (63.16%)	104 (49.06%)	0.0346

Data are mean±SD or n (%). Significant results are in bold type.
*Optimal MTX dose defined as fulfilling the following three criteria: (1) MTX introduction during the first 3 months after inclusion in the ESPOIR cohort; (2) initial dosage ≥10 mg/week; and (3) achieving ≥20 mg/week or 0.3 mg/kg/week MTX with DAS28 >2.6 at 6 months (or any dose with DAS28 <2.6 at 6 months).
†Symptom duration defined from the appearance of the first fixed swollen joint.
‡Baseline C-reactive protein level (normal <10 mg/L); IgM and IgA rheumatoid factor (ELISA, Menarini, France; positive >9 UI/mL) and anti-CCP2 antibodies (ELISA, DiaSorin, France; positive >50 U/mL) were detected in all patients by using the same technique in a central lab (Paris-Bichat).
§At least 7.5 mg/day equivalent prednisone for more than 3 months in the first year.
¶At least 7.5 mg/day equivalent prednisone for more than 3 months in the second year.
ACR, American College of Rheumatology; DAS28, Disease Activity Score in 28 joints; DMARD, disease-modifying antirheumatic drugs; HAQ, Health Assessment Questionnaire; MTX, methotrexate; SHS, Sharp/van der Heijde score; SJC, swollen joint count; TJC, tender joint count.

Among the 288 patients receiving MTX as the first DMARD, 240 (83.3%) were still receiving MTX at 12 months, with a mean dose of 15.33±4.26 vs 13.06±3.63 mg/week with optimal versus non-optimal MTX dose (p<0.0001), and 216 (75%) at 24 months, with a mean dose of 14.37±4.09 vs 13.64±4.0 mg/week with optimal than non-optimal MTX dose (p=0.227).

Optimal MTX dose, comorbidities and toxicities

At baseline, the optimal and non-optimal MTX dose groups did not differ in comorbidities (blood creatinine level, liver tests,

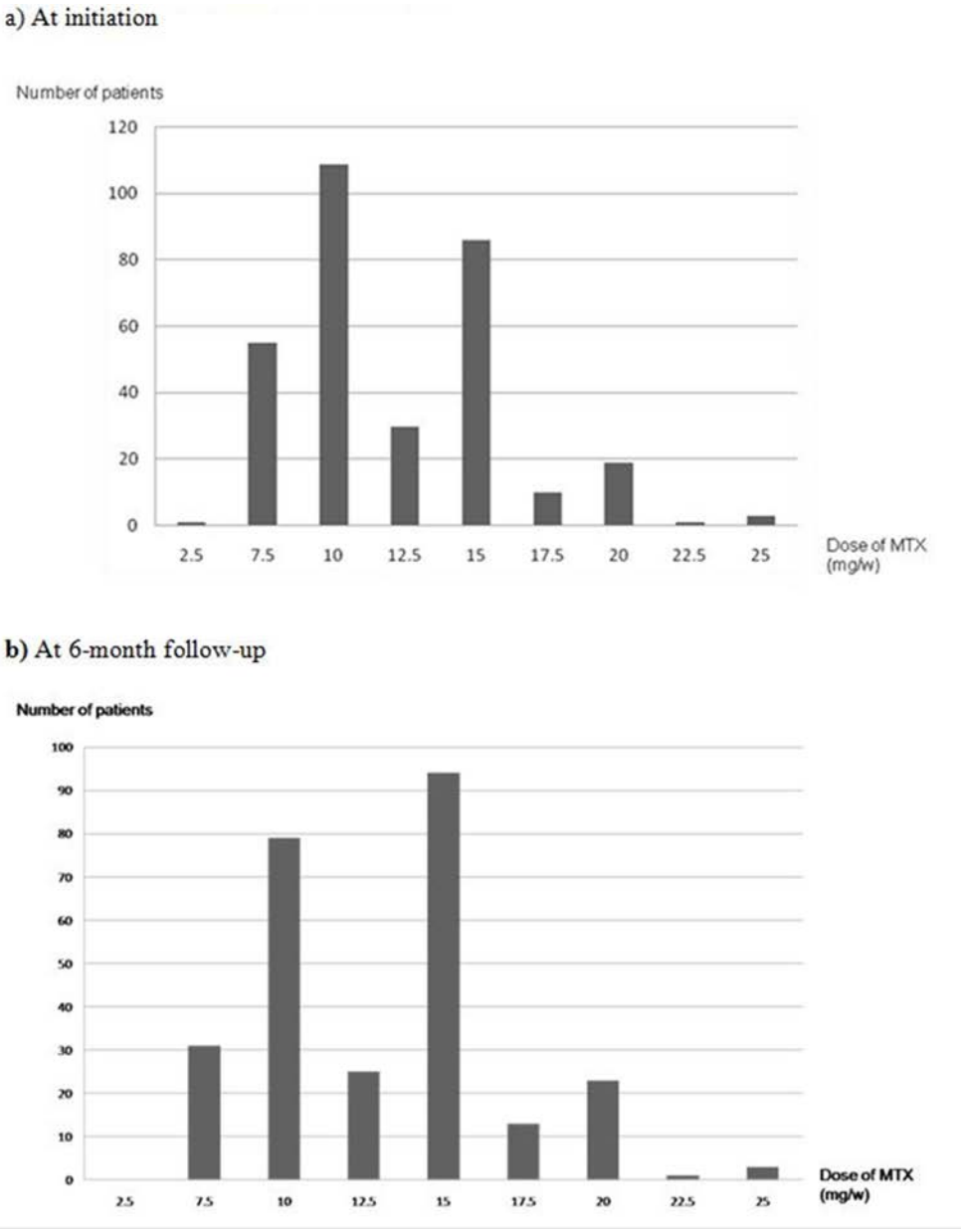

Figure 2 Distribution of the dose of methotrexate (MTX) at initiation (a) and at 6-month follow-up (b).

severe gastrointestinal events, bronchitis and chronic obstructive pulmonary disease). The toxic effects experienced by each group were limited mainly to transaminitis, severe gastrointestinal distress and bronchitis, with a trend to more abnormal level of AST and significantly more abnormal level of ALT at 6 months with optimal than non-optimal MTX dose but no difference in level ≥2 upper limit of normal (ULN) or ≥3 ULN (table 2).

Effect of optimal MTX dose on disease activity and function

On bivariate analysis, optimal MTX dose was associated with being in remission (whatever the definition used: Boolean remission, DAS28 or SDAI) and achieving normal functioning at 1 and 2 years (table 3). After adjustment (on centre, age, CRP level, SJC, positivity for rheumatoid factor (RF) or anti-CCP2 antibodies, presence of erosions, smoking, HAQ score, 1987 ACR criteria), optimal MTX dose was more efficient than non-optimal dose for achieving remission (whatever the definition used: Boolean remission, DAS28 or SDAI) and normal functioning at 1 and 2 years: ACR-EULAR remission at 1 year (OR 4.28 (95% CI 1.86 to 9.86)), normal functioning (HAQ≤0.5; OR at 1 year 4.36 (95% CI 2.03 to 9.39)) (table 3).

Effect of optimal MTX dose on radiological progression

The mean SHS score at baseline was 5.6±7.6 units (median 3 (IQR 8)). The mean change in radiographic progression at 1 year was 4.0±5.1 units (median 2 (IQR 6)). Many patients (n=170, 66.9%) showed radiographic progression (ΔmSHS>1 over 1 year), and 76 (29.9%) showed rapid radiographic progression of at least 5 units over 1 year. The mean change in radiographic progression between 1 and 2 years was 3.01±7.50 (median 0 (IQR 3)). Many patients (64.3%) did not show any radiographic progression over the second year, but 20.7% showed rapid radiographic progression during year 2.

On bivariate analysis, absence of rapid radiographic progression did not differ with optimal and non-optimal MTX dose. Results were similar after adjustment on centre, age, CRP level, SJC, positivity for RF or anti-CCP2 antibodies, presence of erosions, smoking, HAQ score and 1987 ACR criteria (table 3).

Table 2 Optimal MTX dose and comorbidities/toxic effects

Comorbidities/toxicities	Optimal MTX dose* (n=76)	Non-optimal MTX dose (n=212)	p
At baseline			
Transaminitis, n (%)			
AST≤30 U/L	6 (7.9%)	24 (11.5%)	0.514
30 U/L<AST<60 U/L	69 (90.8%)	187 (88%)	0.672
AST≥60 U/L	1 (1.3%)	1 (0.48%)	0.459
AST≥90 U/L	0 (0%)	1 (0.48%)	1
ALT≤35 U/L	7 (9.2%)	24 (11.5%)	0.673
35 U/L<ALT<70 U/L	69 (90.8%)	186 (87.5%)	0.536
ALT≥70 U/L	0 (0%)	2 (0.96%)	1
ALT≥105 U/L	0 (0%)	1 (0.48%)	1
γ-GT≤45 U/L	20 (26.3%)	61 (29.6%)	0.767
γ-GT level, U/L	37.7±42.2	43.3±54.4	0.416
Blood creatinine level, µmol/L	77.0±17.5	72.9±16.8	0.072
Severe gastrointestinal events (haemorrhage, perforation, ulcer)	2 (2.6%)	15 (7.1%)	0.255
Bronchitis and chronic obstructive pulmonary disease	1 (1.3%)	0 (0%)	0.264
At M6			
Transaminitis, n (%)			
AST≤30 U/L	6 (7.9%)	36 (17.2%)	0.059
30 U/L<AST<60 U/L	69 (90.8%)	172 (81.1%)	0.069
AST≥60 U/L	1 (1.3%)	4 (1.91%)	1
AST≥90 U/L	0 (0%)	0 (0%)	1
ALT≤35 U/L	7 (9.2%)	39 (18.6%)	0.069
35 U/L<ALT<70 U/L	69 (90.8%)	167 (78.8%)	**0.023**
ALT≥70 U/L	0 (0%)	6 (2.86%)	0.346
ALT≥105 U/L	0 (0%)	4 (1.9%)	0.576
Severe gastrointestinal events (haemorrhage, perforation, ulcer)	0 (0%)	3 (1.4%)	0.569
Bronchitis and chronic obstructive pulmonary disease	1 (1.3%)	0 (0%)	0.264

*Data are mean±SD or n (%). Significant results are in bold type.
ALT, alanine transaminase; AST, aspartate transaminase; M6, 6 months; MTX, methotrexate; γ-GT, gamma-glutamyltranspeptidase.

Sensitivity analyses

Additional analyses, conducted to test the robustness and validity of the approach, gave similar conclusions: with adjustment based on mSHS score at baseline instead of presence of erosions or on DAS28 at baseline instead of SJC, results were similar (data not shown).

Other analyses were performed with two alternative definitions of MTX optimal dose: (1) initiation of MTX during the first 3 months of follow-up, at ≥10 mg/week, and achieving ≥20 mg/week or 0.3 mg/kg/week for a minimum of 2 months during the first 6 months, with DAS28 >2.6 at M6, or any dose with DAS28 <2.6 at M6; and (2) same with achieving ≥20 mg/week or 0.3 mg/kg/week for a minimum of 3 months during the first 6 months, with DAS28 >2.6 at M6, or any dose with DAS28 <2.6 at M6. Results were similar for 73 patients with optimal MTX dose by the first definition and 72 patients with optimal MTX dose by the second definition.

DISCUSSION

This study is the first to describe the optimal dose of MTX in a large cohort of patients with early RA in daily clinical practice and to evaluate its symptomatic and structural efficacy in a real-life setting over 2 years. We found optimal MTX dose in only 26.4% of 288 patients. The definition of optimal MTX dose (initiation of MTX during the first 3 months of follow-up, at ≥10 mg/week and achieving ≥20 mg/week or 0.3 mg/kg/week at 6 months with DAS28 >2.6, or any dose noted at month 6 with DAS28 <2.6) is based on the available literature data: international guidelines for the use of MTX, starting with ≥10 mg/week orally, escalating with 5 mg/month to 25–30 mg/week, or the highest tolerable dose, with a subsequent switch to subcutaneous administration with insufficient response.[3 8] Because of the very limited number of patients who used a subcutaneous form of MTX in the ESPOIR cohort, we did not use this administration form in defining optimal MTX dose. Of note, patients were included in the ESPOIR cohort between December 2002 and March 2005, before EULAR and 3E initiative guidelines.[1 8]

Table 3 Symptomatic and structural efficacy of MTX optimisation

Outcomes	Optimal MTX dose n=76 n (%) at M12 n (%) at M24	Non-optimal MTX dose n=212 n (%) at M12 n (%) at M24	aOR* M0–M12 (95% CI)	aOR* M12–M24 (95% CI)
ACR-EULAR Boolean remission	20 (27.4) 23 (32.4)	16 (8.0) 34 (18.1)	4.28 (1.86 to 9.86)	2.75 (1.33 to 5.70)
SDAI remission	23 (31.9) 27 (38.0)	27 (10.8) 33 (17.6)	5.14 (2.27 to 11.60)	3.08 (1.54 to 6.14)
DAS28 remission	42 (58.3) 40 (57.1)	57 (28.8) 74 (39.4)	4.09 (2.01 to 8.29)	2.71 (1.35 to 5.45)
Normal functioning†	56 (73.7) 52 (68.4)	107 (50.5) 110 (51.9)	4.36 (2.03 to 9.39)	2.02 (1.03 to 3.96)
Absence of rapid radiographic progression (ΔSHS score <5)	46 (68.7) 46 (75.4)	132 (70.6) 145 (80.6)	1.17 (0.60 to 2.28)	1.70 (0.79 to 3.65)

*aOR, adjusted on age, centre, swollen joint count, C-reactive protein level, positivity for anticitrullinated protein antibody or rheumatoid factor, erosion, smoking, Health Assessment Questionnaire (HAQ) score, 1987 American College of Rheumatology criteria.
†HAQ≤0.5.
ACR, American College of Rheumatology; aOR, adjusted OR; DAS28, Disease Activity Score in 28 joints; EULAR, European League Against Rheumatism; M12, 12 months; M24, 24 months; M0, baseline; MTX, methotrexate; SDAI, Simplified Disease Activity Index; SHS, Sharp/van der Heijde score.

Of note, significantly more patients received folic acid supplementation with optimal than non-optimal MTX dose at 6 months. Side effects that could have been avoided with folate supplementation (stomatitis, gastrointestinal distress, transaminitis, and so on) could have influenced the ability of patients to accelerate MTX. Prescription of at least 5 mg folic acid per week with MTX therapy is strongly recommended.[3 8]

Second, we found more optimal dose of MTX in younger than older patients. This fact could be related to at least two elements: (1) the incidence of comorbidities and comedications increasing with age, which can lead to a bias of indication and a risk of undertreatment of older patients, and (2) increased MTX toxicity in older patients limiting the ability to increase the weekly dose.

Third, in daily practice, optimal MTX dose was more efficacious than non-optimal MTX dose in terms of remission and function in early RA but had no impact on radiographic progression over 2 years. In a previous study,[19] we confirmed the 6-month symptomatic and 12-month structural efficacy of MTX in early RA in daily clinical practice despite the suboptimal use of MTX. With optimal MTX dose, the additional benefit is essentially for clinical remission and function more than structure. However, the structural progression in the ESPOIR cohort was quite low, which may explain the lack of benefit of MTX optimisation on structural damage progression and a limited number of patients in the optimal MTX dose group may indicate lack of power.

One of the strengths of this study is that we included a wide spectrum of patients with early RA. The ESPOIR cohort aimed to include all patients with early arthritis regardless of disease level, age and sex, so our study reveals the performance of optimal MTX dose in a real-life setting. Because of the large number of baseline variables available in the ESPOIR cohort, the possibility of failure to include confounding covariates was reduced.

Our study has some limitations. We tried to include all baseline covariates associated with treatment assignment and/or that affect the outcome. However, ensuring that some confounders were not omitted is difficult. Because of a change in patient condition, data at baseline do not necessarily represent their condition at the time of the treatment decision. With a predetermined visit for follow-up, the information on DAS28 between two visits when the treatment is optimised is lacking. We extrapolated that if the patient was not in DAS28 remission at 6 months, the probability was high that the remission was not achieved before and so the MTX dose should be escalated to ≥20 mg/week or 0.3 mg/kg/week at month 6.

The description of the toxic effects experienced by each group was limited mainly to transaminitis, severe gastrointestinal distress and bronchitis, with no difference between groups except for moderate transaminitis <2 ULN at 6 months. Data on stomatitis, rash, brain fog and non-severe gastrointestinal distress were not collected, so determining to what extent common MTX toxic effects affected the ability to increase the weekly dose was difficult. However, whatever the reason of not achieving MTX optimal dose, our study is clearly showing that optimal MTX dose is more efficacious than non-optimal dose for remission and function in early arthritis in daily practice.

The proportion of sDMARD combinations used was greater with optimal than non-optimal MTX dose during the first year, including only one triple therapy with MTX+sulfasalazine+hydroxychloroquine. However, none of these combinations have clearly shown superiority to MTX monotherapy without being combined with corticosteroids.

We found no previous study concerning the use and clinical and structural efficacy of MTX optimisation in early RA in a real-life setting. Observational studies,[16 18 20 21] analysed the effect of early treatment on early RA outcome. Wiles *et al*[18 21] suggested that early MTX treatment (within 6 months of symptom onset) had a beneficial effect on long-term radiographic progression (progression of Larsen score at 5 years) and disability (HAQ score ≥1 at 5 years). However, MTX was used in only 7 of 219 patients, with sulfasalazine being prescribed in 60.7%. In a large patient population with RA, Kyburz *et al* showed that radiographic progression over 5 years was significantly lower for patients with than without early initiation of DMARDs (within the first year of symptom onset). [20] In a study of the ESPOIR cohort, Lukas *et al* showed that in daily clinical practice, a rapid (within 3 months) DMARD start (including MTX, sulfasalazine, leflunomide, tumour necrosis factor inhibitors) reduced 12-month radiographic progression.[16] Recently, Hazlewood *et al*[22] compared the effectiveness of starting with oral versus subcutaneous MTX over the first year in a cohort of 666 patients with early RA (417 oral MTX, 249 subcutaneous MTX). Initial treatment with subcutaneous MTX was associated with lower rates of treatment change, no difference in toxicity and some improvement in disease control as compared with oral MTX over the first year in these patients (lower mean DAS28 scores: mean difference (−0.38 (95% CI −0.64 to −0.10)) and small difference in DAS28 remission (OR 1.2 (95% CI 1.1 to 1.3))).[22] In the CONCERTO trial, increasing doses of MTX in combination with adalimumab demonstrated a statistically significant trend in improved clinical outcomes in patients with early RA.[23]

The results of our study help close the gap in evidence that optimal MTX dose in patients with early RA in a real-life setting is more favourable than non-optimal dose in terms of remission and function over 2 years. Such data suggest that efforts are needed to achieve a better use of MTX for early RA (initiation during the first 3 months and at optimal dose). By enhancing our knowledge of the use of MTX for RA, we will be able to optimise the use of this anchor drug in clinical practice and improve the well-being of our patients.

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Contributors CGV, MD, BC, BF: design of the study. CGV, NR: statistical analysis. CGV, MD, BC, BF, NR: interpretation of the results. CGV: drafting the work. MD, BC, BF, NR: revising the article. CGV, NR, MD, BC, BF: final approval of the version submitted.

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CONCISE REPORT

Successful treatment of arthritis induced by checkpoint inhibitors with tocilizumab: a case series

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ABSTRACT

Background Immune checkpoint inhibitors (ICIs) have significantly improved outcomes for patients with numerous cancers. However, these therapies are associated with immune-related adverse events (irAEs), which are inflammatory side effects potentially affecting any organ. Cases of ICI-induced inflammatory arthritis have also been reported. In general, mild irAEs are treated with corticosteroids, while tumour necrosis factor-α (TNFα) inhibitors are reserved for refractory cases. However, prolonged use of TNFα inhibitor (TNFαi) can induce widespread, significant immunosuppression, which can negatively impact the antitumour efficacy of ICI therapy. Therefore, in clinical scenarios where patients develop severe immunotherapy-induced irAEs, an unmet need exists for alternative therapeutic strategies that are effective and without immune dampening effects.

Case reports The anti-interleukin (IL)–6 receptor antibody, tocilizumab, is a biological agent Food and Drug Administration approved for the treatment of rheumatoid arthritis and juvenile idiopathic arthritis. Here, we report on three patients who developed severe polyarthritis while receiving ICI therapy and were treated with tocilizumab. All three patients demonstrated significant clinical improvement; one patient maintained a durable antitumour response derived from checkpoint inhibition.

Conclusions These three cases suggest that anti-IL-6 receptor antibody may be an effective alternative to corticosteroids or TNFαi for the treatment of arthritis irAEs.

INTRODUCTION

Immune checkpoint inhibitors (ICIs) targeting programmed death protein-1 (PD-1) and cytotoxic-T-lymphocyte-antigen-4 (CTLA-4) have revolutionised cancer treatment[1]; however, ICI therapy can cause immune-related adverse events (irAEs), inflammation of multiple organs.[2] Clinical studies demonstrated that inflammatory arthritis can be induced by ICI treatment.[3–6] Current guidelines suggest using corticosteroids for mild irAEs, and high-dose corticosteroids or tumour necrosis factor-α inhibitors (TNFαi) for severe irAEs.[7 8] However, both corticosteroid and TNFαi can result in serious adverse events.[8] In addition, preclinical data demonstrated that TNFα inhibition may dampen ICI therapy antitumour benefits.[9] Considering these limitations, alternative therapies are needed for irAEs.

Interleukin (IL)-6 is a cytokine with various biologic activities, including inflammation, immune responses and haematopoiesis.[10] Notably, IL-6 is a potent inducer of Th17 cells from naïve $CD4^+$ T cells. Th17, a helper T-cell subset that secretes IL-17, has been reported as a key mediator of many autoimmune diseases including rheumatoid arthritis, inflammatory bowel disease and colitis irAE and there has been recent interest in selectively blocking Th17 induction as a novel treatment strategy for patients with autoimmunity or irAEs.[11–13]

Here, we report three patients with metastatic melanoma who developed severe arthritis on ICI therapy. Owing to concerns over potentially abrogating the priming phase of antitumour immune response with prolonged TNFαi and considering the growing body of evidence suggesting the pathogenic role of Th17 cells in autoimmunity and irAE development, we treated patients with the anti-IL-6 receptor antibody, tocilizumab.[14] Each patient demonstrated significant arthritis improvement.

CASE DESCRIPTIONS

Case 1

A 71-year-old man developed metastatic melanoma in the right parotid gland in August 2014 and underwent parotidectomy with lymph node dissection followed by adjuvant radiation therapy. In April 2015, two recurrent melanoma lesions were found in the left pelvis. Treatment was initiated with ipilimumab (anti-CTLA-4 antibody) and palliative cryoablation to the painful pelvic lesions. After completing four ipilimumab treatments, he achieved a complete response (CR).

Subsequently, the patient developed fatigue with severe bilateral shoulder and hip pain. Physical examination revealed mild lethargy without active synovitis. Rheumatological workup was unremarkable except for a weakly positive rheumatoid factor (RF) (27 IU/ml, ULN: 15.9 IU/mL). His polyarthralgia and fatigue with recent ipilimumab exposure suggested arthritis irAE development, and the patient was started on prednisone (50 mg/day; weekly taper by 10 mg). This resulted in symptomatic improvement. However, shortly after the prednisone was tapered off, the patient's symptoms recurred.

Prednisone was restarted at 40 mg/day. Unfortunately, the patient developed paroxysmal atrial fibrillation and prednisone was discontinued. In January 2016, physical examination revealed tenderness over the bilateral wrists, metacarpophalangeal joints (MCPs), proximal interphalangeal

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joints (PIPs) and distal interphalangeal joints (DIPs), with active synovitis on all MCPs. To avoid potential antitumour immunosuppression with TNFαi, we initiated tocilizumab (162 mg subcutaneous injection every 2 weeks). Two months later, the patient's arthritis completely resolved. At 5 months, the patient developed bilateral MCP tenderness which responded to increasing the frequency of tocilizumab to weekly dosing (figure 1). Currently, his arthritis remains in remission with weekly tocilizumab. Prednisone has been tapered and he is currently taking 5 mg/day. Furthermore, he remains in durable CR and does not require further anticancer therapy over 18 months.

Case 2

A 65-year-old man presented with metastatic melanoma of the left elbow in December 2009. He underwent surgical resection followed by adjuvant interferon-α therapy. In May 2012, he developed recurrent disease in the left ileum and received biochemotherapy (dacarbazine, vinblastine, cisplatin and IL-2) followed by radiation therapy resulting in a CR. In August 2014, recurrent disease developed in the chest wall, right thigh and left hip. The patient received four doses of ipilimumab, but experienced disease progression.

Thereafter, patient was started on pembrolizumab (anti-PD-1 antibody). Shortly after receiving the second infusion, the patient developed severe pain and swelling in multiple joints. Examination revealed severe tenderness over the bilateral wrists, MCPs, PIPs and knees with active synovitis in the wrists and knees. Antinuclear antibody (ANA), RF, anti-cyclin citrullinated antibody (anti-CCP) were negative. The patient started prednisone 40 mg/day and his symptoms improved. However, the patient failed to taper prednisone below 20 mg/day and tocilizumab (every 2 weeks) was initiated in September 2015. Eight weeks later, his arthritis significantly improved and prednisone was discontinued (figure 1).

In November 2015, the patient developed progressive tumorous disease, and was started on a clinical trial which required discontinuing tocilizumab. Shortly after, the patient experienced an arthritis flare; tocilizumab was resumed with symptom improvement (figure 1). Currently, 15 months after initiating tocilizumab, his arthritis remains well controlled. He continues to receive tocilizumab while receiving an investigational melanoma therapy.

Case 3

A 46-year-old woman developed melanoma on the lower back in 2009, and underwent surgical resection. In June 2014, she developed recurrent melanoma involving the chest wall, adrenal gland and lung. Mutational analysis revealed a BRAF-V600E mutation and she was started on BRAF-targeted therapy. In March 2015, she developed progressive disease and switched to pembrolizumab. Thereafter, patient developed right adrenal gland haemorrhage and underwent an adrenalectomy.

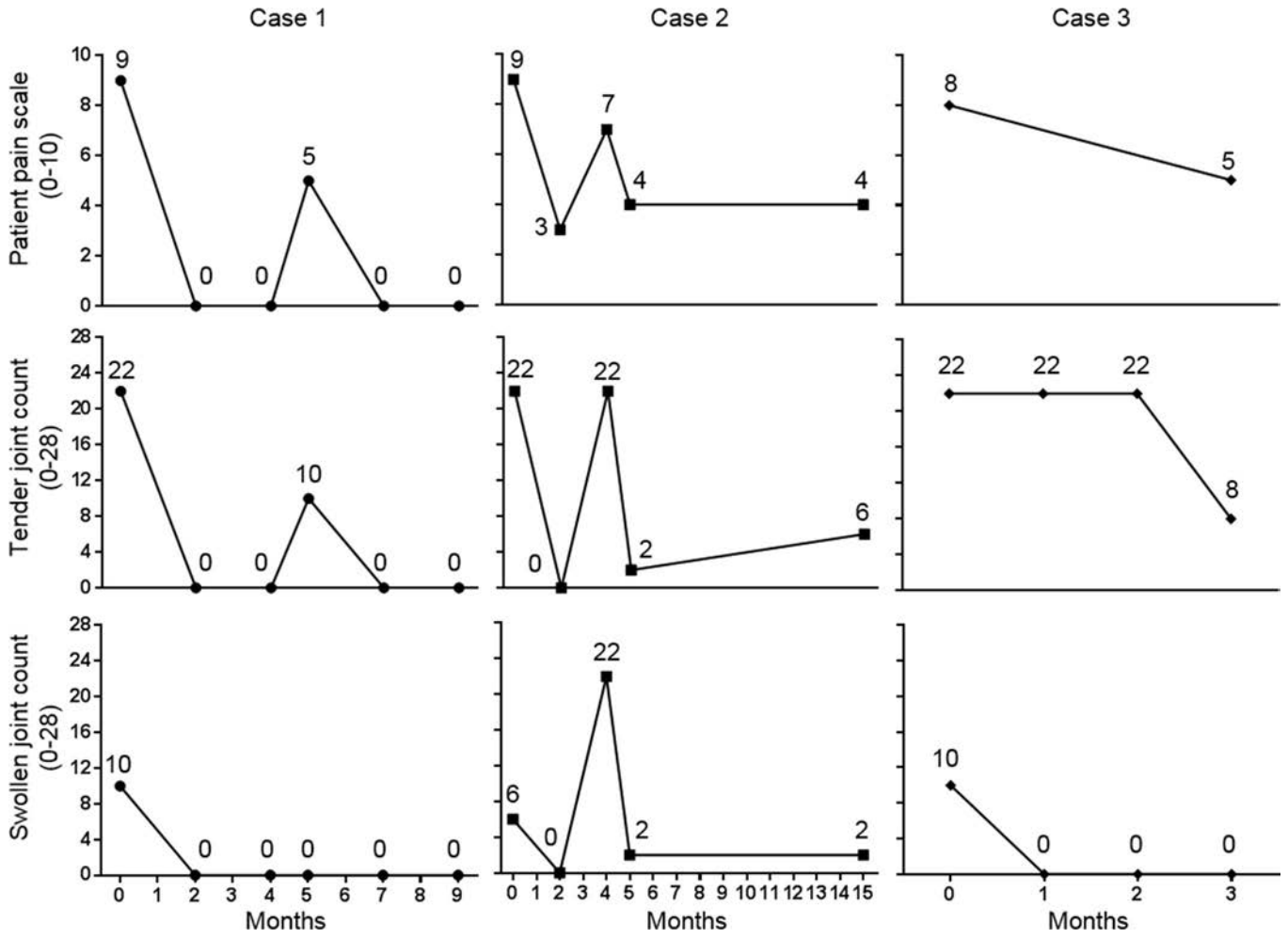

Figure 1 Patient pain scale, tender joint counts, swollen joint counts before (baseline) and after tocilizumab treatment. Measurements performed by a rheumatologist.

Table 1 Demographic and clinical characteristics of each case

Patient	1	2	3
Age, years	71	65	46
Sex	Male	Male	Female
Race	Caucasian	Caucasian	Caucasian
Tumour	Metastatic melanoma	Metastatic melanoma	Metastatic melanoma
ICIs	Ipilimumab	First: Ipilimumab Second: Pembrolizumab	First: Pembrolizumab Second: Ipilimumab
Tumour response to ICIs at present (RECIST 1.1)	Complete remission	Progressive disease	Progressive disease
Onset of arthritis irAEs	After the fourth infusion	After the second infusion of pembrolizumab	After the third infusion of Pembrolizumab
Pattern of arthritis	Symmetric polyarthritis involving small joints	Symmetric polyarthritis involving small and large joints	Symmetric polyarthritis involving small joints
Autoantibody results	ANA, CCP neg RF: 27 IU/ml (UNL: 15.9 IU/ml)	ANA, CCP, RF neg	CCP, RF, Ro, dsDNA neg ANA 1:640 (homogenous) La positive
Duration of tocilizumab (months)	11 (ongoing)	15 (ongoing)	3 (discontinued)

ANA, antinuclear antibody; CCP, anti-cyclin citrullinated antibody; dsDNA, anti–double-stranded DNA antibody; ICI, immune checkpoint inhibitor; La, anti-SSB antibody; RF, rheumatoid factor; Ro, anti-SSA antibody; UNL, upper normal limit.

While on pembrolizumab, the patient developed diarrhoea secondary to colitis irAE and was treated with budesonide with symptomatic improvement. In January 2016, she developed disease progression in the left adrenal gland. At the time, the patient also reported progressive pain and swelling in multiple joints associated with morning stiffness while on pembrolizumab. Physical examination revealed tenderness on both wrists, MCPs, PIPs and ankles, with mild synovitis on PIPs. Two possible incipient erosions on hand X-rays were reported by two different radiologists in left second MCP and right third DIP joints. Laboratory evaluation revealed a positive ANA (1:640 with homogenous pattern) and positive anti-La antibody. RF and anti-CCP antibody were negative. Subsequently, tocilizumab (every 2 weeks) was initiated.

Due to melanoma progression, patient switched therapy to ipilimumab, while remaining on tocilizumab. After 3 months of therapy with tocilizumab, the patient's joint symptoms improved. Physical examination only showed minimal tenderness over the bilateral second to fourth MCPs without active synovitis (figure 1). Interestingly, while on ipilimumab and tocilizumab, the patient denied any symptoms of colitis/diarrhoea despite being off budesonide. In November 2016, the patient underwent a left adrenalectomy due to haemorrhage. Tocilizumab was held and the patient initiated steroid supplementation for adrenal insufficiency. Repeat hand X-rays showed no radiographic progression. Unfortunately, recent imaging demonstrated melanoma progression and the patient has restarted BRAF-targeted therapy.

Demographic and clinical characteristics of each patient and the effect of tocilizumab on the arthritis irAEs are shown in table 1 and figure 1. Notably, all patients tolerated tocilizumab well without adverse events.

DISCUSSION

ICI therapy is approved for multiple cancers and our experience with them continues to evolve. While irAEs are currently managed with corticosteroids and/or TNFαi,[7] these agents have limitations.

Corticosteroids are known to suppress the 'priming' aspect of the immune response and, if used for a prolonged time, may abrogate the ICI-mediated antitumour benefits. In addition, high-dose and/or long-term use of corticosteroids can cause severe systemic toxicity. Despite this, corticosteroids remain the mainstay of therapy for acute irAEs.

The effects of TNFαi on ICI-mediated antitumour benefits are more complicated. Our group (unpublished data) and others have observed that one to two doses of TNFαi for colitis irAE result in faster diarrhoea resolution without negative impacts on overall survival or antitumour response.[15] However, effective treatment of arthritis irAE requires prolonged TNFαi, which can potentially dampen the antitumour benefit of ICI therapy.[9] Taking these therapy drawbacks into consideration, alternative strategies for treating irAEs are needed.

Although the immune mechanisms underlying irAEs have not been fully elucidated, studies suggest that Th17 cells play a prominent pathogenic role in some autoimmune diseases that resemble irAEs, such as colitis. Importantly, IL-6 promotes Th17 induction and IL-6 inhibition may rebalance the altered Th17-Treg axis without inhibiting the Th1-$CD8^+$ T-cell subsets that govern antitumour immunity.[16] We recently reported a patient with metastatic melanoma and concurrent Crohn's disease who received pembrolizumab and tocilizumab.[17] The patient achieved a melanoma CR without exacerbation of the underlying Crohn's disease. Lastly, although outside the scope of this report, it is worth mentioning that the IL-6 signalling pathway has been shown to play a role in the tumorigenesis of multiple cancers, associated with worse prognosis, and resistance to chemotherapy/immunotherapy.[18–21] Therefore, targeting this pathway for cancer treatment is an area of active investigation.[22]

In conclusion, to our knowledge, this case series is the first report describing that arthritis irAE can be safely managed with tocilizumab while possibly preserving ICI therapy benefits. We recognise that this is a small case series; therefore, these observations are descriptive and we cannot reliably draw definitive conclusions. However, our hypothesis that the IL-6-Th17 pathway plays a significant pathogenic role in arthritis irAE and possibly other irAEs remains intriguing and deserves further investigation in larger patient samples, and ultimately in prospective and controlled studies.

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Competing interests None declared.

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CONCISE REPORT

Allopurinol dose escalation to achieve serum urate below 6 mg/dL: an open-label extension study

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ABSTRACT

Objectives To determine the long-term safety and efficacy of allopurinol dose escalation (DE) to achieve target serum urate (SU) in gout.

Methods People, including those with chronic kidney disease, who completed the first 12 months of a randomised controlled trial continued into a 12-month extension study. Participants randomised to continue current dose for the first 12 months began allopurinol DE at month 12 if SU was ≥6 mg/dL (control/DE). Immediate DE participants who achieved target SU maintained allopurinol dose (DE/DE). The primary endpoints were reduction in SU and adverse events (AEs) at month 24.

Results The mean (SE) change in SU from month 12 to 24 was −1.1 (0.2) mg/dL in control/DE and 0.1 (0.2) mg/dL in DE/DE group (p<0.001). There was a significant reduction in the percentage of individuals having a gout flare in the month prior to months 12 and 24 compared with baseline in both groups and in mean tophus size over 24 months, but no difference between randomised groups. There were similar numbers of AEs and serious adverse events between groups.

Conclusions The majority of people with gout tolerate higher than creatinine clearance-based allopurinol dose and achieve and maintain target SU. Slow allopurinol DE may be appropriate in clinical practice even in those with kidney impairment.

Trial registration number ACTRN12611000845932

INTRODUCTION

Although other urate-lowering therapies (ULT) are available for gout, allopurinol is the mainstay of therapy due to its low-cost and widespread availability. Despite allopurinol being registered to 800 mg/day by the Food and Drug Administration and 900 mg/day in Europe, doses above 300 mg/day are used infrequently.[1 2]

Use of allopurinol at higher than creatinine clearance (CrCL) based doses remains controversial due to concerns over increased risk of adverse events (AEs), particularly allopurinol hypersensitivity syndrome (AHS). While European League Against Rheumatism (EULAR) advocates changing ULT if target serum urate (SU) is not achieved with CrCL-based allopurinol,[3] the American College of Rheumatology (ACR) advocates gradual escalation above CrCL-based doses to achieve target SU even in those with chronic kidney disease (CKD).[4] We report the results of the 12-month open-label extension (OLE) of a previously reported randomised controlled trial of allopurinol dose escalation (DE).[5] The aims were to determine whether target SU can be safely maintained over time, and the effects of DE to target SU strategy on clinical outcomes.

METHODS

This paper reports the 12-month OLE of a previously published 12-month randomised, controlled, parallel-group, comparative clinical trial.[5] Detailed study design and methods are available in online supplementary text 1.

RESULTS

Demographics

Of the 183 participants (control/DE (n=93), DE/DE (n=90)) who entered the study, 73/93 (78.5%) control/DE and 70/90 (78.9%) DE/DE participants completed the month 12 and 68/93 (73.1%) control/DE and 69/90 (76.7%) DE/DE participants completed the month 24 (see online supplementary figure S1). Complete baseline demographics have been reported previously[5] (see online supplementary table S1). Between baseline and month 24, two control/DE and five DE/DE participants discontinued allopurinol; of these only three in the DE/DE group discontinued allopurinol between months 12 and 24. Eighteen control/DE participants were not dose escalated after entering the DE phase as SU was <6 mg/dL during months 12–24.

Efficacy

Primary endpoint

The mean (SE) change in SU from month 12 to 24 was −1.1 (0.2) mg/dL in the control/DE group and 0.1 (0.2) mg/dL in the DE/DE group (p<0.001); mean difference 1.3 mg/dL (95% CI 0.8 to 1.7, p<0.001). The mean (SE) change in SU from baseline to 24 months was −1.4 (0.1) mg/dL in the control/DE group and −1.7 (0.1) mg/dL in the DE/DE group (p=0.14); mean difference −0.3 mg/dL (95% CI −0.7 to 0.1, p=0.14). In the control/DE group, mean (SE) SU was 7.13 (0.16) mg/dL at baseline and 5.7 (0.2) mg/dL at final visit, and 7.18 (0.2) mg/dL and 5.4 (0.1) mg/dL in the DE/DE group (figure 1A).

Secondary endpoints

SU was <6 mg/dL at the final visit in 69.1% of the control/DE group and 79.7% in the DE/DE group (p=0.16); OR 1.8 (95% CI 0.8 to 3.8) (figure 1B). Of those not at target at month 24,

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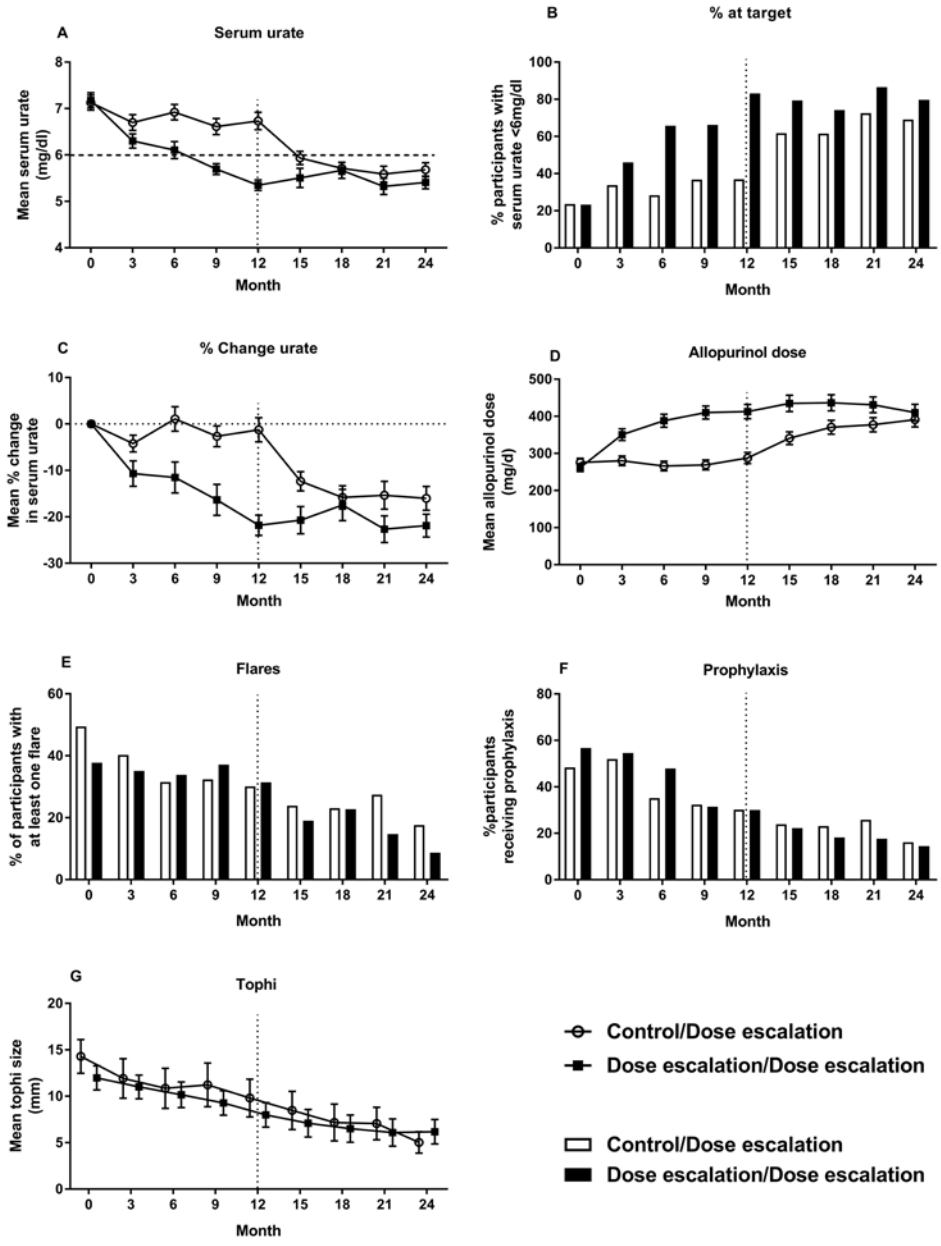

Figure 1 Mean (SEM) serum urate (A), percentage of participant achieving target serum urate (B), mean (SEM) percentage change in serum urate over the 24-month study period (C), mean (SEM) allopurinol dose in control/DE and DE/DE groups (D), percentage of participants reporting at least one gout flare in the preceding month (E), percentage of participants receiving anti-inflammatory prophylaxis (F) and mean (SD) size in tophi at each study visit (for those with measurable tophus at baseline) (G). The vertical line represents the start of the open-label extension phase of the study. DE, dose escalation.

nine (six control/DE and three DE/DE) had been at target for all other visits from month 12.

The mean (SE) percentage change in SU from month 12 to final visit was −13.6% (2.6%) in the control/DE group compared with 3.4% (2.6%) in the DE/DE group (p<0.001); mean difference 17.0% (95% CI 9.8% to 24.1%) (figure 1C). The mean percentage change in SU from baseline to final visit was −16.0% in the control/DE group compared with −21.9% in the DE/DE group (p=0.10); mean difference −5.9% (95% CI −12.9% to 1.2%) (figure 1C).

Between 12 and 24 months, there was a significantly higher time-adjusted area under the curve ($AUC_{adj\text{-}t}$) in the control/DE group compared with the DE/DE group (0.83 mg/dL vs −0.22 mg/dL; p<0.001); mean difference 1.06 mg/dL (95% CI 0.69 to 1.43). Between baseline and 24 months, there was a significantly lower $AUC_{adj\text{-}t}$ in the control/DE group compared with the DE/DE group (0.61 mg/dL vs 1.29 mg/dL; p=0.004); mean difference 0.68 mg/dL (95% CI 0.22 to 1.14).

The mean (range) allopurinol dose at month 24 was 391 mg/day (0–600 mg/day) in the control/DE and 410 mg/day (0–900 mg/day) in the DE/DE (figure 1D).

Gout flares and other outcomes

There was a significant reduction in the percentage of participants having a gout flare in the month prior to month 12 and

month 24 in both groups compared with the month prior to baseline (p<0.001), but no difference between randomised groups (p=0.29) (figure 1E, see online supplementary table S2). There was a significant reduction in the percentage of individuals having gout flares between baseline and month 24 in both groups (p<0.001) (figure 1E, see online supplementary table S2). There was no difference in the flare reduction between groups (p=0.78).

There was a significant reduction in the percentage of individuals using prophylaxis between months 12 and 24 in both groups (p<0.03), but no significant difference between randomised groups (p=0.84) There was a significant reduction in the use of prophylaxis over the 24-month period in both groups (p<0.001) but no significant difference between randomised groups (p=0.71) (figure 1F, see online supplementary table S2).

Of those with a tophus at baseline, 6/37 (16.2%) of the control/DE group and 4/31 (12.9%) of the DE/DE group had complete resolution of all tophi between months 12 and 24 (p=0.75). Between baseline and month 24, of those with measurable tophi, 13/45 (28.9%) of the control/DE group and 11/38 (28.9%) of the DE/DE group had complete resolution of all tophi (p=1.0). In the entire group, there was a significant decline in the mean (SEM) tophus size over the 24 months (13.1±1.0 mm baseline vs 6.6±1.2 mm month 24; p<0.001) (figure 1G). There was no difference in the change in tophus size between randomised groups (p=0.27) (figure 1G).

There was no significant difference in the mean change from month 12 to month 24 or from baseline to month 24 between randomised groups for Health Assessment Questionnaire, pain visual analogue scale, swollen joint count or tender joint count (see online supplementary table S2).

Adverse events

Serious adverse events

From month 12 to 24, there were 38 serious adverse events (SAEs) in 14 control/DE participants and 33 SAEs in 22 DE/DE participants (table 1, see online supplementary table S3). None were considered related to allopurinol. Four control/DE and three DE/DE participants died between months 12 and 24. None of the deaths were attributed to allopurinol. In the control/DE group, deaths were attributed to infection (n=1) and heart failure (n=3) and in the DE/DE group acute coronary syndrome (n=1) and infection (n=2).

Non-laboratory AEs

From month 12 to 24, there were 279 non-laboratory AEs in 65 control/DE and 208 in 62 DE/DE participants (table 1, see online supplementary table S4). The number of participants experiencing at least one non-laboratory AE in each CTCAE (Common Terminology Criteria for Adverse Events) category is shown in table 1. Between months 12 and 24, seven control/DE participants developed rash; one was probably related to allopurinol, which was discontinued, and two were possibly related. In months 12–24, seven control/DE participants developed pruritus, one considered possibly related to allopurinol. In months 12–24, four DE/DE participants developed rash, none considered related to allopurinol, and four DE/DE participants developed pruritus, one possibly allopurinol related.

Laboratory AEs

Between months 12 and 24, the majority of elevations in aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were CTCAE grade 1 (figure 2A–D). For gamma-glutamyltransferase (GGT), 15 control/DE and 14 DE/DE participants had treatment emergent AEs between months 12 and 24, of which one control/DE and three DE/DE participants had increases over two CTCAE grades. For ALT, two DE/DE participants had increases by >1 CTCAE grade and ALP in one DE/DE participant increased by >1 CTCAE grade between months 12 and 24. There were no instances of AST increasing by >1 CTCAE grade.

For creatinine, an increase from baseline value was used to determine CTCAE grade. Between months 12 and 24, there were 154 events in 61 control/DE participants and 139 events in 53 DE/DE participants; the majority were CTCAE grade 1 (>1–10.5× above baseline) (figure 2E). Twenty-three control/DE and 19 DE/DE participants experienced more than a 20% decrease in CrCL between months 12 and 24 (figure 2F).

Haematological treatment emergent or worsening AEs are shown in online supplementary table S5 and supplementary figure S1.

Improvement in laboratory variables is available in online supplementary text 2.

DISCUSSION

These results provide further evidence that allopurinol DE is well tolerated and effective in people with gout including in those who have not been at target SU for some months. Once achieved, target SU can be maintained with ongoing allopurinol use.

While there were a number of AEs/SAEs, only a few were considered related to allopurinol. The number and type of AEs/SAEs seen in those who dose escalated in months 12–24 were similar to those dose escalated in the first 12 months. During months 12–24, there was no obvious increase in AEs or SAEs in those dose escalated in the first 12 months. A number of laboratory AEs were noted, in particular GGT increases. This has been reported previously[2] and the clinical significance is uncertain. Minor fluctuations in creatinine resulted in a large number of creatinine CTCAE grade 1 AEs (creatinine >1–10.5× baseline). Approximately 10%–20% of participants had a decrease in CrCL >20% at some point during the study with ~10% having an improvement in CrCL. No new safety signal was identified.

The questionable safety of allopurinol above CrCL-based doses led to EULAR not supporting a DE strategy.[3] Most of the concern relates to the increased risk of AHS and poorer outcomes associated with AHS in people with CKD.[6] While no cases of AHS occurred during the study, it is important to note that AHS typically occurs in the first 8 weeks after commencing allopurinol[7] and has been associated with a higher starting dose.[8] The current study enrolled participants after the early risk period and was not powered to detect such a rare AE. Our study provides support for the approach advocated by the ACR, showing that for individuals tolerating the CrCL-based allopurinol dose, escalation to achieve target appears safe and effective.

There was a reduction in the number of participants having gout flares over the study compared with prior to the study and a reduction in mean tophi size in both groups over the 24-month study period with no difference between randomised groups. The reduction in flares occurred between months 12 and 24, with no difference between baseline and month 12.[5] This delay in flare reduction has been observed in other clinical trials[9] and reflects the time it takes to deplete total body urate once target SU is achieved. The lack of difference between randomised groups raises several important issues. Flares are an important clinically relevant outcome, particularly from the patient's perspective.

Table 1 Number (%) of participants with treatment emergent serious and non-adverse events in each category during year 1 and year 2

	Serious adverse events months 0–12		Serious adverse events months 12–24		Non-laboratory treatment emergent adverse event months 0–12		Non-laboratory treatment emergent adverse event months 12–24	
	Control/DE (n=93)	DE/DE (n=90)	Control/DE (n=93)	DE/DE (n=90)	Control/DE (n=93)	DE/DE (n=90)	Control/DE (n=93)	DE/DE (n=90)
Number of participants with at least one adverse event	25 (27%)	22 (24%)	14 (15.1%)	22 (24.4%)	80 (86%)	73 (81%)	65 (70%)	62 (69%)
Cardiac disorders	8 (9%)	11 (12%)	7 (7.5%)	7 (7.8%)	9 (10%)	5 (6%)	3 (3%)	3 (3%)
Gastrointestinal disorders	6 (7%)	3 (3%)	5 (5.4%)	4 (4.4%)	21 (23%)	18 (20%)	14 (15%)	14 (16%)
General disorders	1 (1%)	1 (1%)	2 (2%)	1 (1%)	47 (51%)	48 (53%)	31 (33%)	30 (33%)
Hepatobiliary disorders	0	1 (1%)	0	0	0	2 (2%)	0	2 (2%)
Infections and infestations	8 (9%)	3 (3%)	6 (6.5%)	7 (7.8%)	18 (19%)	14 (16%)	17 (18%)	11 (12%)
Injury, poisoning and procedural complications	2 (2%)	1 (1%)	4 (4.3%)	1 (1%)	15 (16%)	24 (27%)	12 (13%)	10 (11%)
Metabolism and nutrition	0	0	0	0	4 (4%)	1 (1%)	2 (2%)	4 (4%)
Musculoskeletal	1 (1%)	1 (1%)	1 (1%)	1 (1%)	27 (29%)	24 (27%)	24 (26%)	30 (33%)
Nervous system disorders	3 (3%)	1 (1%)	2 (2%)	4 (4.4%)	10 (11%)	11 (12%)	13 (14%)	4 (4%)
Renal and urinary disorders	5 (5%)	2 (2%)	0	3 (3.3%)	0	2 (2%)	1 (1%)	0
Respiratory, thoracic and mediastinal disorders	2 (2%)	2 (2%)	0	0	16 (17%)	15 (17%)	11 (12%)	11 (12%)
Skin and subcutaneous tissue disorders	1 (1%)	1 (1%)	0	0	20 (22%)	23 (26%)	18 (19%)	14 (16%)
Ear and labyrinth	0	0	0	0	3 (3%)	3 (3%)	1 (1%)	3 (3%)
Endocrine	0	0	0	0	0	1 (1%)	0	1 (1%)
Eye	0	0	0	0	4 (4%)	3 (3%)	1 (1%)	1 (1%)
Immune system	0	0	0	0	1 (1%)	1 (1%)	0	1 (1%)
Neoplasms benign, malignant and unspecified	0	0	0	0	4 (4%)	4 (4%)	11 (12%)	3 (3%)
Psychiatric disorders	0	0	1 (1%)	1 (1%)	5 (5%)	4 (4%)	3 (3%)	4 (4%)
Reproductive and breast disorders	0	0	0	0	2 (2%)	0	1 (1%)	2 (2%)
Surgical and medical procedures	0	0	0	0	2 (2%)	2 (2%)	1 (1%)	2 (2%)
Vascular disorders	0	0	0	1 (1%)	8 (9%)	10 (11%)	4 (4%)	12 (13%)
Venous disorders	0	0	0	0	1 (1%)	0	1 (1%)	0
Allopurinol specific adverse events								
Allopurinol hypersensitivity syndrome					0	0	0	0
Rash					11 (12%)	8 (9%)	7 (7%)	4 (4%)
Pruritus					5 (5%)	10 (11%)	7 (7%)	4 (4%)
Nausea/vomiting					9 (10%)	6 (7%)	6 (6%)	5 (6%)
Abdominal pain					5 (5%)	6 (7%)	2 (2%)	3 (3%)

DE, dose escalation.

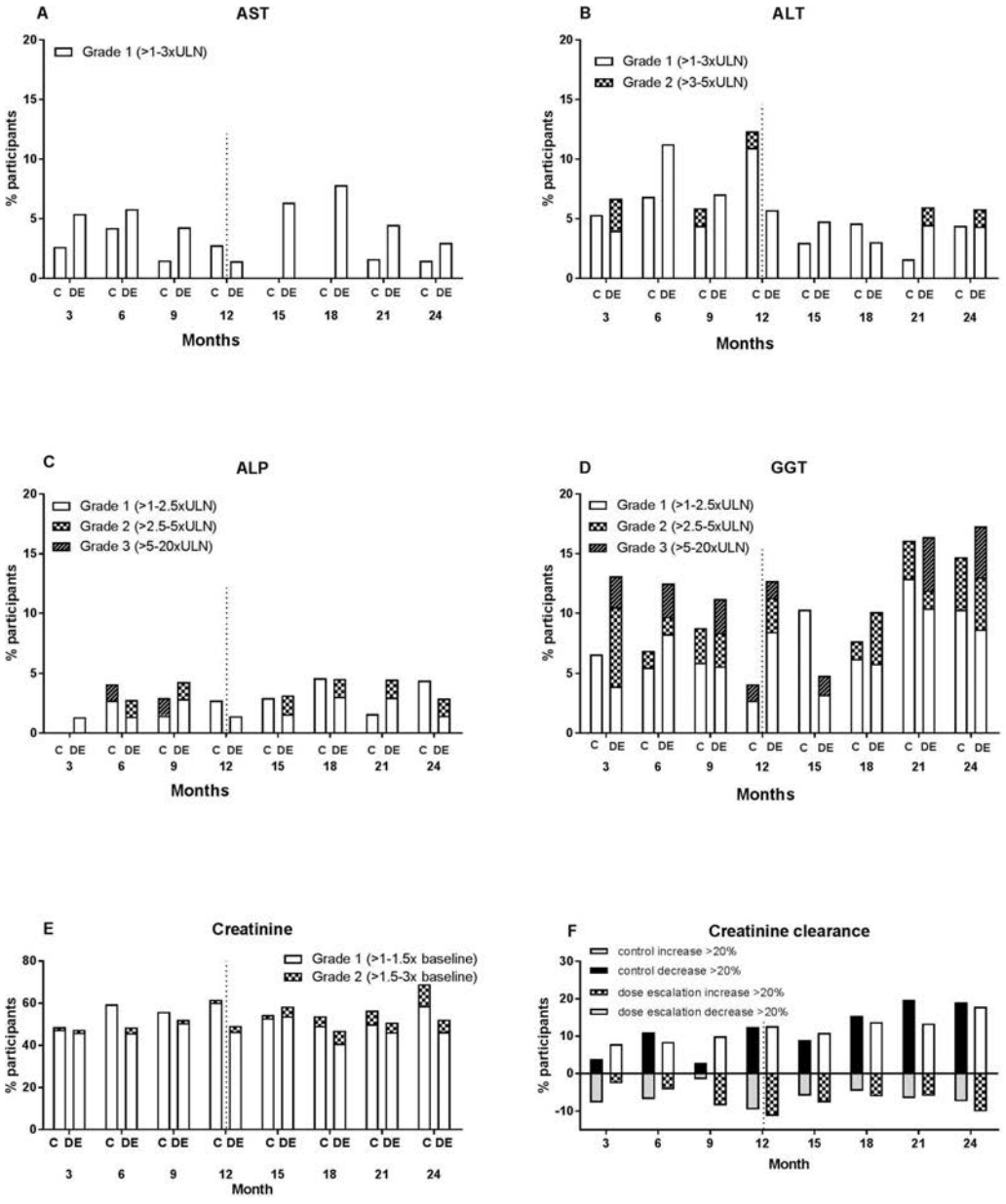

Figure 2 Treatment emergent or worsening laboratory adverse events. (A-D) Liver function over the 24-month study period by CTCAE grade in control/DE and DE/DE groups. (E) Percentage of participants with increase in creatinine over baseline and (F) percentage of participants with more than a 20% decrease (worsening) or increase (improvement) in CrCL from baseline. ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; C, control; CrCL, creatinine clearance; CTCAE, Common Terminology Criteria for Adverse Events; DE, dose escalation; GGT, gamma-glutamyltransferase; ULN, upper limit of normal.

Despite this, SU has been the primary efficacy endpoint for most ULT clinical trials.[9–11] The use of SU as the primary outcome measure allows for shorter, cheaper trials but relies on SU being a 'biomarker' for clinically important outcomes such as flares. While there is a sound scientific rationale for assuming a reduction in SU below the point where monosodium urate crystals form, there have been concerns over whether SU is a true 'biomarker'. SU fulfils many of the required characteristics for a biomarker[12] and work is under way to determine whether it can fulfil more sophisticated criteria.[13] The use of SU as a treatment target and outcome measure in clinical trials has become controversial with the American College of Physician Gout Guidelines advocating a 'treat-to-symptom' rather than a 'treat-to-target SU' approach to gout management.[14]

Whether the target SU of <6 mg/dL, with <5 mg/dL for those with tophi, is the most appropriate remains to be determined. There is a linear relationship between SU and speed of tophus size reduction.[15] In clinical trials of pegloticase, participants who maintained SU <6 mg/dL for ≥80% of the time were more likely to have complete remission of tophus at 6 months.[16] In the Febuxostat Compared with Allopurinolin Patients with Hyperuricemia and Gout (FACT) study, the proportion of patients with gout flare between weeks 49 and 52 was lower among those with postbaseline SU <6 mg/dL than those with postbaseline SU ≥6 mg/dL (6% vs 14%; p=0.005).[9] These studies did not examine whether one specific target SU is superior to another and there are no randomised controlled trials comparing clinical outcomes with different SU targets.

There are a number of limitations with this study. The open label design introduces bias; however, this was minimised by the use of SU as the primary endpoint. Attribution of AEs/SAEs to allopurinol may have been more likely during the DE period. One of the key strengths of this study is the 'real-life' population recruited who had a significant number of significant comorbidities, including CKD.

In conclusion, we have shown that allopurinol DE above CrCL-based doses is effective in maintaining SU at treatment target and is well tolerated. Gradual allopurinol DE with appropriate monitoring of kidney and liver function is an alternative to changing to an alternate ULT in people with gout.

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

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Patient consent Obtained.

Ethics approval Multiregional ethics committee of New Zealand.

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CONCISE REPORT

Remission and Low Disease Activity Status (LDAS) protect lupus patients from damage occurrence: data from a multiethnic, multinational Latin American Lupus Cohort (GLADEL)

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ABSTRACT

Objective To evaluate disease activity statuses' (DAS') impact on systemic lupus erythematosus (SLE) outcomes.

Materials and methods Four DAS were defined: remission off-therapy: SLE Disease Activity Index (SLEDAI)=0, no prednisone or immunosuppressive drugs (IS); remission on-therapy: SLEDAI=0, prednisone ≤5 mg/day and/or IS (maintenance); low (L) DAS: SLEDAI ≤4, prednisone ≤7.5 mg/day and/or IS (maintenance); non-optimally controlled: SLEDAI >4 and/or prednisone >7.5 mg/day and/or IS (induction). Antimalarials were allowed in all. Predefined outcomes were mortality, new damage (increase of at least one Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) damage index (SDI) point) and severe new damage (increase of at least 3 SDI points). Univariable and multivariable Cox regression models were performed to define the impact of DAS, as time-dependent variable, on these outcomes.

Results 1350 patients were included, 79 died during follow-up, 606 presented new and 177 severe new damage. In multivariable analyses, remission (on/off-therapy) was associated with a lower risk of new (HR 0.60; 95% CI 0.43 to 0.85), and of severe new damage (HR 0.32; 95% CI 0.15 to 0.68); low disease activity status (LDAS) was associated with a lower risk of new damage (HR 0.66; 95% CI 0.48 to 0.93) compared with non-optimally controlled. No significant effect on mortality was observed.

Conclusions Remission was associated with a lower risk of new and severe new damage; LDAS with a lower risk of new damage after adjusting for other damage confounders.

INTRODUCTION

Treat to target strategy (T2T) has been proposed for several chronic diseases as means of improving patients' outcomes. For example, in rheumatoid arthritis, early combination therapy plus either prednisone or infliximab strategy resulted in patients achieving the target earlier than those on sequential monotherapy or step-up strategy.[1]

Recently, T2T strategy has been proposed for patients with systemic lupus erythematosus (SLE). However, there is no consensus about the definition of these possible outcomes.[2] The Definitions Of Remission In SLE international task force proposed that remission should require the use of a validated index which could be supplemented with a physician's global assessment (PGA). Additionally, two categories of remission should be considered: off-therapy (only antimalarials) and on-therapy (including prednisone ≤5 mg/day, immunosuppressives (IS) or biologics on maintenance dose).[3] Given that remission is achieved infrequently, low disease activity status (LDAS) was considered an alternative outcome; there have been three definitions of LDAS: (1) Asian Pacific Lupus Consortium (APLC): SLE Disease Activity Index (SLEDAI)−2K≤4, no activity in any major organ, no new disease activity features, PGA ≤1, prednisone ≤7.5 mg/day and IS (maintenance dose)[4]; (2) Lupus Clinical Trials Consortium (LCTC): SLEDAI ≤4, PGA <1, prednisone ≤7.5 mg/day and IS (maintenance)[5]; and (3) Toronto Cohort: SLEDAI <3 with only one of these manifestations present: rash, alopecia, mucosal ulcers, pleurisy, pericarditis, fever, thrombocytopenia or leucopenia.[6]

The impact of these targets on SLE outcome, however, has not been fully evaluated. We aimed at determining the impact of remission and LDAS in two defined lupus outcomes, damage and mortality.

METHODS

Patients

Grupo Latino Americano De Estudio de Lupus (GLADEL) is an observational inception cohort study started in 1997 in 34 centres from nine Latin American countries. A common protocol, consensus definitions and outcome measures were established. The general characteristics and composition of the 1480 GLADEL cohort patients have been described in detail elsewhere.[7] The study was performed in accordance with the Declaration of Helsinki for the

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Table 1 Baseline characteristics of patients evaluated

Characteristic	n (%) or median (IQR)
Gender, female	1211 (89.7)
Age at baseline, years	28.0 (21.0–37.0)
Ethnicity	
Caucasian	564 (41.8)
Mestizo	577 (42.7)
African Latin American	158 (11.7)
Other	51 (3.8)
Educational level, years	9.0 (6.3–12.0)
Socioeconomic status	
High	132/1345 (9.8)
Medium	391/1345 (29.1)
Low	822/1345 (61.1)
Medical coverage	806/1337 (60.3)
SLE Disease Activity Index at baseline	8.5 (7.0–15.0)
SDI at baseline	0.0 (0.0–1.0)
Disease duration at baseline, years	0.3 (0.0–0.9)
Follow-up, years	2.4 (0.7–5.6)

SDI, SLICC/ACR damage index.

conduct of research in humans and following local institutional review board's regulations.

For these analyses, we evaluated the intervals between visits which were defined as the period between two SLEDAIs or between one SLEDAI and the end of follow-up. Only patients with at least two intervals were included.

Variables

Disease activity was ascertained using the SLEDAI,[8] and it was assessed, per protocol, twice a year. Each interval was classified as one of four disease activity statuses:

- Remission off-therapy: SLEDAI=0 without prednisone or IS.
- Remission on-therapy: SLEDAI=0 and a prednisone dose ≤5 mg/day and/or IS (maintenance dose).
- LDAS: SLEDAI ≤4, a prednisone dose ≤7.5 mg/day and/or IS (maintenance dose).
- Non-optimally controlled status: SLEDAI >4 and/or prednisone dose >7.5 mg/day and/or IS (induction dose).

Antimalarials were allowed in all groups.

Disease damage was ascertained using the Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) damage index (SDI)[9] and was measured, per protocol, once a year.

Predefined outcomes were mortality (any cause), new damage (an increase of at least one point in the SDI) and severe new damage (an increase of at least three points in the SDI).

Table 2 The best and last statuses achieved by the 1350 patients evaluated

Status	Best n (%)	Last n (%)
Non-optimally controlled	885 (65.6)	1034 (76.6)
Low disease activity status	192 (14.2)	149 (11.0)
Remission on-therapy	223 (16.5)	141 (10.4)
Remission off-therapy	50 (3.7)	26 (1.9)

Statistical analyses

Categorical variables were summarised as frequencies and percentages while continuous variables are presented as medians and IQR. Cox regression models were used to derive unadjusted and adjusted HRs and 95% CIs quantifying the association between disease activity status as a time-dependent variable and outcomes. Non-optimally controlled status was considered the reference level. Adjustment variables were age at baseline, gender, socioeconomic status, ethnicity, educational level, medical coverage and SDI at baseline. Due to the small number of intervals, both remission groups were combined. Additionally, exploratory analyses for glucocorticoids-related damage (cataracts, myocardial infarction, angina, ventricular dysfunction, osteoporosis with fracture, osteonecrosis and diabetes mellitus) were performed.

Statistical analyses were performed using SAS V.9.4.

RESULTS

There were 5672 intervals from 1350 patients. These patients' baseline characteristics are depicted in table 1. The median number of intervals per patient was 4 (IQR 2–7), and the median length of the intervals was 7.1 months (5.1–11.7). The best and last status achieved by each patient is depicted in table 2.

New damage was present in 606 (44.7%) patients, severe new damage in 177 (13.1%); 79 (5.8%) patients died during the follow-up.

Of the intervals examined, the most frequent status was non-optimally controlled, with 4446 (78.4%) intervals, followed by LDAS 566 (10.0%), remission on-therapy 553 (9.7%) and remission off-therapy 107 (1.9%) intervals.

The impact of remission and LDAS on new damage, severe new damage and mortality is depicted in table 3. In multivariable analyses, remission was associated with a lower risk of new damage (HR 0.60; 95% CI 0.43 to 0.85; p=0.0042) and a lower risk of severe new damage (HR 0.32, 95% CI 0.15 to 0.68; p=0.0033); LDAS was associated with a lower risk of new damage (HR 0.66; 95% CI 0.48 to 0.93; p=0.0158). No association between disease activity status and mortality was observed. Furthermore, achieving remission and LDAS was associated with a lower risk of new and severe new damage accrual unrelated to glucocorticoids in both univariable and multivariable analyses. That was not the case for glucocorticoids-related damage.

DISCUSSION

Using GLADEL's longitudinal data, a multiethnic, multinational inception cohort, we have evaluated if achieving LDAS or remission was associated with a lower risk of damage accrual and mortality. Remission was associated with a lower risk of new and severe new damage occurrence, whereas LDAS was only for new damage. Neither status impacted on mortality.

Remission off-therapy in the GLADEL cohort was rare with only 3.7% of the patients achieving it at least once, lower than reported in other cohorts; using the same definition 1.7% of Toronto Cohort's patients achieved remission for at least 5 years, 10.2% for at least 1 year.[10] In an Italian cohort, 7.1% of the patients achieved remission for at least 5 years[11] whereas 12.8% did so in a Netherlands' cohort[12]; 24% achieved remission for at least 1 year in a Spaniard cohort. Including PGA in the definition, 5.4% of LCTC patients were on remission for at least 1 year.[5]

Remission on-therapy was achieved in 16.8% of our patients, similar to other cohorts. In the Toronto Cohort remission, regardless of therapy, occurred for at least 5 years in 1.8% of the

Table 3 Impact of disease activity statuses on mortality, new damage and severe new damage: univariable and multivariable analyses

	Remission (on/off therapy)		LDAS	
	HR (95% CI)	p Value	HR (95% CI)	p Value
Mortality				
Unadjusted	0.46 (0.17 to 1.27)	0.1330	0.65 (0.26 to 1.60)	0.3454
Adjusted*	0.56 (0.20 to 1.55)	0.2623	0.81 (0.32 to 2.02)	0.6476
New damage†				
Unadjusted	0.53 (0.38 to 0.75)	0.0003	0.61 (0.44 to 0.85)	0.0032
Adjusted*	0.60 (0.43 to 0.85)	0.0042	0.66 (0.48 to 0.93)	0.0158
Severe new damage‡				
Unadjusted	0.31 (0.15 to 0.64)	0.0014	0.48 (0.26 to 0.92)	0.0260
Adjusted*	0.32 (0.15 to 0.68)	0.0033	0.54 (0.28 to 1.03)	0.0614
New damage (non-GC)§				
Unadjusted	0.45 (0.31 to 0.66)	<0.0001	0.56 (0.40 to 0.80)	0.0013
Adjusted*	0.51 (0.35 to 0.75)	0.0006	0.62 (0.43 to 0.87)	0.0067
Severe new damage (non-GC)¶				
Unadjusted	0.25 (0.10 to 0.62)	0.0028	0.30 (0.12 to 0.74)	0.0091
Adjusted*	0.31 (0.12 to 0.75)	0.0101	0.35 (0.14 to 0.85)	0.0206
New damage (GC)**				
Unadjusted	1.06 (0.59 to 1.92)	0.8476	1.24 (0.69 to 2.23)	0.4682
Adjusted*	0.99 (0.53 to 1.84)	0.9697	1.34 (0.74 to 2.42)	0.3333

*Adjusted by age at baseline, gender, ethnicity, socioeconomic status, years of instruction, medical coverage and first SLICC/ACR damage index (SDI).
†One-point increment in the SDI.
‡Three-point increment in the SDI.
§One-point increment in the damage unrelated to glucocorticoids.
¶Three-point increment in the damage unrelated to glucocorticoids.
**One-point increment in the damage glucocorticoids-related damage.
LDAS, low disease activity status; reference group for HRs: non-optimally controlled.

patients and in 18.9% for at least 1 year[10] while 7.6% of patients achieved remission on-therapy for at least 1 year.[5]

LDAS was achieved in 14.4% of our patients, similar to LCTC patients (14.9%),[5] but less frequently than in APLC where 88.5% of their patients achieved it at least once.[4] Using the APLC definition 76.0% of the Netherland patients achieved LDAS at least once, and 64.5% were on it in at least 50% of the observations.[12]

Our data clearly show that remission on/off-therapy is protective of new and severe new damage, consistent with the Toronto Cohort's data; patients who achieved remission off therapy accrued less damage than those with active disease (1.1 vs 1.6; p=0.03); furthermore, damage accrual among patients on remission, either off-therapy or on-therapy, was comparable.[13] Likewise, in the Italian cohort, patients with unremitted disease had a two times higher risk of accruing damage compared with those on remission; the remission group included complete remission (remission off-therapy) and clinical remission (with or without serological activity, on/off-therapy). Furthermore, within the remission group, those patients on prednisone developed glucocorticoid-related damage more frequently than those not on it; glucocorticoid-unrelated damage was, however, similar in all patients in remission.[11] In the same cohort, being 1 year in remission was not sufficient for preventing damage; and in those on remission for at least 5 years, being on prednisone was associated with a higher damage accrual than not being on it.[14] In the Netherlands cohort, using the same definition than in the Italian cohort, prolonged remission (complete or clinical remission for at least 5 years) was associated with reduced damage accrual.[12]

LDAS was found to be protective of new damage accrual, similar to the report from APLC in which patients with at least 50% of the observation time on LDAS had a twofold reduction in the risk of new damage compared with those on LDAS for less of that time.[4] In the Toronto Cohort, patients on LDAS had, after 2 years of follow-up, similar prognosis than those on remission in terms of flares, damage accrual, mortality or need for immunosuppressive drugs. Both groups together (LDAS and remission) had, after 2 years of follow-up, a better prognosis than those with high disease activity (SLEDAI >6) in terms of damage accrual, mortality, IS use, dose of prednisone and numbers of flares.[6]

Our study has some limitations. First, the relatively short follow-up (and consequently, few events) precluded us from finding an impact of remission or LDAS on mortality (resulting in wide CIs). Second, as there are no uniform remission and LDAS' definitions, it is possible had we used different definitions, our results could have been different; however, similar definitions have been used in other studies, and they are considered reliable.[5 10 11 15] Third, we used an alternative definition of LDAS given that the PGA had not been assessed in the GLADEL patients; however, in the SLE Response Index, which was developed for the belimumab's trials,[16] the SLEDAI was the variable with the highest impact on the definition of response. Thus the modified definition of LDAS we have used is entirely valid.

Despite these limitations, our data, from a very large multiethnic, multinational lupus cohort, emphasise the importance of achieving remission or LDAS in the prognosis of patients with SLE. In conclusion, remission diminished the risk of new and severe new damage, and LDAS diminished the risk of new damage after adjusting for other well-known risk factors of damage. These data support the use of these outcomes as targets in the treatment of patients with SLE.

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Contributors All authors were involved in drafting or critically revising this manuscript for important intellectual content, and all authors approved the final version to be published. MFU-G, DW, GSA and BAP-E have full access to all the study's data and take responsibility for their integrity and the accuracy of the analyses performed.

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OPEN ACCESS

EXTENDED REPORT

Chemotaxis of Vδ2 T cells to the joints contributes to the pathogenesis of rheumatoid arthritis

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ABSTRACT

Objectives To explore the role of Vδ2 T cells in the pathogenesis of rheumatoid arthritis (RA).

Methods Sixty-eight patients with RA, 21 patients with osteoarthritis and 21 healthy controls were enrolled in the study. All patients with RA fulfilled the 2010 American College of Rheumatology/European League Against Rheumatism criteria for RA. Peripheral Vδ2T population, chemokine receptor expression and proinflammatory cytokine secretion were quantified by flow cytometry. The infiltration of Vδ2 T cells within the synovium was examined by immunohistochemistry and flow cytometry. The effect of tumour necrosis factor (TNF)-α and interleukin (IL)-6 on Vδ2 T migration was determined by flow cytometry and transwell migration assay.

Results Peripheral Vδ2T cells, but not Vδ1 T cells, were significantly lower in patients with RA, which was negatively correlated with disease activity gauged by Disease Activity Score in 28 joints. Vδ2 T cells from RA accumulated in the synovium and produced high levels of proinflammatory cytokines including interferon-γ and IL-17. Phenotypically, Vδ2 T cells from RA showed elevated chemotaxis potential and expressed high levels of chemokine receptors CCR5 and CXCR3, which was driven by increased serum TNF-α through nuclear factor kappa B signalling. In vivo, TNF-α neutralising therapy dramatically downregulated CCR5 and CXCR3 on Vδ2 T cells and repopulated the peripheral Vδ2 T cells in patients with RA.

Conclusions High levels of TNF-α promoted CCR5 and CXCR3 expression in Vδ2 T cells from RA, which potentially infiltrated into the synovium and played crucial roles in the pathogenesis of RA. Targeting Vδ2 T cells might be a potential approach for RA.

INTRODUCTION

γδ T cells are a subset of T cell with distinctive T cell receptor (TCR), which is composed of one γ chain and one δ chain. γδ T cells mainly accumulate in mucosal tissues such as gut, and consist the minor population of peripheral lymphocytes (2%–5%).[1 2] Two main subsets of γδ T cells have been defined, namely Vγ9/Vδ2 and Vδ1. Vγ9/Vδ2 T cells, the major population of peripheral blood γδ T cells, express TCR variable regions Vγ9 and Vδ2 and produce high levels of interferon (IFN)-γ and tumour necrosis factor (TNF)-α. They also participate in host defence against intracellular pathogens and haematological malignancies.[3–6] Vδ1 T cells, which differ from Vγ9/Vδ2 T cells, mainly reside in the mucosal-associated lymphoid tissues and consist approximately 10%–30% of peripheral γδ T cells.[7] Vδ1 cells have lower cytotoxicity compared with Vδ2 T cells, have regulatory potential[8] and produce a broader set of cytokines, including interleukin (IL)-4 and IL-17.[3 9]

γδ T cells are implicated in many infectious diseases and tumours. In recent years, growing evidence has implicated γδ T cells in human autoimmune disorders such as diabetes, arthritis and multiple sclerosis. Vδ2-expressing circulatory γδ T cells significantly accumulated in the brains of patients with multiple sclerosis.[10] In addition, γδ T cells were found to induce Ig secretion in B cell lines and induce autoantibody production in peripheral B cells from patients with systemic lupus erythematosus.[11]

Rheumatoid arthritis (RA) is a chronic inflammatory disease that causes severe joint destruction and deformity. RA is characterised with serum autoantibodies as well as extensive lymphocytes infiltration in the synovia, including T and B cells. $CD4^+$ T cells play crucial roles in the pathogenesis of RA. However, accumulating evidence suggests that γδ T cells are also involved in RA.[12 13] In collagen-induced arthritis, an experimental model of RA, preventive depletion of γδ T cells ameliorated the disease severity in DBA1/J mice.[12] In human RA, synovial effusions (SF) and synovial membranes have been found to contain a high number of T cells bearing the γδ TCR.[14 15] Their percentages in SFs were between twofold and fourfold higher compared with peripheral blood.

In light of this evidence, we investigated Vδ2 T cells in peripheral blood, SF and synovium from patients with RA and their contribution to the pathogenesis of RA.

METHODS

Patients and controls

This study was approved by the Institutional Review Board of Peking Union Medical College Hospital. Written informed consent was obtained from each participating patient and healthy control (HC). Peripheral blood samples were collected from 15 patients with RA before and after treatment with TNF-α antagonist or IL-6 receptor antagonist.

Detection of phosphorylation

Peripheral blood mononuclear cells (PBMCs) were maintained for 24 hours in RPMI 1640 medium

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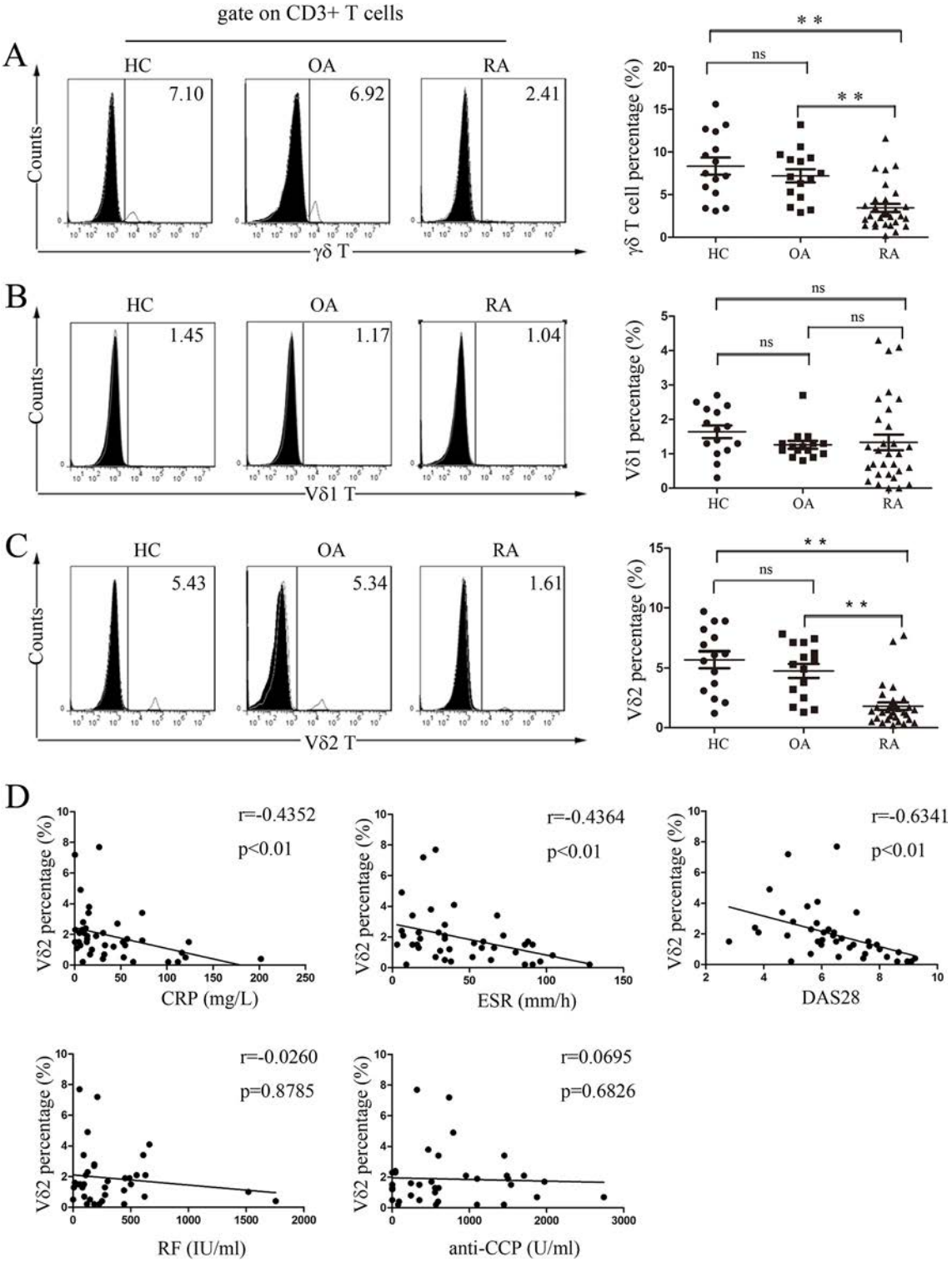

Figure 1 Peripheral Vδ2 T cells were lower in patients with RA. Peripheral blood mononuclear cells obtained from patients with RA, patients with OA and HCs were stained with anti-CD3, anti-γδ TCR, anti-Vδ1 or anti-Vδ2 mAb followed by flow cytometry. The solid plots represent isotype controls, and the open plots represent indicated staining. The left panels show flow cytometry data of (A) γδ T cells, (B) Vδ1 T cells or (C) Vδ2 T cells. The right panels show bar graphs of the percentage of positively stained cells. Representative data of RA (n=30), HC (n=15) and OA (n=15) are shown. (D) The percentage of peripheral Vδ2 T cells in RA is negatively correlated with CRP, ESR and DAS28 (n=42). Results are expressed as mean±SEM. ns, no significance; **p<0.01 by one-way analysis of variance with Tukey-Kramer post-hoc test. Correlations are calculated using Spearman correlation analysis. Anti-CCP, anti-cyclic citrullinated peptide; CRP, C reactive protein; DAS 28, Disease Activity Score in 28 joints; ESR, erythrocyte sedimentation rate; HC, healthy control; OA, osteoarthritis; RA, rheumatoid arthritis; RF, rheumatoid factor; TCR, T cell receptor.

containing 0.1% serum. To activate cytokine-induced signalling, PBMCs were treated in RPMI 1640 containing TNF-α (100 ng/mL) for 5–30 min at 37°C. Then the cells were fixed and permeabilised according to the instructions of BD Phosflow Protocol. The treated and untreated cells were stained with antibody for 1 hour at room temperature. Stained cells were acquired

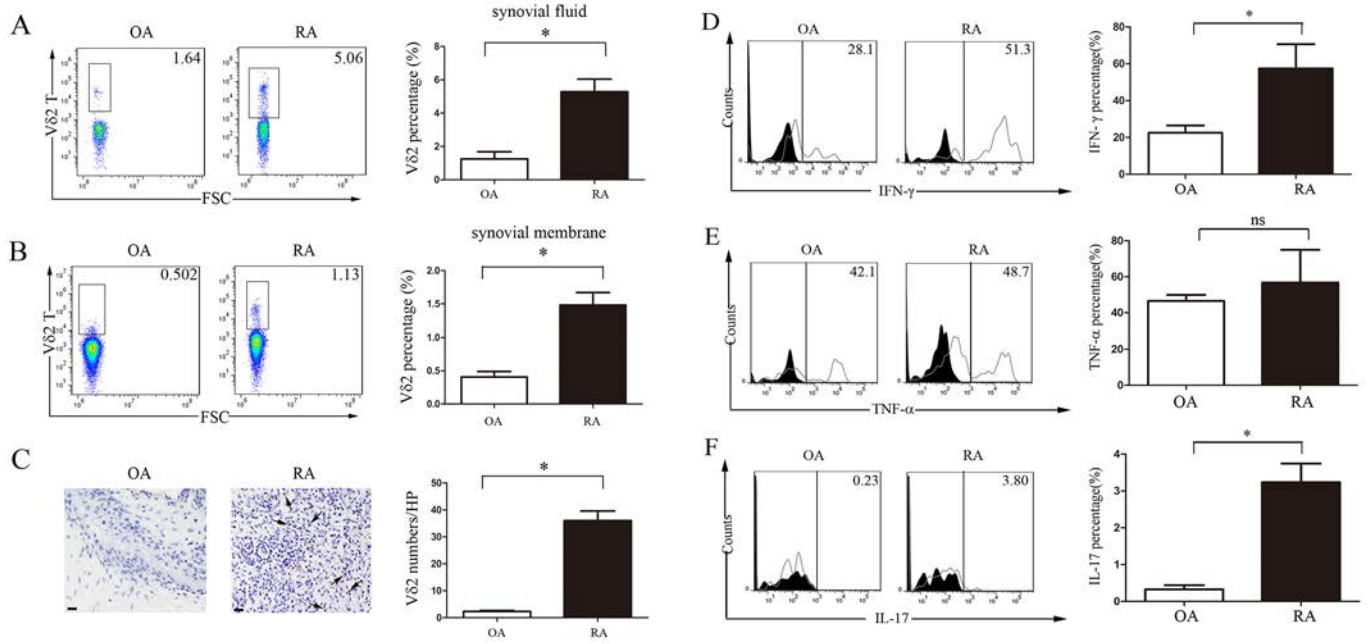

Figure 2 Vδ2 T cells accumulated at the affected joints of RA and secreted high levels of IFN-γ and IL-17. (A,B) The percentage of Vδ2 T cells in (A) SF and (B) enzyme-digested fresh synovium analysed by flow cytometry. Representative data of OA (n=4) and RA (n=4) are shown. (C) Infiltrations of Vδ2 T cells in the knee joint synovium of RA and OA were examined by immunohistochemical staining. Representative data of OA (n=3) and RA (n=3) are shown. Scale bars represent 50 µm. (D–F) Flow cytometry analyses of the intracellular staining of (**D**) IFN-γ, (**E**) TNF-α and (**F**) IL-17 in Vδ2 T cells from RA and OA synovium were performed. Data are representative of three independent experiments. The right panels show bar graphs of the percentage or the average number of positively stained cells. Results are expressed as mean±SEM. *p<0.05 by Student's t-test. FSC, forward scattering; IFN-γ, interferon-γ; IL-17, interleukin-17; OA, osteoarthritis; RA, rheumatoid arthritis; SF, synovial effusion; TNF-α, tumour necrosis factor-α.

by flow cytometry on a BD Accuri C6 flow cytometer (Becton Dickinson) and analysed using the FlowJo software (Tree Star).

Statistics

All data were analysed using SPSS V.17.0 software. One-way analysis of variance (ANOVA) with Tukey-Kramer post-hoc test was used to compare data displaying a normal distribution and homogeneity of variance. Two-way ANOVA was used to examine the influence of two different categorical independent variables. Student's t-test was used to compare differences between two groups, and paired t-test was used to compare differences before and after treatment. Correlations were calculated using Spearman correlation analysis.

Other experimental procedures were included in the 'online supplementary file'.

RESULTS

Peripheral blood Vδ2 T cells were lower in patients with RA and negatively correlated with disease activity

To systematically investigate the roles of γδ T cells in the pathogenesis of RA, we first compared the subpopulations of peripheral γδ T cells in RA, patients with osteoarthritis (OA) and HCs. The results showed a significant decrease of peripheral total γδ T cells in RA (3.45%±0.48% vs HC 8.35±1.00% vs OA 7.21%±0.77%; p<0.01) (figure 1A), which resulted from *significant* reduction of peripheral Vδ2 T cells (1.80%±0.32% vs HC 5.68±0.70% vs OA 4.75%±0.59%; p<0.01) but not Vδ1 T cells (figure 1B,C). In addition, the percentages of peripheral Vδ2 T cells of RA were negatively correlated with the levels of inflammatory markers, including C reactive protein, erythrocyte sedimentation rate as well as the Disease Activity Score in 28 joints (r=−0.6341, n=42, p<0.01; figure 1D). However, no correlation was observed between peripheral Vδ2 T cells and the titres of rheumatoid factor or anticyclic citrullinated peptide antibodies (figure 1D). Taken together, these results suggest peripheral Vδ2 T cells were closely related to RA, which suggested a role in the pathogenesis of RA.

Vδ2 T cells accumulated in RA synovium and were proinflammatory

We then set out to investigate the mechanisms that led to the lower population of peripheral Vδ2 T cells in RA. We found that the proliferation rate of Vδ2 T cells in RA was comparable with that in OA or HC (RA 90.03±7.81% vs HC 82.53±14.97% vs OA 84.77%±6.51%; p>0.05) (online supplementary figure S1A). Also, the apoptosis rates of Vδ2 T cells in RA, OA and HC did not show any significant difference (RA 0.68±0.22% vs HC 0.88±0.56% vs OA 0.96%±0.37%; p>0.05) (online supplementary figure S1B). Therefore, the peripheral reduction of Vδ2 T cells in RA did not result from abnormal proliferation or apoptosis capacity.

Given the previous observation of accumulated γδ T cells in RA SF,[16] we then examined the infiltration of Vδ2 T cells in the joints of RA. Consistently, we found a significantly higher percentage of Vδ2 T cells in RA SF compared with OA SF (5.29%±0.76% vs 1.25±0.44%; p<0.05 (figure 2A). In addition, we found a significantly higher infiltration of Vδ2 T cells in RA than in OA synovium when examining the cells from enzyme-digested fresh synovium (1.48%±0.19% vs 0.41±0.08%; p<0.05 (figure 2B), as well as immunohistochemical staining of the synovium (36.00%±3.60% vs 2.33±0.33%; p<0.05) (figure 2C). These findings suggested that peripheral Vδ2 T cells in RA potentially migrated and accumulated in the synovium.

Similar to natural killer cells, Vδ2 T cells possess highly cytotoxic activity and produce proinflammatory cytokines, including IFN-γ and TNF-α (online supplementary figure S2D,E).[3 4] We

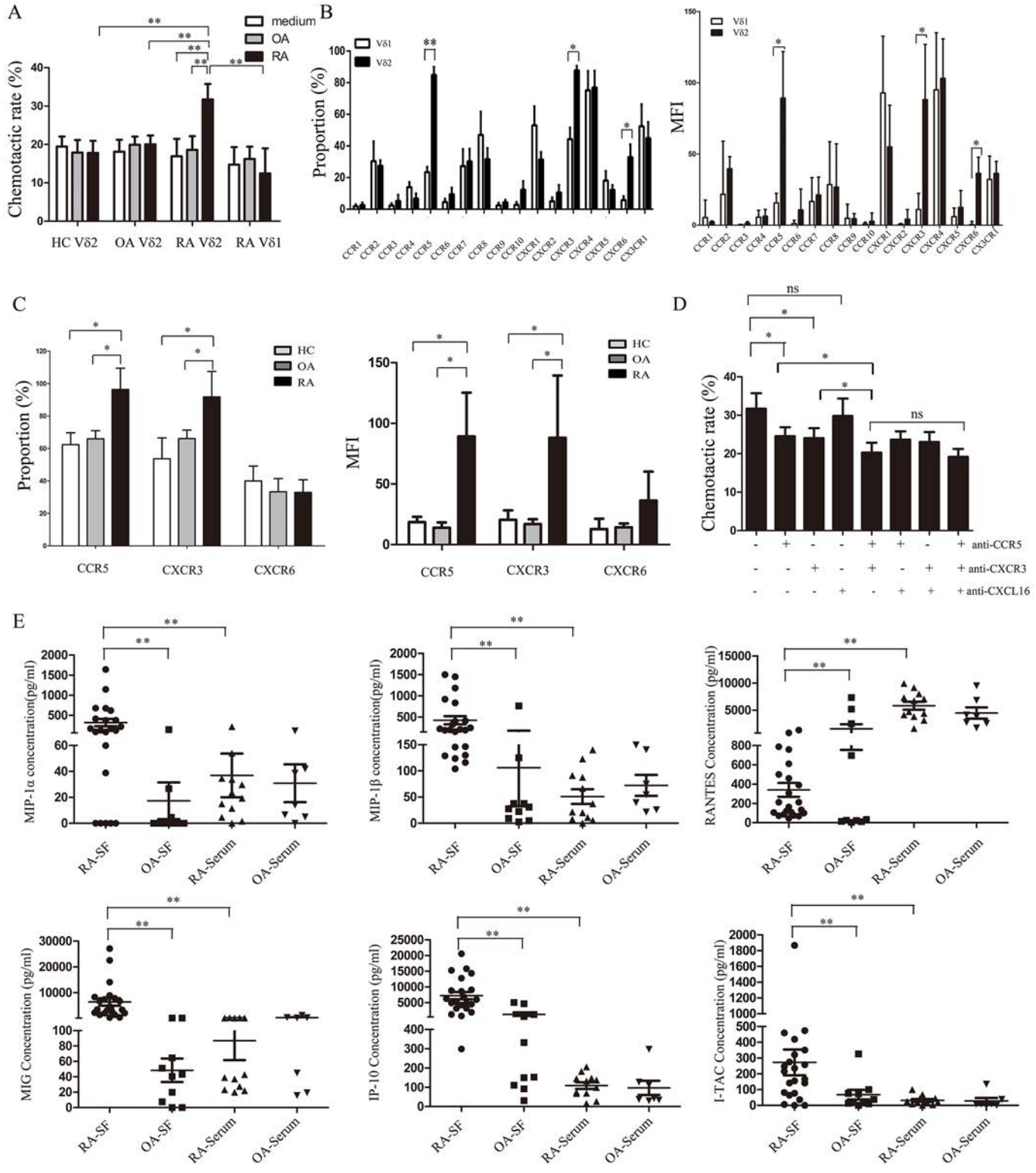

Figure 3 CCR5 and CXCR3 upregulation promoted Vδ2 T cell chemotaxis in RA. (A) Transwell migration assay: freshly isolated peripheral blood mononuclear cells from HC, OA and RA were loaded in the upper chamber, and SFs of OA, RA or medium were loaded in the lower chamber in the transwell invasion model. (B) The percentage and MFI of indicated chemokine receptors on Vδ1/Vδ2 T cells of RA. (C) Comparison of the proportion and MFI of indicated chemokine receptors in Vδ2 T cells from RA, HC and OA. (D) Vδ2 T cell migration assay with RA serum in the presence or absence of neutralising antibodies against CCR5, CXCR3 and CXCR6. (E) The concentration of known ligands of CCR5 and CXCR3 in SF of RA (n=22) and OA (n=10), and serum of RA (n=12) and OA (n=7). Data were pooled from three independent experiments (A,D) or five independent experiments (B,C). Results are expressed as mean±SEM. *p<0.05, **p<0.01 by one-way analysis of variance with Tukey-Kramer post-hoc test (A,C,D,E) and Student's t-test (B). HC, healthy control; MFI, mean fluorescence intensity; OA, osteoarthritis; RA, rheumatoid arthritis; SF, synovial effusion.

found that Vδ2 T cells from RA synovium produced higher levels of IFN-γ (57.43%±7.63% vs 22.60±2.26%; p<0.05) (figure 2D) and IL-17 (3.23%±0.30% vs 0.33±0.06%; p<0.05) (figure 2F) compared with Vδ2 T cells from OA synovium, although no significant difference in TNF-α production was observed in Vδ2 T cells between RA and OA (56.77%±10.51% vs 46.57±4.90%; p>0.05) (figure 2E). Similarly, peripheral Vδ2 T cells from RA synthesised more IFN-γ and IL-17 compared with those from OA and HC (online supplementary figure S2A–C). In addition, Vδ2 cells produced more TNF-α and IFN-γ than $CD3^+$ T cells that were depleted of $Vδ2^+(CD3^+Vδ2^-)$ as well as total $CD3^+$ T cells. Moreover, $CD3^+Vδ2^-$ cells produced approximately 20%–35% less TNF-α and 25%–50% less IFN-γ than total $CD3^+$ cells from both PBMC and synovial fluid of patients with RA (online supplementary figure S2D–E). These data suggest Vδ2 T cells from RA aberrantly secrete high levels of IFN-γ and IL-17, and potentially contribute to the pathogenesis of RA.

Chemotaxis of Vδ2 T cells to synovium was driven by CCR5 and CXCR3

To elucidate the mechanism of Vδ2 T cells accumulation in RA synovium, we performed in vitro transwell assay to examine the capacity of Vδ2 T cells chemotaxis in RA. As expected, we found that RA SF significantly promoted the recruitment of RA but not OA Vδ2 T cells (p<0.01, figure 3A). In contrast, neither RA nor OA SFs promoted the recruitment of RA Vδ1 T cells. These results suggest specific recruitment of Vδ2 T cells to the synovium in RA.

The recruitment of leucocytes to target tissues is regulated by chemokines and corresponding receptors. We performed chemokine receptor expression profile screening, and found the expressions of CCR5, CXCR3 and CXCR6 on Vδ2T cells were significantly higher compared with those on Vδ1T cells (p<0.05, figure 3B). Moreover, the expressions of CCR5 and CXCR3 on RA Vδ2T cells were significantly higher than HC or OA Vδ2T cells (p<0.05, figure 3C). Furthermore, we applied neutralising antibody assay to verify the effect of these upregulated chemokine receptors on Vδ2 T cells recruitment, and we demonstrated that neutralising CCR5 and CXCR3 completely abrogated the migration capacity of RA Vδ2 T cells (figure 3D). Next, we examined the SF and serum levels of the known ligands of CCR5 (MIP-1α, MIP-1β, regulated on activation, normal T cell expressd and secreted (RANTES)) and CXCR3 (MIG, IP-10, I-TAC). We found all ligands except RANTES were significantly elevated in RA SF compared with OA SF and serum (p<0.01, figure 3E). Taken together, these data indicated that CCR5 and CXCR3 upregulation on Vδ2 T cells, in combination with high levels of their ligands in SF, work cooperatively to promote the recruitment of Vδ2 T cells to the affected joints in patients with RA.

TNF-α and IL-6 upregulated the expression of CCR5 and CXCR3 on Vδ2 T cells

To explore the triggering factors responsible for CCR5 and CXCR3 upregulation in RA, we cultured HC Vδ2 T cells with RA, OA and HC serum. Not surprisingly, we found the expressions of CCR5 and CXCR3 were significantly increased in the presence of RA serum (p<0.05, figure 4A), which were abrogated by administration of neutralising antibody against TNF-α, but not IL-17 (p<0.05, figure 4B). To support this finding, we demonstrated that TNF-α alone could significantly upregulate the expressions of CCR5 and CXCR3 on HC Vδ2 T cells (p<0.05, figure 4C). In addition, similar phenotypes were observed in IL-6-neutralised RA serum or IL-6-treated HC Vδ2 T cells (online supplementary figure S3). These results taken together suggest that TNF-α and IL-6 potentially play a role in the upregulation of CCR5 and CXCR3 expression on RA Vδ2 T cells.

NF-kB signalling pathway was involved in TNF-α-mediated upregulation of chemokine receptors in RA

TNF-α is a multifunctional cytokine involved in apoptosis, cell survival, inflammation and a variety of immune responses via two distinct receptors[17]: TNFR-1 (p60) on all cells, and TNFR-2 (p80) that are mainly expressed on immune cells.[18] The major signalling pathway of TNFR-associated factor 2 (TRAF-2) is activation of nuclear factor kappa B (NF-κB) via NF-κB-inducing kinase and inhibitor of nuclear factor kappa-B kinase (IKK) complex. In addition, TRAF-2 also phosphorylates mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinase (JNK) to activate c-Fos/c-Jun transcription factors.[19] To find out which signalling pathway participates in the upregulation of chemokine receptors on Vδ2T cells, we detected phosphorylation of NF-κB p65 (pS529), JNK1/2 (pT183/pY185) and p38 MAPK (pT180/pY182) in TNF-α-treated Vδ2T cells by Phosflow at different time points. The results showed that NF-κB, but not JNK or MAPK, was phosphorylated on TNF-α stimulation (figure 5A). Furthermore, blocking NF-κB signalling with its specific inhibitor (QNZ) completely abrogated the upregulation of CCR5 and CXCR3 on Vδ2 T cells by TNF-α (figure 5B). Together, these findings indicate that NF-κB signalling was involved in the TNF-α regulated expression of CCR5 and CXCR3 on Vδ2 T cells.

TNF-α antagonist therapy restored Vδ2 T cells in patients with RA in vivo

To investigate whether TNF-α regulated the chemotaxis of Vδ2 T cells in vivo, we examined the peripheral Vδ2 T cells from patients with RA treated with etanercept, a kind of TNF-α receptor fusion protein. We found that the percentage of Vδ2 T cells could be restored in patients with RA after treatment with etanercept (figure 6A). Furthermore, TNF-α antagonist treatment downregulated the expression of CCR5 and CXCR3 on Vδ2 T cells in patients with RA (figure 6B,C). We also detected the effect of IL-6 receptor antagonist on modulating peripheral Vδ2 T cell population and the expression of CCR5 and CXCR3. However, no significant effect was observed (online supplementary figure S4). Taken together, these findings further support the conclusion that TNF-α specifically regulates peripheral Vδ2 T cell trafficking to RA synovium by modulating the expressions of CCR5 and CXCR3 in vivo.

DISCUSSION

All the data in this study collectively suggested the hypothesis that in patients with RA, peripheral Vδ2 T cells potentially infiltrated into the synovium and secreted high levels of proinflammatory cytokines, which contributed to the pathogenesis of RA. Mechanistically, we further showed that elevated level of serum TNF-α in patients with RA induced high expressions of CCR5 and CXCR3 on Vδ2T cells, which promoted Vδ2 T chemotaxis, and NF-kB signalling pathway was involved in this process. More strikingly, anti-TNF-α therapy restored the peripheral Vδ2 T cells population as well as the expression of CCR5 and CXCR3 in patients with RA.

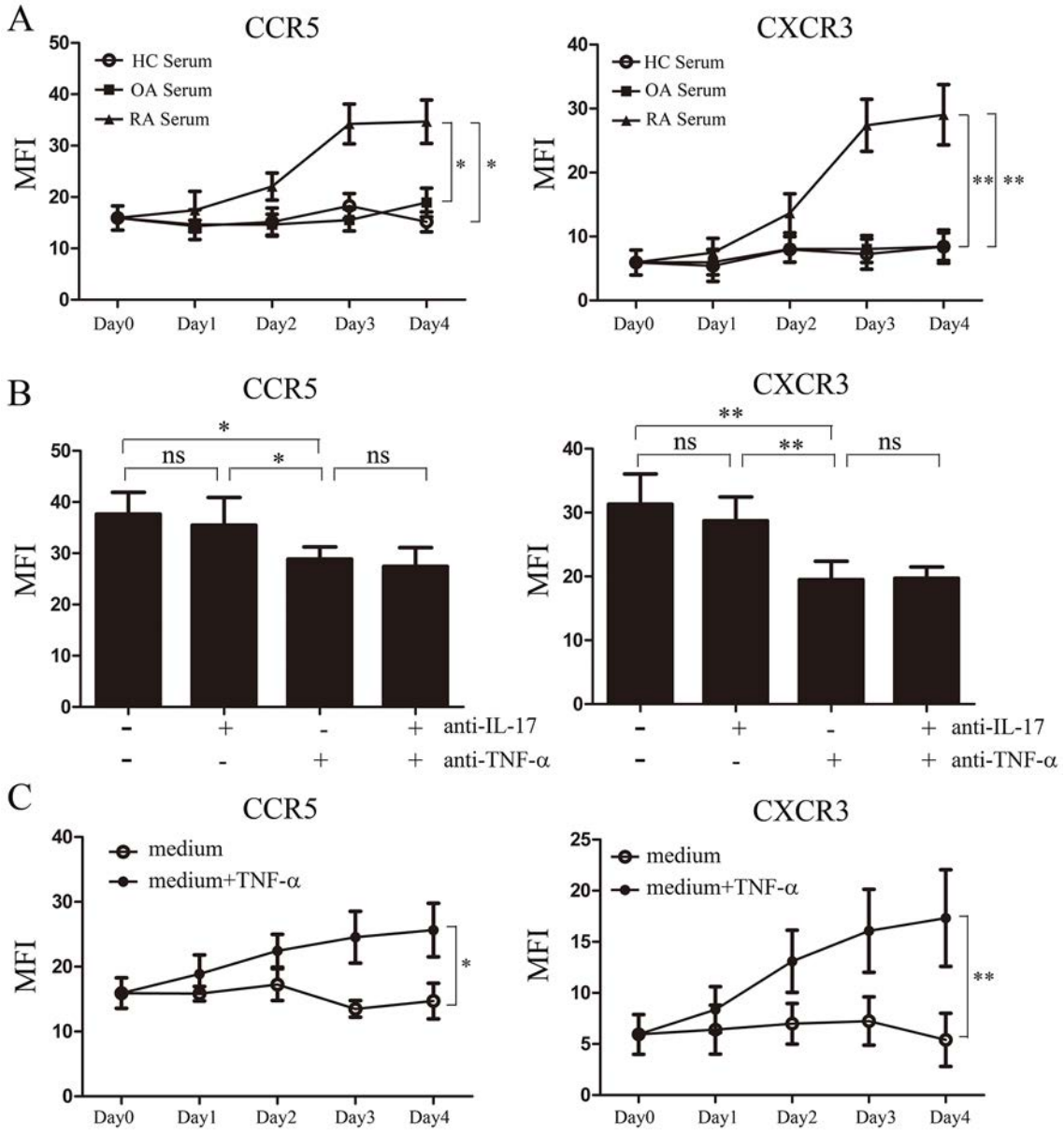

Figure 4 TNF-α augmented the expression of CCR5 and CXCR3 on Vδ2 T cells. Flow cytometry analysis of (A) CCR5 and CXCR3 expression on Vδ2 T cells at indicated time points in the presence of HC, OA or RA serum; or (B) RA serum in combination with neutralising antibodies against TNF-α or IL-17 for 3 days; or (C) with medium in the presence or absence of TNF-α for indicated days. Data were pooled from three independent experiments. Results are expressed as mean±SEM. ns, no significance; *p<0.05, **p<0.01 by two-way ANOVA (A,C) or one-way ANOVA (B). ANOVA, analysis of variance; HC, healthy control; IL-17, interleukin-17; MFI, mean fluorescence intensity; OA, osteoarthritis; RA, rheumatoid arthritis; TNF-α, tumour necrosis factor-α.

Previous reports show a higher proportion of γδT cells in RA SFs and synovium compared with peripheral blood.[20 21] In our study, a significant lower percentage of peripheral Vδ2 T cells in patients with RA was noted, which might be caused by intensive accumulation into synovial tissues but not abnormal cell apoptosis or proliferation potential. Additionally, both Vδ2 cells from peripheral blood and from SF of patients with RA showed proinflammatory phenotype, which produced higher levels of IFN-γ and IL-17 compared with controls. Consistently, peripheral blood and SF from patients with RA contained heterogeneous γδT cells dominated with effector memory Vγ9/Vδ2 T cells producing inflammatory cytokines including IFN-γ and IL-17.[22] Animal studies also show that γδT cells are the major source of IL-17 in joints and their increasing numbers are correlated with disease activity.[16 23 24] In collagen-induced arthritis model, preventive depletion of γδ T cells significantly delayed the onset and severity of arthritis.[12 24] Moreover, depletion of Vγ4+ cells, the counterpart of Vδ2 T cells in human and the major population of γδ T cells in mice, significantly attenuates arthritis severity, incidence of arthritis and anticollagen antibodies production. Taken together, our data suggest Vδ2 T cells are involved in the pathogenesis of RA, and targeting Vδ2 T cells might be a promising approach to treat RA.

We further explored the underlying mechanism of abnormal accumulation of Vδ2 T cells in synovial tissues. Chemokine receptors are expressed in various types of cells and interact with chemokines to promote 'homing' of cells to target tissues. By profiling chemokine receptors, we found CXCR3 and CCR5,

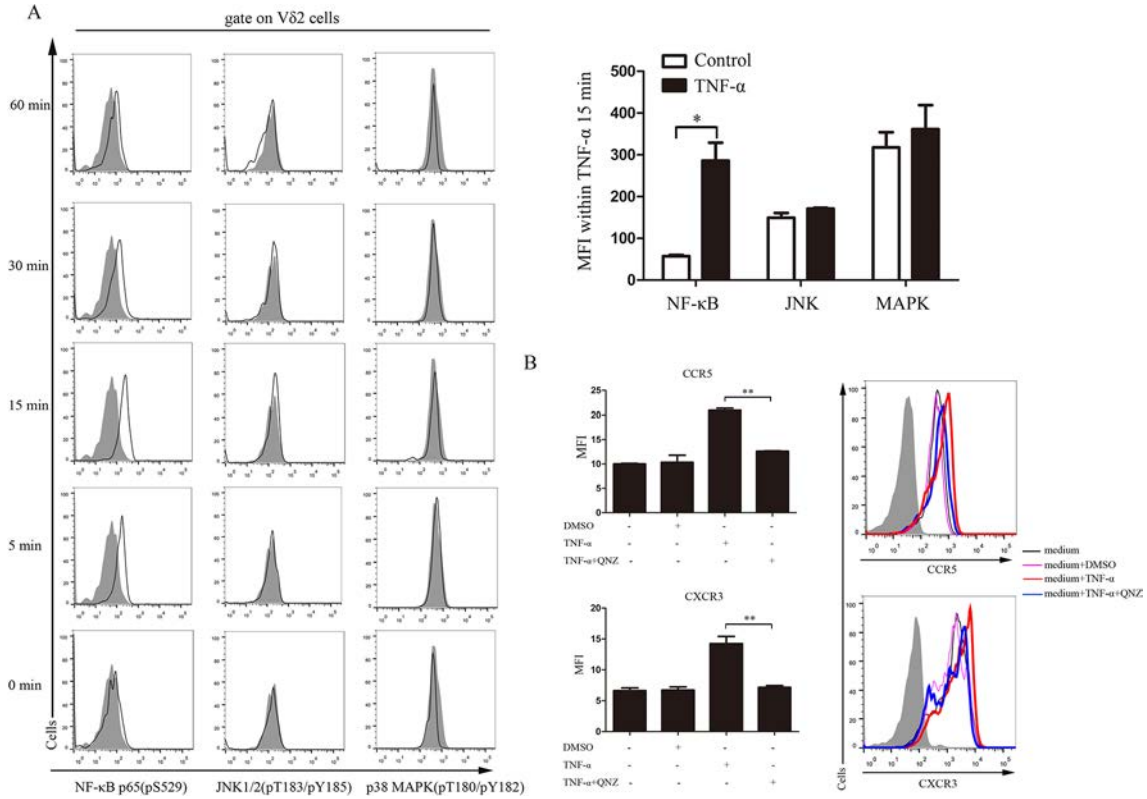

Figure 5 NF-κB signalling pathway was involved in the expression of CCR5 and CXCR3 on Vδ2 T cells. (A) Vδ2 T cells were treated with TNF-α (100 ng/mL) for indicated time. The cells were permeabilised and stained with antibodies against NF-κB p65 (pS529), JNK1/2 (pT183/pY185) or p38 MAPK (pT180/pY182). The data represent one of three independent experiments. The right panels show bar graphs of MFI of Vδ2 T cells stimulated with TNF-α in 15 min. (B) Flow cytometric analysis of chemokine receptor expressions on TNF-α-stimulated HC Vδ2 T cells pretreated with QNZ (5 μM) or dimethyl sulfoxide (DMSO) for 1 hour. The solid plots represent isotype controls, and the open plots represent indicated staining. Results are expressed as mean±SEM. *p<0.05, **p<0.01 by paired t-test (A) and one-way analysis of variance with Tukey-Kramer post-hoc test (B). HC, healthy control; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MFI, mean fluorescence intensity; NF-κB, nuclear factor kappa B; TNF-α, tumour necrosis factor-α.

the chemokine receptors preferentially expressed on IFN-γ-producing Th1 cells,[25–28] were highly expressed on Vδ2 T cells and were essential for their migration to synovial tissues. Additionally, we confirmed that the corresponding agonistic ligands except RANTES were significantly elevated in SF of RA[29 30] and promoted migration of circulating γδT cells.[31] These chemokines are produced locally by synovial fibroblasts and TNF-α stimulation promotes their production.[32] Collectively, these findings suggest upregulated CXCR3 and CCR5 in Vδ2 T cells potentially orchestrated with elevated chemokines to promote Vδ2 T migration.

Monocytes, macrophages and synovial fibroblasts produce high levels of cytokines including TNF-α and IL-1 on stimulation in RA.[33] TNF-α, one of the major proinflammatory cytokines in RA, is a potent stimulator of synovial fibroblasts, osteoclasts and chondrocytes to release matrix metalloproteinases, which ultimately lead to joint destruction and bone degradation. In contrast, blocking TNF-α with its antagonist significantly reduces the production of matrix metalloproteinases. Additionally, neutralising TNF-α reduced production of other proinflammatory cytokines including IL-1, IL-6, IL-8 and gannulocyte-macrophage colony stimulating factor (GM-CSF).[34–36] In our study, neutralising TNF-α in vivo dramatically restored the Vδ2 T population in patients with active RA. Mechanically, TNF-α regulates CCR5 and CXCR3 expression on Vδ2 T cells via NF-kB signalling, which is an important pathway of many inflammatory process. Additionally, p65(RelA), a member of NF-kB/Rel family, is a potent activator of the CCR5 promoter.[32 37] Collectively, we suggest a novel alternative mechanism of action of TNF-α antagonist: anti-TNF-α therapy downregulates CCR5 and CXCR3 expression of Vδ2 T cells, and subsequently reduces Vδ2 T accumulation in synovial tissues and ameliorate arthritis.

Intriguingly, despite anti-IL-6 in RA serum abolished upregulation of CCR5 and CXCR3 in Vδ2 T in vitro and IL-6R antagonist therapy ameliorated RA disease in vivo, IL-6R antagonist did not rescue the Vδ2 T population in vivo. Elevated level of IL-6 after tocilizumab therapy[38 39] might account for the attenuated repopulation of Vδ2 T cells.

The impact of Vδ2T cells on bone metabolism remains elusive. We showed Vδ2T cells of RA produced high levels of IFN-γ and IL-17, especially in synovial tissues. IL-17 and receptor activator for nuclear factor-kappa-B ligand (RANKL) are the major cytokines promoting osteoclast differentiation and activation, which ultimately lead to bone erosion. IL-17 stimulates osteoblasts to produce RANKL, which in turn induces differentiation of osteoclast progenitors into mature osteoclasts.[40] Additionally, IL-17-secreting γδ T cells prime αβ T cells to produce IL-17 and enhance the function of Th17 cells.[41] In contrast, the role of IFN-γ in osteoclast genesis is controversial. Although animal studies report that IFN-γ inhibits osteoclast formation,[42] clinical studies have failed to demonstrate the efficacy of IFN-γ administration in bone loss prevention.[43–46] The implication of elevated IFN-γ of Vδ2 T cells is yet to be further elucidated.

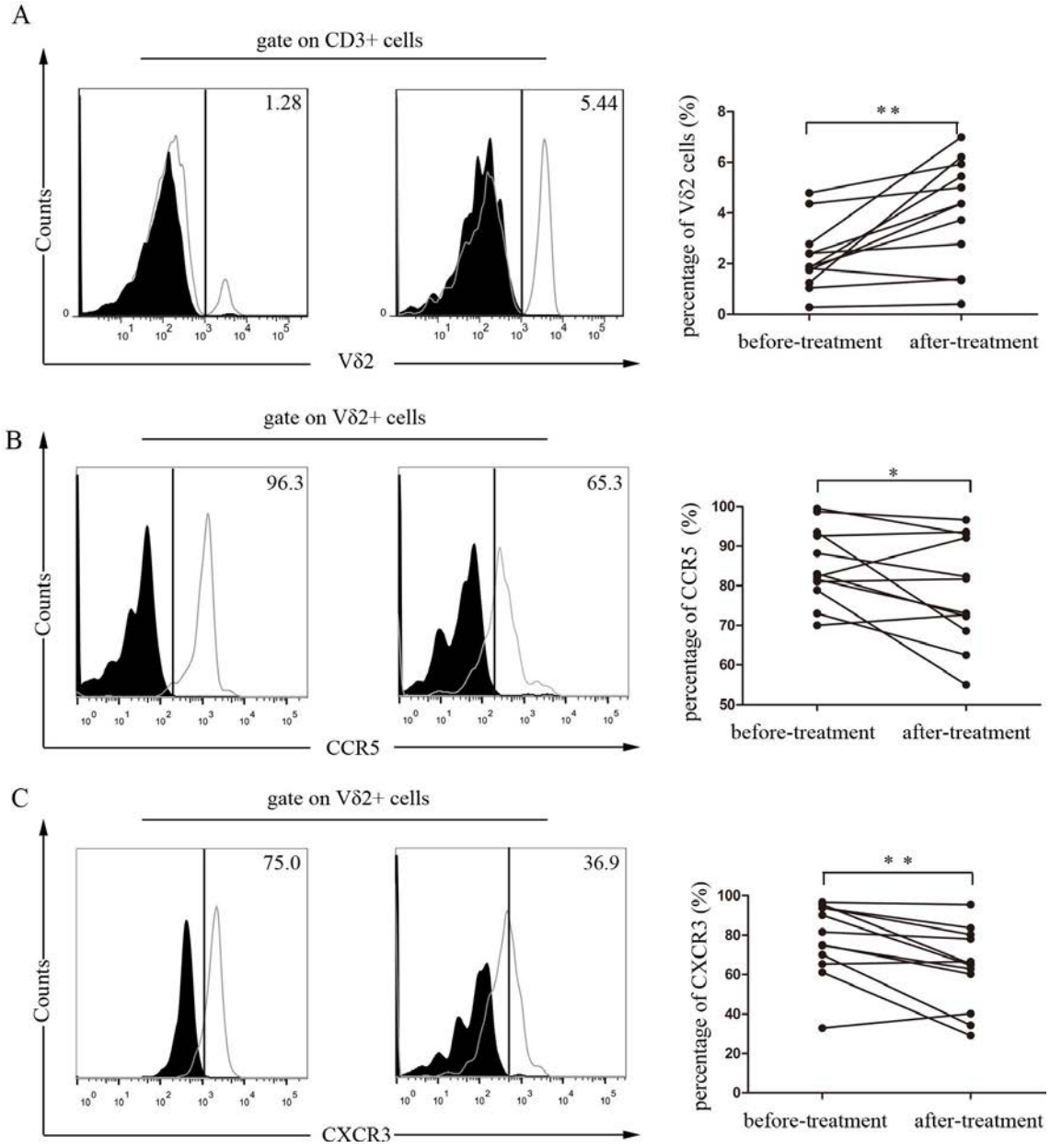

Figure 6 TNF-α antagonist therapy repopulated peripheral Vδ2 T cells and downregulated CCR5 and CXCR3 expressions in patients with RA. Treatment-naïve patients with RA (n=12) were treated with etanercept in combination with methotrexate for 3 months. Flow cytometry was performed for the analysis of (A) the percentage of peripheral Vδ2 T cells and the expressions of (B) CCR5 and (C) CXCR3 on Vδ2 T cells before and after treatment. The solid plots represent isotype control, and the open plots represent Vδ2 T cells staining. *p<0.05, **p<0.01 by paired t-test. RA, rheumatoid arthritis; TNF-α, tumour necrosis factor-α.

Moreover, Vδ2 T cells potentially contribute to RA pathogenesis in other ways. The majority of adult Vγ9/Vδ2 T cells express the CD45RO memory phenotype[2] with memory CD45RA-CD27+ subset and effector CD45RA-CD27− subset.[47] The effector memory Vγ9/Vδ2 T cells exhibit phenotypic characteristics of specific antigen-presenting cells, including high human leukocyte antigen DR (HLA-DR) and CD80/86 expression, which promotes B cell activation and polarises adaptive immunity towards a Th1 immune response in patients with RA.[22] Both circulating Vδ2 cells and residential Vδ2T cells produce a variety of cytokines and chemokines including MIP, RANTES and IL-8,[48 49] inducing macrophage aggregation and activated T lymphocytes migration,[50] which may also be involved in the pathogenesis of RA.

In summary, we demonstrate that Vδ2T cells were lower in peripheral blood and accumulated in the RA joint and secreted increasing amounts of proinflammatory cytokines that are involved in the pathogenesis of RA, which resulted from its upregulation of CCR5 and CXCR3 induced by TNF-α via NF-kB signalling pathway. Elucidation of the roles of Vδ2 T cells in RA advances our knowledge in understanding the complex pathogenetic mechanism of RA, and provides an alternative mechanism of biological agents to develop new promising biomarker of therapy, and exploring potential Vδ2 cell-targeted therapy.

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Contributors W-XM, S-SY and HC contributed equally to this manuscript. XZ and WH conceptualised and designed the project and supervised the project. W-XM, S-SY and HC performed all the experiments and wrote the manuscript with contributions from all authors. J-MZ revised the manuscript. CZ, L-FH, L-DZ, Y-YF, H-XY and WZ participated in the sample collection and clinical analysis. All authors read and approved the manuscript.

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Competing interests None declared.

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EXTENDED REPORT

A novel Pyrin-Associated Autoinflammation with Neutrophilic Dermatosis mutation further defines 14-3-3 binding of pyrin and distinction to Familial Mediterranean Fever

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ABSTRACT

Objective Pyrin-Associated Autoinflammation with Neutrophilic Dermatosis (PAAND) is a recently described monogenic autoinflammatory disease. The causal p.S242R *MEFV* mutation disrupts a binding motif of the regulatory 14-3-3 proteins within pyrin. Here, we investigate a family with clinical features consistent with PAAND in whom the novel p.E244K *MEFV* mutation, located in the +2 site of the 14-3-3 binding motif in pyrin, has been found.

Methods Multiplex cytokine analyses were performed on p.E244K patient and control serum. Peripheral blood mononuclear cells were stimulated ex vivo with lipopolysaccharide (LPS). In vitro, inflammasome complex formation was evaluated by flow cytometry of Apoptosis-associated Speck-like protein containing a Caspase recruitment domain (ASC) specks. Interleukin-1β (IL-1β) and IL-18 production was quantified by ELISA. The ability of the p.E244K pyrin mutation to interact with 14-3-3 was assessed by immunoprecipitation.

Results PAAND p.E244K patient serum displayed a different cytokine profile compared with patients with Familial Mediterranean Fever (FMF). In overexpression models, p.E244K pyrin was associated with decreased 14-3-3 binding and increased ASC speck formation. THP-1 monocytes expressing PAAND pyrin mutations demonstrated spontaneous caspase-1-dependent IL-1β and IL-18 secretion, as well as cell death, which were significantly greater than those of wild-type and the FMF-associated mutation p.M694V.

Conclusion In PAAND, disruption of the +2 position of a 14-3-3 binding motif in pyrin results in its constitutive activation, with spontaneous production of IL-1β and IL-18, associated with inflammatory cell death. The altered serum cytokine profile may explain the different clinical features exhibited by PAAND patients compared with those with FMF.

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INTRODUCTION

Pyrin-Associated Autoinflammation with Neutrophilic Dermatosis (PAAND) is a recently described monogenic autoinflammatory condition caused by a heterozygous mutation in the *MEFV* gene resulting in the p.S242R substitution in pyrin.[1] The dominant clinical phenotype of prolonged fever and neutrophilic dermatosis (eg, acne, pyoderma gangrenosum), and potentially the mechanism of disease, differs from the classical pyrin-associated disease, Familial Mediterranean Fever (FMF).

The p.S242 site of pyrin forms a 14-3-3 binding motif.[1 2] Although there are a number of variations of 14-3-3 recognition motifs reported, all contain a phosphorylated serine or threonine residue.[3 4] In its inactive state, pyrin is phosphorylated by serine-threonine kinases PKN1 and PKN2 at residues p.S208 and p.S242, and is bound to 14-3-3 proteins.[5] When triggered in response to RhoGTPase modifications, such as those induced by the pathogen *Clostridium difficile*, there is dephosphorylation of pyrin at p.S208 and p.S242 residues and loss of 14-3-3 binding.[1 5 6] In vitro models show that the p.S242R pyrin mutation is constitutively dephosphorylated, with reduced 14-3-3 binding.[1] The resulting increased pyrin inflammasome activation and enhanced IL-1β production appear to drive the pathology in PAAND.[1]

Here, we report a novel mutation in the *MEFV* gene in a family with clinical features of PAAND that results in an altered 14-3-3 binding motif and constitutive activation of pyrin. We also confirm phenotypic differences and identify cytokine differences between PAAND and FMF.

METHODS

Patients

We investigated three symptomatic patients in one family. We used patients with homozygous p.M694V FMF as disease controls, and blood donors as healthy controls. This study was approved by the Hospital Clinic-IDIBAPS Ethics Committee.

Patient cell stimulation and analysis

Fresh serum samples were collected from patients and controls, and cytokine quantification was performed by Luminex multiplex assay. PAAND patients had active clinical features at the time of collection. For human IL-18 and IL-18BP, serum was assayed in multiplex on a Luminex Magpix system (Bio-Rad, Hercules, California, United States). Bio-Rad group II cytokine standard was used for IL-18, whereas recombinant human IL-18BPa-Fc

chimeric protein (R&D Systems, Minneapolis, Minnesota, United States) was used as standard for IL-18BP.

Peripheral blood mononuclear cells (PBMCs) were isolated using Histopaque-1077 (Sigma-Aldrich, St Louis, Missouri, United States) and treated with *Escherichia coli* LPS serotype 055:B5 (Sigma-Aldrich; 1 µg/mL, 2 hours at 37°C) or left untreated. IL-1β was measured on cell supernatants by ELISA (eBioscience, San Diego, California, United States) while other cytokine quantification was performed by Luminex multiplex assay as described above. Cells were fixed with 2% paraformaldehyde and stained for the detection of Apoptosis-associated Speck-like protein containing a Caspase recruitment domain (ASC) specks by Time of Flight Inflammasome Evaluation using the rabbit polyclonal antibody anti-ASC (N-15)-R (Santa Cruz Biotechnology, Dallas, Texas, United States) as previously described.[7] Alternatively, for the detection of active caspase-1, PBMCs were incubated for 20 min with Fluorochrome Inhibitor of Caspases (FLICA)660 reagent (ImmunoChemistry Technologies, Bloomington, Minnesota, United States) and fixed following manufacturer's recommendations. Monocytes were detected with the APC-vio770 mouse anti-human CD33 antibody (Miltenyi Biotech, Bergisch Gladbach, Germany) and with the APC-Cy7-conjugated anti-human CD14 antibody (TONBO Biosciences, San Diego, California, United States). Stained cells were acquired on a BD FACSCanto cytometer.

Heat maps representing cytokine expression profiles were created using Morpheus software (Broad Institute, Cambridge, Massachusetts, United States).

Cell culture

HEK293T cells were cultured in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS) and transfected with mCherry-pyrin or GST-pyrin,[8] GFP-ASC,[9] or V5-Proline Serine Threonine Phosphatase-Interacting-Protein 1 (PSTPIP1) (HsCD00438559, DNASU Plasmid repository) constructs using Lipofectamine (Life Technologies) according to manufacturer's instructions. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 techniques were used for generation of *MEFV* KO and *CASP1* KO THP-1 cells, as has been described.[1 10] These cells were cultured in Roswell Park Memorial Institute medium (RPMI) supplemented with 10% FCS.

Lentiviral infection of THP-1 cells

MEFV KO THP-1 cells were reconstituted with pyrin by lentiviral transduction. A lentiviral construct was generated through ligation of *MEFV* cDNA into *Bam*HI and *Age*I restriction sites on the pFUGW backbone after performing site directed mutagenesis of the *Bam*HI restriction site within *MEFV*. Lentivirus was produced as previously described.[10] One million THP-1 cells were seeded per well in six-well plates with 3.5 mL of virus and 24 µg of polybrene. A total of 6 million THP-1 cells were seeded per condition. Plates were centrifuged at 840 g for 3 hours and then incubated at 37°C overnight. Cells were collected the following day, washed in phosphate buffered saline (PBS), reseeded in fresh media and incubated at 37°C overnight. After a further 24 hours, live and dead cells were separated using Ficoll density gradient centrifugation (GE Healthcare, Chicago, Illinois, United States). Live cells were seeded for experiments. Supernatant was harvested after 24 hours for cytokine analysis by ELISA for IL-1β and IL-18 (DY201 and DY008, R&D Systems). Cytokines from cell culture supernatant were also quantified using Bio-Plex Pro Assay (Bio-Rad). Cell death was analysed by flow cytometry using propidium iodide (Sigma-Aldrich) staining at 1 µg/mL. Where indicated, priming of cells was performed with Pam3CSK4, a synthetic TLR1/2 agonist (InvivoGen, San Diego, California, United States). Cells were also lysed using radioimmunoprecipation assay buffer to assess expression of pyrin by western blotting.

Site-directed mutagenesis

Site-directed mutagenesis was performed using the QuickChange Lightning Kit (210519–5, Agilent Technologies, Santa Clara, California, United States) according to manufacturer's instructions. Mutations were introduced to pyrin-expressing constructs using the following oligonucleotide primers:

p.E244K 5'-TAGAAATGGTGACCTTAAGGCTTCTAG GTCGCATC-3'
5'- GAT GCGA CCTA GAAG CCTT AAGG TCAC CATTTCTA-3'
p.M694V 5'-GGTACTCATTTTCCTTCACCATTATCA CCACCCAGTAG-3'
5'- CTA CTGG GTGGTGATAATG GTGA AGGA AAAT GAGTACC-3'
p.S242R 5'-GAAATGGTGACCTCAAGCCTTCTAGGT CGCATCTT-3'
5'- AAG ATGC GACC TAGA AGGC TTGA GGTC ACCATTTC-3'
p.E244P 5'-GATGCGACCTAGAAGCCTTCCGGTCAC CATTTCTACAG-3'
5'- CTGTAGAAATGGTGACCGGAAGGCTTCTAGG TCGCATC-3'
p.E244D 5'-CGACCTAGAAGCCTTGATGTCACCATT TCTACAGG-3'
5'- CCT GTAG AAAT GGTG ACAT CAAG GCTT CTAGGTCG-3'
p.E244R 5'AGATGCGACCTAGAAGCCTTAGGGTCA CCATTTCTACAGG-3'
5'- CCT GTAG AAAT GGTG ACCC TAAG GCTT CTAG GTCGCATCT-3'
R39R(Δ*Bam*HI) 5'-GGAGCACTCCAGAATCCCC CGGAGC-3'
5'-GCTCCGGGGGATTCTGGAGTGCTCC-3'
I666I(Δ*Bam*HI) 5'-CAGGCTCCCAGTATCCATGCTGT CTTGTCTCC-3'
5'-GGAGACAAGACAGCATGGATACTGGGAGCCTG-3'

Fluorescence microscopy and flow cytometry

HEK293T cells were transfected with 25 ng wild type (WT) or mutant mCherry-MEFV and 5 ng GFP-ASC, and ASC specks were quantified 16 hours later using flow cytometry, as previously described.[7] Colocalisation experiments were performed using mCherry-MEFV and GFP-ASC transfected into 1×10^5 HEK 293 T cells seeded in ibidi chamber slides (ibidi GmbH, Munich, Germany). Images were taken with a Zeiss LSM 780 Confocal microscope and were processed using FIJI software (National Institutes of Health, Bethesda, Maryland, United States).

Immunoprecipitation and western blotting

HEK293T cells (3×10^6 cells) were transfected with 5 µg of GST-tagged WT or mutant pyrin, with or without WT PSTPIP1. Where indicated, cells were treated with *Clostridium difficile* Toxin B protein (TcdB, 5 µg/mL, ab124001, Abcam, Cambridge, United Kingdom) 16 hours before harvesting. Cell lysates were generated 48 hours after transfection using 1% NP-40 lysis buffer

supplemented with protease inhibitors and sodium orthovanadate. Immunoprecipitation of pyrin was performed using glutathione sepharose 4B (GE Healthcare). After washing, bound proteins were eluted from beads using 2x sodium dodecyl sulphate (SDS) buffer and boiling at 90°C. Immunoblots were prepared using 4%–12% Novex SDS-Polyacrylamide gel electrophoresis (Invitrogen, Carlsbad, California, United States) gels in MES running buffer, followed by transfer on to nitrocellulose membranes. Membranes were blocked with tween/tris-buffered saline (TBST) +3% bovine serine albumin (BSA) at room temperature and subsequently probed overnight at 4°C with antibodies against pan-14-3-3 (1:500 Santa Cruz #sc-629-G), 14-3-3τ (1:500 Santa Cruz #sc-59414), 14-3-3ε (1:1000 Biorbyt #orb6357), pSer 14-3-3 binding motif (1:500 Cell signalling #9601), pyrin (1:500 AdipoGen #AL196), p10 Caspase-1 (1:200 Santa Cruz #sc-515), IL-1β (1:1000 R&D #AB-401-NA), GST (1:1000 in-house), PSTPIP1 (1:500 Abnova #H00009051) and actin (1:5000 Santa Cruz #sc-1616). All antibodies were prepared in TBST +1% BSA.

Statistical analysis

Mann-Whitney non-parametric test was used for the analysis of data in figure 2. Two-tailed t-tests were performed in other analysis using Prism software (GraphPad Software, La Jolla, California, United States). Data are represented as mean+/−SEM unless otherwise specified (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

RESULTS

PAAND family with a novel mutation in *MEFV*

The index patient is a 43-year-old woman of Spanish descent with a 30-year history of chronic and severe pustular acne, severe hidradenitis suppurativa, recurrent pyoderma gangrenosum, recurrent long-lasting febrile episodes, neutrophilic panniculitis as well as polyarthralgia and oligoarthritis of small and large joints (figure 1A). Raised C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and peripheral blood neutrophil count persisted despite treatment with corticosteroids and the IL-1 receptor antagonist (IL-1Ra), anakinra

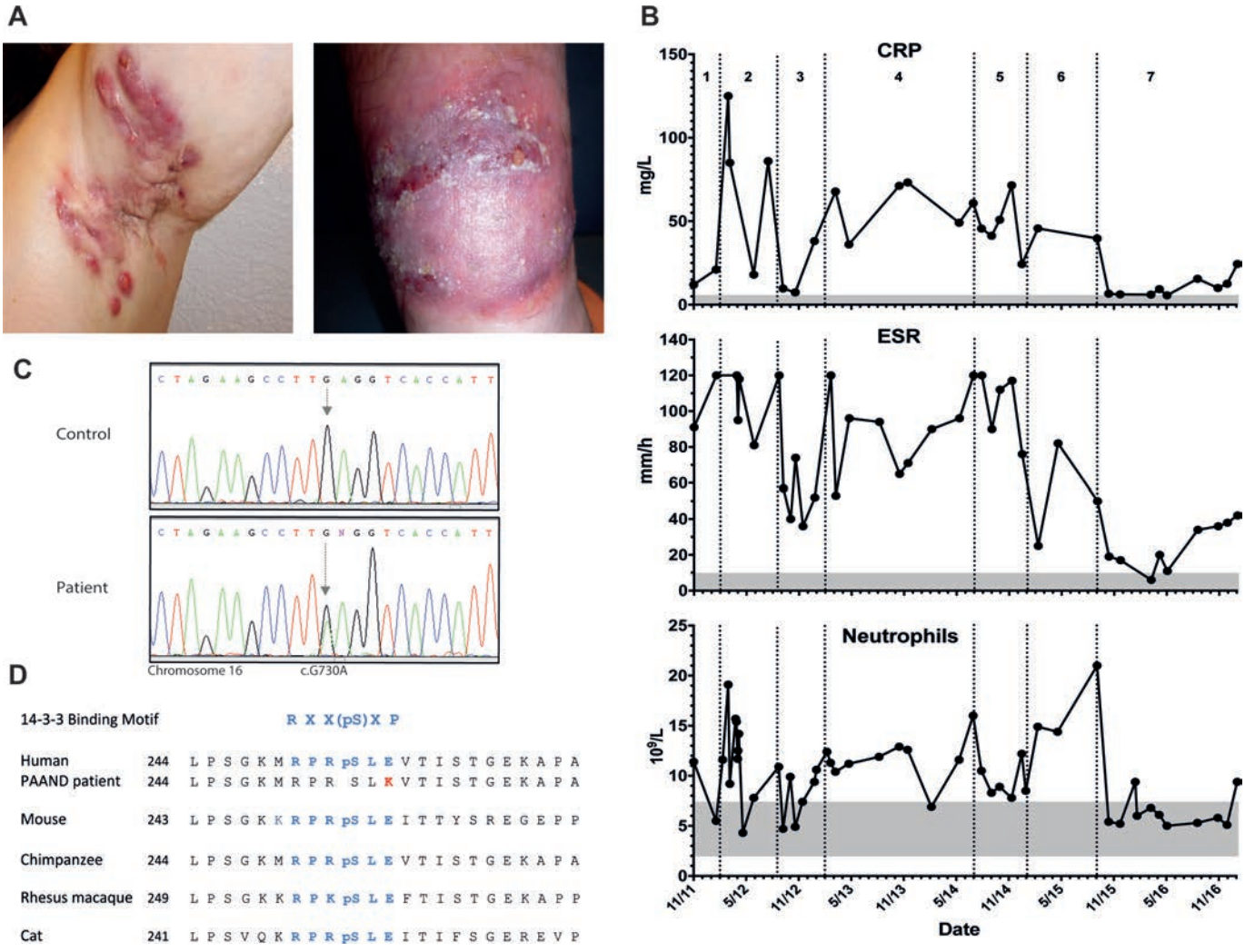

Figure 1 Clinical features of Pyrin-Associated Autoinflammation with Neutrophilic Dermatosis (PAAND). (A) Representative macroscopic images of dermatological manifestations in index case (left: hidradenitis suppurativa axillae; right: pyoderma gangrenosum lower leg). (B) Acute phase reactants and neutrophils over time, with treatment periods 1. infliximab+methotrexate; 2. prednisolone+ciclosporin A; 3. prednisolone+infliximab; 4. prednisolone+doxycycline; 5. prednisolone+anakinra+ clindamycin; 6. prednisolone+clindamycin+ moxifloxacin+dapsone; 7. prednisolone+clindamycin+ moxifloxacin+dapsone+ adalimumab. (top: C-reactive protein (CRP); middle: erythrocyte sedimentation rate (ESR); bottom: peripheral blood neutrophil count). (C) DNA chromatogram showing the heterozygous G-to-A transition at position corresponding to c.730 *MEFV.* (D) p.E244 pyrin is highly conserved across species. The glutamate is in position +2 of a 14-3-3 binding motif.

(figure 1B). Long-lasting (8 years) clinical benefit was seen with the chimeric anti-tumor necrosis factor (TNF)-α monoclonal antibody infliximab. However, loss of efficacy of infliximab was observed and necessitated switching to the human anti-TNF-α monoclonal antibody adalimumab when symptoms recurred. Although a clinical diagnosis of pyogenic arthritis, pyoderma gangrenosum and acne (PAPA) syndrome was suspected, genetic testing of *PSTPIP1* failed to reveal a pathogenic mutation. Pathogenic mutations in *NCSTN*, reported in familial cases of hidradenitis suppurativa, were absent.[11] The recent description of PAAND, a condition with significant clinical overlap with PAPA syndrome, prompted exon 2 *MEFV* sequencing in this patient, which revealed the heterozygous c.730G>A transition in the *MEFV* gene encoding for the p.E244K mutation (figure 1C). This mutation was absent from the 1000 Genomes Project, Exome Aggregatium Consortium, Exome Variant Server and 250 Spanish healthy controls. Furthermore, it had not been reported on the INFEVERS database.[12–14] The locus is highly conserved across species (figure 1D) and the amino acid substitution predicted to be damaging using MutationTaster,[15] Sorting Intolerant from Tolerant[16] and Polymorphism Phenotyping v2.[17] Evaluation of the patient's mother and brother, both of whom have had dermatitis and long-lasting (>30 years) severe nodulocystic acne affecting the face and trunk respectively, revealed the mutation of interest, suggesting an autosomal dominant disease with variable penetrance (see online supplementary figure S2).

PAAND family has a cytokine profile distinct from FMF patients

Serum cytokine analysis of the proband, mother and brother revealed a unique profile when compared with FMF patients (n=5) and healthy controls (n=7), highlighted on a heat map of relative values (figure 2A). The increased serum IL-18 was explored further with the measurement of IL-18 binding protein (IL-18BP). IL-18BP has a high affinity for IL-18, and renders it biologically inactive.[18] Free IL-18, rather than total, correlates better with disease activity in IL-18-driven conditions, such as haemophagocytic lymphohistiocytosis.[19] Interestingly, in our PAAND patients, IL-18BP was significantly elevated when compared with healthy controls, and the ratio of total IL-18 to IL-18BP was similar (figure 2B). Therefore, analysis of free IL-18 revealed no significant increase (data not shown).

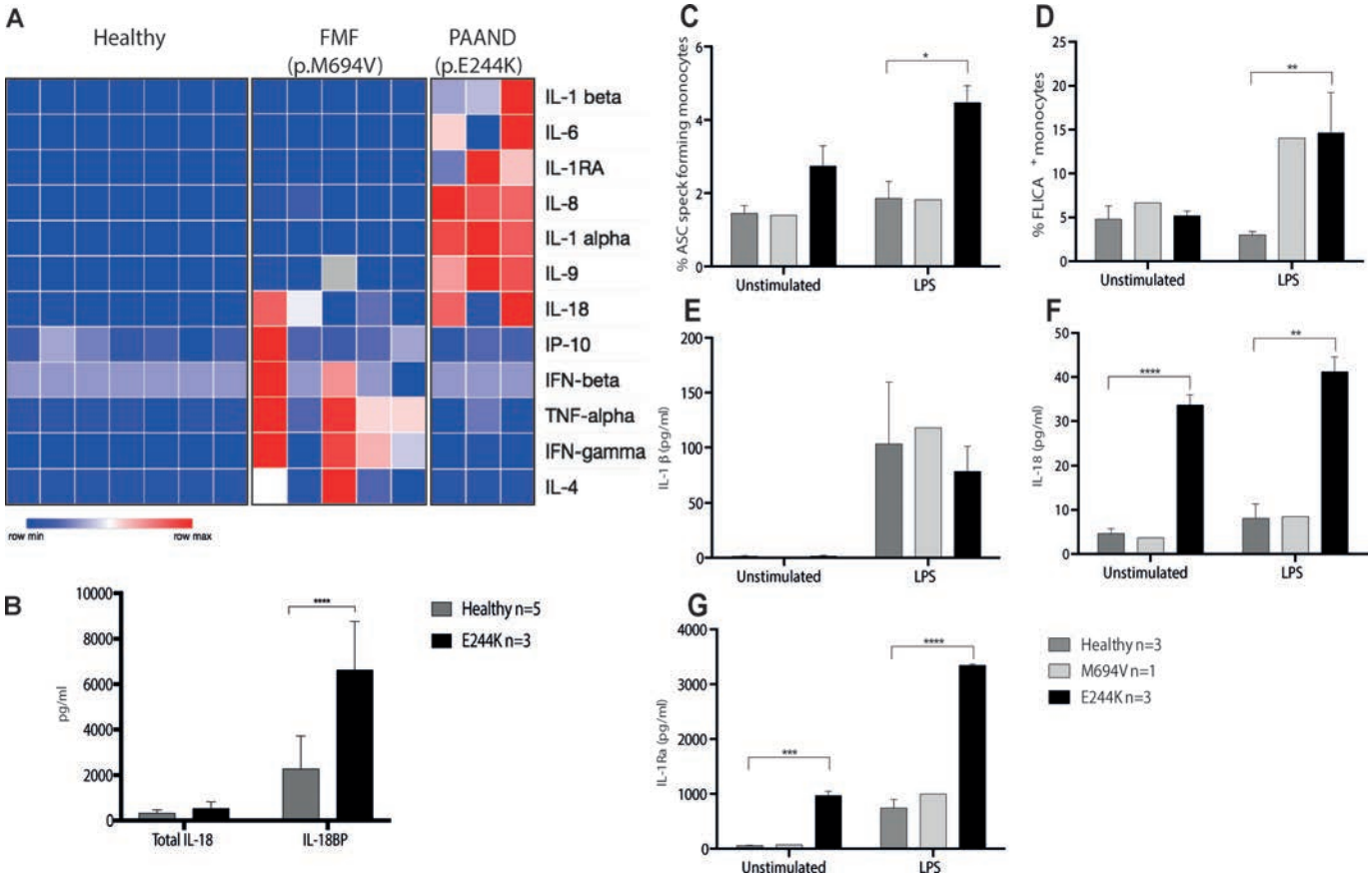

Figure 2 Pyrin-Associated Autoinflammation with Neutrophilic Dermatosis (PAAND) (p.E244K pyrin) has a distinct cytokine and inflammasome profile compared with Familial Mediterranean Fever (FMF). (A) Heat map of serum cytokine analysis of healthy controls, patients with FMF and genetically confirmed homozygous p.M694V *MEFV* mutation or patients with PAAND carrying the heterozygous p.E244K *MEFV* mutation. Representative of relative values of minimum and maximum concentrations measured per cytokine. (B) Serum total IL-18 analysis compared with IL-18BP. Assessment of (C) Apoptosis-associated Speck-like protein containing a Caspase recruitment domain (ASC) speck forming monocytes by flow cytometry and (D) active caspase-1 by YVAD-Fluorochrome Inhibitor of Caspases (FLICA) staining. Peripheral blood mononuclear cell IL-1β (E), IL-18 (F), and IL-1Ra (G) cytokine production at baseline and with Lipopolysaccharide (LPS) stimulation in healthy controls, FMF and PAAND patients. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

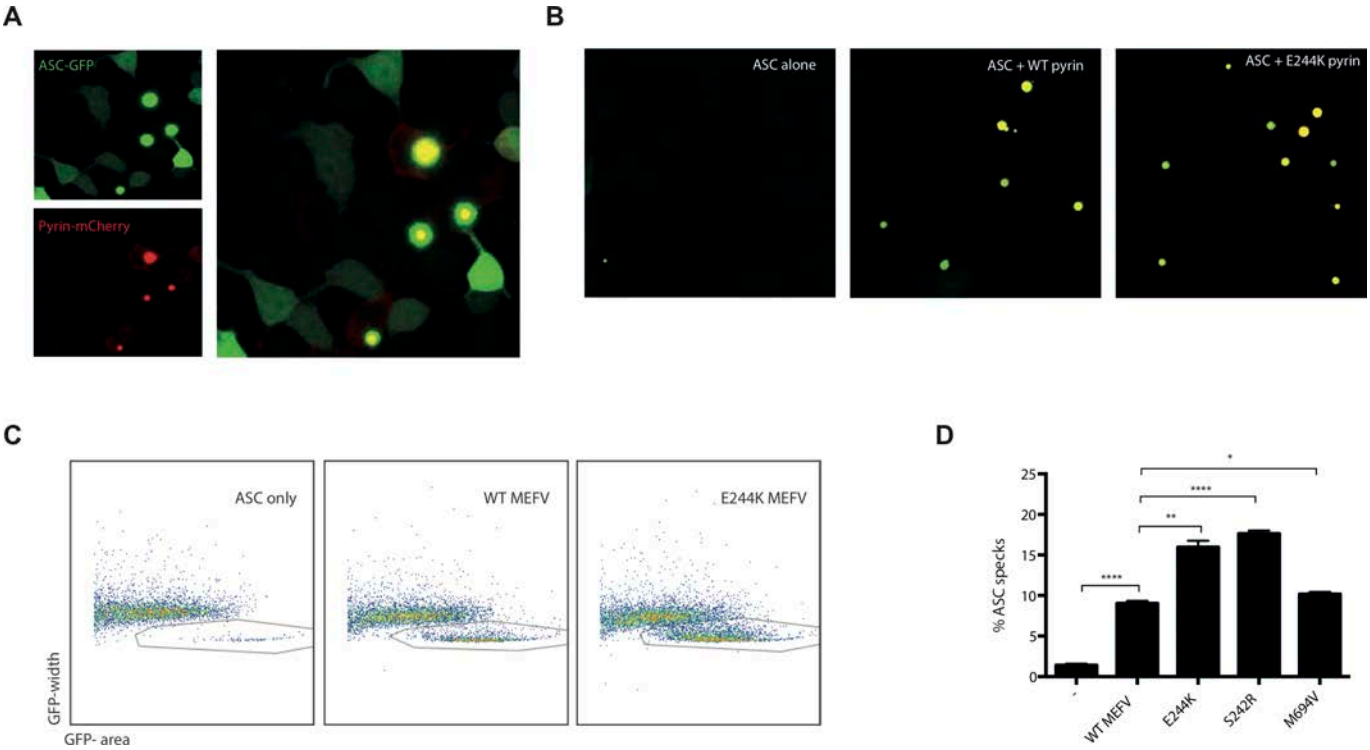

Figure 3 Increased inflammasome activation by p.E244K pyrin. (A) Confocal microscopy showing colocalisation of mCherry-tagged pyrin and GFP-tagged Apoptosis-associated Speck-like protein containing a Caspase recruitment domain (ASC) transfected into HEK293T cells. (B) Increase in spontaneous ASC speck formation in p.E244K pyrin compared with wild type (WT) pyrin or ASC alone control. (C) Fluorescence-activated cell sorting (FACS) analysis of HEK293T cells with mCherry-tagged pyrin and GFP-tagged ASC constructs. After 16 hours, cells were selected by forward scatter (FSC) and side scatter (SSC), expression of both constructs (mCherry and GFP) and finally GFP area versus width. (D) Flow cytometric quantification of ASC speck formation for WT and various pyrin mutants. Data pooled from three independent experiments. *p<0.05, **p<0.01, ****p<0.0001.

When activated, most inflammasome forming proteins, including pyrin, associate with the adaptor protein ASC to form a platform for procaspase-1 activation and cleavage of pro-IL-1β and pro-IL-18 to the mature forms.[20 21] Monocytes isolated from PAAND patients showed increased ASC speck formation with LPS exposure, and there was a trend toward an increase at baseline (figure 2C). Caspase-1 activity as measured by YVAD-FLICA staining was increased in PAAND monocytes when treated with LPS (figure 2D). Given these results, it was surprising that IL-1β production from PAAND patient PBMCs in response to LPS was unaltered (figure 2E). Nevertheless, the total IL-18 secreted by PBMCs was increased compared with healthy controls, both at baseline and following LPS stimulation, as were levels of IL-1Ra (figure 2F,G).

p.E244K pyrin is associated with increased ASC speck formation

To determine whether the above results were indeed caused by the novel p.E244K pyrin mutation, we assessed ASC speck formation in vitro as a surrogate marker for inflammasome formation. Colocalisation experiments were performed by expression of both mCherry-tagged pyrin and GFP-ASC in HEK293T cells (figure 3A). There was minimal spontaneous ASC speck formation. As expected, WT pyrin augmented this response, but p.E244K pyrin did so further (figure 3B). This was quantified using flow cytometry (see online supplementary Figure S3), with p.E244K pyrin resulting in a similar percentage of cells with ASC speck formation compared with the other known PAAND mutation p.S242R, both of which were greater than WT and p.M694V pyrin (figure 3C,D).

p.E244K pyrin is associated with increased IL-1β, IL-18 and pyroptosis

Further functional studies were performed using THP-1 monocytes. *MEFV* KO or *CASP1* KO THP-1 cells were reconstituted with *MEFV* using lentiviral transduction of WT or mutant cDNA. Even without stimulation, *MEFV* KO THP-1 cells expressing p.E244K pyrin displayed increased cell death (figure 4A), as well as IL-1β and IL-18 release (figure 4B,C). Surprisingly, this phenotype was present without 'priming' the inflammasome, which is usually required to induce pro-IL-1β expression.[22] Interestingly, IL-1β production in both p.E244K and p.S242R pyrin-expressing *MEFV* KO THP-1 cells was significantly higher than cells expressing FMF associated p.M694V pyrin (figure 4C). Genetic deletion of caspase-1 prevented p.E244K and p.S242R pyrin-induced cytokine production as well as cell death, suggesting the caspase-1 dependent inflammatory cell death (pyroptosis) (figure 4A–C). However, genetic deletion of caspase-1 did not affect Pam3CSK4-induced priming of pro-IL-1β (figure 4D). These in vitro data support the hypothesis that inflammasome activation in p.E244K pyrin patients is responsible for excessive cytokine release and pyroptosis.

p.E244K pyrin does not alter PSTPIP1 binding

In PAPA syndrome, mutant PSTPIP1 is hyperphosphorylated and binds more strongly to pyrin.[23] Given the clinical similarities between PAAND and PAPA syndrome, binding of PSTPIP1 to pyrin with and without PAAND mutations was assessed. Both GST-pyrin and PSTPIP1 were transfected into HEK293T cells and GST-immunoprecipitation performed. When comparing the

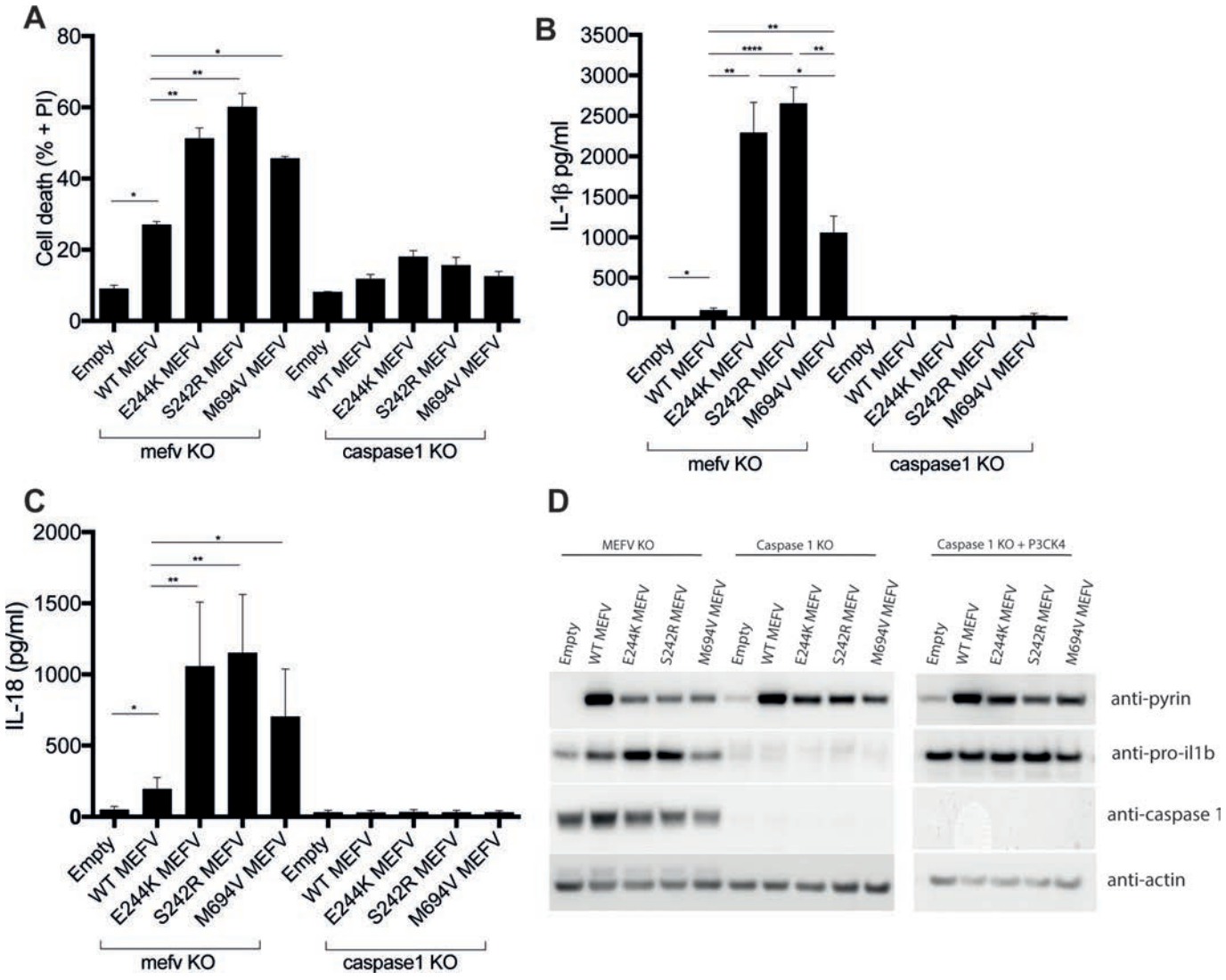

Figure 4 Pyroptosis and cytokine production by p.E244K pyrin. Monocytic THP-1 cells with pyrin or caspase-1 deleted by CRISPR were reconstituted with wild type (WT) and mutant pyrin using lentiviral vectors. (A) Cell death was measured by propidium iodide (PI) staining and flow cytometry, and (B) IL-1β and (C) IL-18 measured by ELISA 48 hours after lentiviral infection. The increased cell death (A), IL-1β (B) and IL-18 (C) seen in the pyrin mutants was abrogated in the caspase-1 KO THP1 cells. (D) Whole cell lysate was prepared from THP-1 cells and western blotting was performed, probing for pro-IL-1β to determine the mechanism of the IL-1β response. *CASP1* KO cells were further treated with Pam3CSK4 to look at a physiological priming response to ensure that pro-IL-1β could be generated in this cell line. Data pooled from three independent experiments. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

binding of WT PSTPIP1 to WT, PAAND and FMF associated pyrin, no significant difference was observed. This suggests that the mechanism of this disease is not related to increased PSTPIP1 binding (see online supplementary figure S4).

p.E244K pyrin has reduced phosphorylation of 14-3-3 binding motif and reduced 14-3-3 binding

The initial report of PAAND showed that the mechanism of increased inflammasome activation was loss of 14-3-3 binding to pyrin and subsequent loss of autoinhibition.[1] Given that p.E244 is the +2 position of a 14-3-3 binding motif (figure 1D), preliminary experiments were conducted to examine 14-3-3 binding to p.E244K pyrin. Serine residues at positions p.S208 and p.S242 have previously been shown to interact with 14-3-3[1,2] and were used as comparators. Immunoprecipitation was performed using GST-tagged WT and mutant pyrin transfected into HEK293T cells. This revealed reduced binding of an antibody that recognises phosphorylated serine in the 14-3-3 binding motif in mutants p.E244K and p.S242R pyrin, but not in the FMF-associated p.M649V pyrin (figure 5A). Binding of 14-3-3 to pyrin was also affected, following the same pattern. Further evaluation of binding of the 14-3-3τ and 14-3-3ε isoforms to these mutants, as well as p.S208A and p.S208A/S242R pyrin, showed no differences, suggesting both isoforms behave similarly (figure 5B). These data suggest that PAAND pyrin mutations result in reduced phosphorylation of the 14-3-3 binding motif and reduced 14-3-3 binding to pyrin.

The p.E244 position is important in 14-3-3 binding to pyrin

Phosphorylated serine in specific motifs is important for 14-3-3 binding. Previous reports had suggested that proline was required at the +2 position of the motif for interaction between 14-3-3s and their target protein, documented as RXX(pS)XP (figure 1D). However, subsequent reports show that proline in +2 position is present in only 50% of 14-3-3 binding motifs.[24] To explore the importance of the +2 position in 14-3-3 binding and pyrin

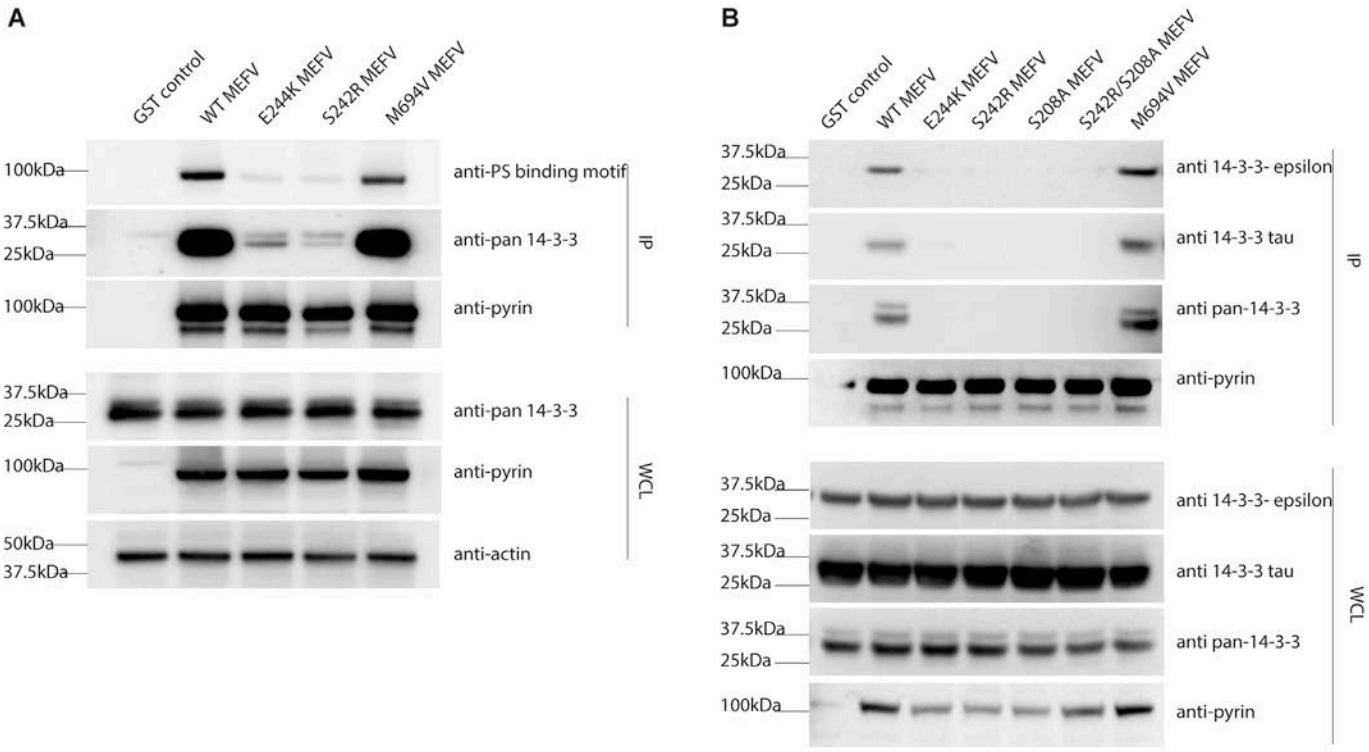

Figure 5 Reduced 14-3-3 binding by p.E244K pyrin. HEK293T cells were transfected with GST-tagged pyrin, with or without Pyrin-Associated Autoinflammation with Neutrophilic Dermatosis (PAAND) and Familial Mediterranean Fever (FMF) mutations, and immunoprecipitation performed. (A) Western blot was performed to compare phosphoserine (PS) 14-3-3 binding motif, pan-14-3-3 binding and pyrin expression in immunoprecipitate (IP) and whole cell lysate (WCL). Comparison was made to p.S242R and p.M694V pyrin. (B) Western blot was performed to compare pan-, τ or ε 14-3-3 binding and pyrin expression in IP and WCL. Comparison was made to p.S242R, p.S208A, p.S208A/S242R and p.M694V pyrin. Representative of three independent experiments.

regulation, p.E244 pyrin was mutated to various amino acids. Glutamate (E) was substituted by aspartate (D) or arginine (R) to explore charge and polarity, respectively, or proline (P) to explore the effect of the canonical 14-3-3 binding motif. Flow cytometric analysis of these mutants showed an increase in ASC speck formation in p.E244R pyrin mutant, while p.E244D and p.E244P pyrin mutations did not further activate pyrin in this assay (figure 6A). Immunoprecipitation showed that p.E244R pyrin had reduced binding to 14-3-3 when compared with WT, similar to p.E244K (figure 6B). Interestingly, p.E244P pyrin had increased 14-3-3 binding, suggesting that this mutation could potentially suppress pyrin activation. To test this hypothesis, cells were treated with the RhoGTPase inhibitor, TcdB, to activate pyrin. Although p.E244P increased binding of 14-3-3 to pyrin, this was insufficient to prevent activation by TcdB (figure 6C). Furthermore, the double mutant p.E244P/M694V had no effect on this, highlighting again a distinct pathophysiological mechanism of FMF and PAAND (figure 6C).

DISCUSSION

The initial clinical suspicion of PAPA syndrome in the index patient highlights the striking clinical overlap between PAAND and PAPA syndrome, as noted in the original description of PAAND.[1] Compared with the initial report, our family is distinct in suffering from polyarthritis as well as severe hidradenitis suppurativa, suggesting that even within the PAAND diagnosis, there is variability in clinical presentation, consistent with a spectrum of pyrin-associated features. Our results agree with the original description of PAAND, namely that excessive IL-1β is pyrin dependent. Although PAPA syndrome is also pyrin dependent,[23] the exact mechanisms underlying the similar clinical presentations of PAAND and PAPA syndrome have not been elucidated. We suggest that patients with clinically suspected PAPA syndrome who test negative for *PSTPIP1* mutations should undergo genetic evaluation of *MEFV*, with particular attention to the bases in exon 2 encoding 14-3-3 binding motifs.

The role of 14-3-3 in controlling the activation of pyrin is highlighted by this novel mutation causing PAAND. Reduced binding of 14-3-3 to pyrin was seen with both p.E244K and p.S242R pyrin, but not in the FMF-associated p.M694V mutation (figure 5). The loss of 14-3-3 binding following stimulation with TcdB suggests that 14-3-3 is required to maintain pyrin in an auto-inhibited state and reduced 14-3-3 binding to PAAND-associated pyrin leads to its auto-activation. We propose that with the same expression of pyrin across the mutants examined in our model, the PAAND pyrin is likely to be more active, with increased pyroptosis and availability of pro-IL-1 for cleavage. It is possible that PAAND is at one spectrum of pyrin-associated disorders in terms of severity, with PAAND pyrin being spontaneously active and FMF pyrin having a lower threshold for activation than WT pyrin.

The 14-3-3 binding motif of pyrin differs from the canonical RXX(pS)XP motif with a highly conserved glutamate at the +2 position. Substituting glutamate for proline or aspartate,

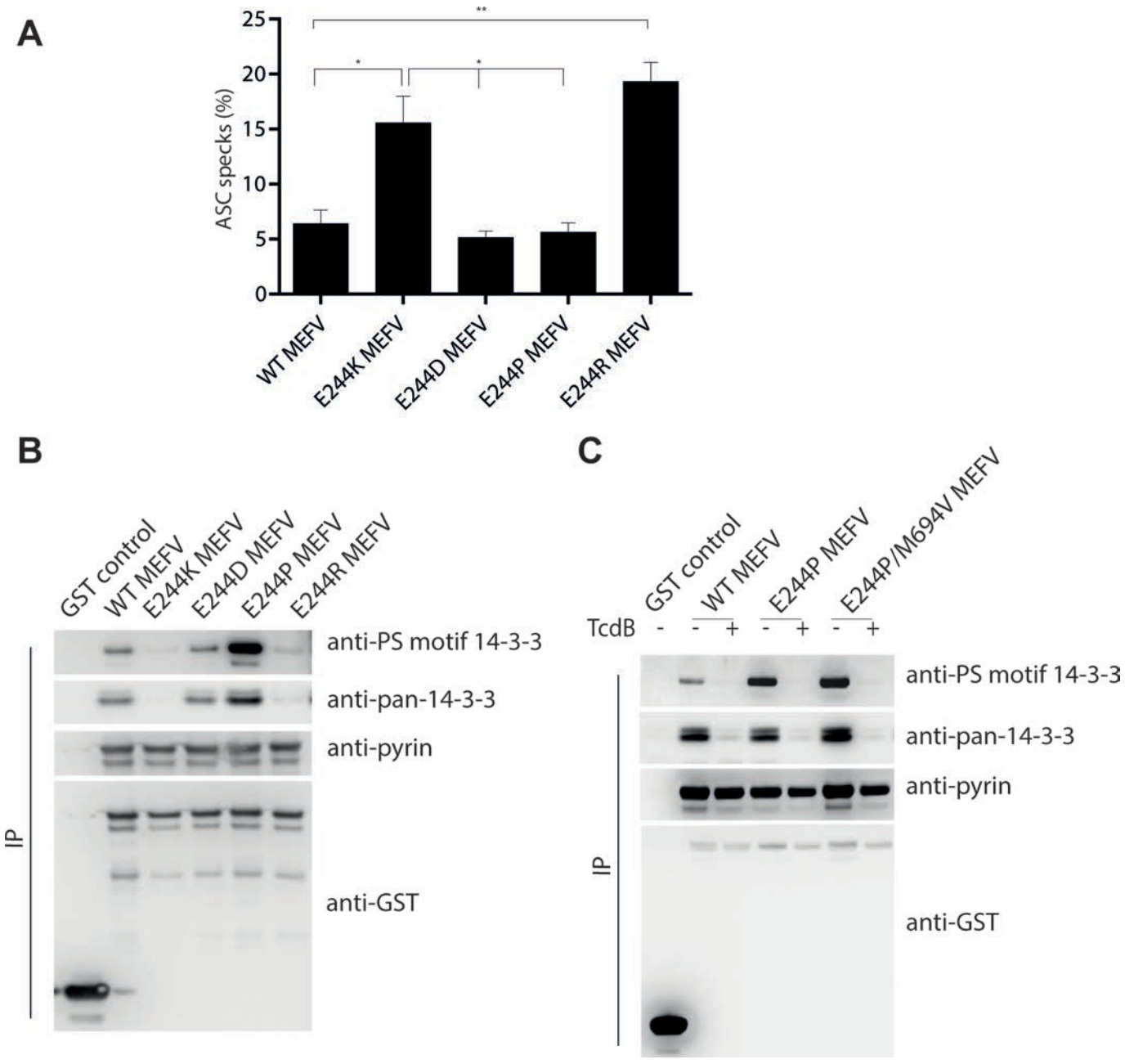

Figure 6 +2 position of 14-3-3 binding site is important in regulation of pyrin activation. (A) Flow cytometric analysis of Apoptosis-associated Speck-like protein containing a Caspase recruitment domain (ASC) speck formation performed on HEK293T cells transfected with mCherry-tagged pyrin with various mutations at position p.E244 and GFP-tagged ASC. Data pooled from three independent experiments. (B) HEK293T cells were transfected with GST-tagged pyrin with various mutations at position p.E244, and then immunoprecipitated and blotted with antibodies to detect the phosphorylated 14-3-3 binding motif, pan-14-3-3, pyrin or the GST tag. (C) Immunoprecipitation was performed as described above, but with *Clostridium difficile* Toxin B protein (TcdB) stimulation for 16 hours, to assess phosphorylation of 14-3-3 binding sites and 14-3-3 binding. Representative of three independent experiments. *p<0.05, **p<0.01. IP= immunoprecipitate.

non-polar and negatively charged amino acids, respectively, retained 14-3-3 binding to pyrin, whereas substitutions to lysine or arginine, both positively charged amino acids, do not appear to be tolerated. The structure of this region of pyrin has not been elucidated, making it difficult to predict the effect of amino acid substitutions. However, we demonstrate that the +2 position of the 14-3-3 binding motif is important, and that substitution at this site can alter the ability for 14-3-3 to bind to pyrin.

Although M694V pyrin results in increased inflammasome formation[25], the mechanism of auto-activation still remains to be elucidated. We saw no discernible difference between WT and p.M694V pyrin with regards to 14-3-3 binding, and Van Gorp *et al* documented unaltered phosphorylation at position p.S242 in p.M694V pyrin transfected HEK293T cells, which is required for 14-3-3 binding.[26] Interestingly, Park *et al* did see reduced 14-3-3ε binding in FMF-associated mutations.[5] It is possible that subtle differences in the experimental approach may influence this result, and given that PAAND is a more severe disease, we would expect FMF mutations to have a smaller mechanistic effect on 14-3-3 binding.

In addition to 14-3-3 binding, the clinical presentation, mode of inheritance and biochemical status of PAAND differ from

FMF. Although this study focuses on only two generations of one family, the heterozygous mutation and variable phenotype suggest a dominant disorder with variable penetrance, compared with the typically autosomal recessive inheritance of FMF. All members of the PAAND family have marked dermatological manifestations, further differentiating this condition from FMF.

Another distinction between these pyrin-associated conditions is evident from the serum cytokine profile, and cytokine production by PBMCs, both at baseline and after LPS-priming. Although the FMF patients were asymptomatic, there was evidence of systemic inflammation with raised CRP in four of the five controls (see online supplementary table S1). Furthermore, their serum cytokine profile was distinct from healthy controls as well as PAAND patients, suggesting that there are indeed differences that are not accounted for by symptom control.

Despite elevated IL-1β in these analyses, one patient with the p.E244K mutation did not improve with a trial of anakinra. Interestingly, the elevated IL-1Ra levels in this individual may explain why a recombinant IL-1Ra did not provide further benefit. Our FMF patients did not have elevated IL-1Ra levels, and a number of recent publications suggest that colchicine-resistant FMF can be adequately treated with IL-1 antagonism.[27–29] Despite elevated IL-18 levels in PAAND PBMC secreted at baseline and in response to LPS (figure 2F), an increase in IL-18BP levels (figure 2B) suggests that targeting this pathway may not be as effective as shown for patients with activation of the Nod-Like Receptor CARD containing protein 4 (NLRC4) inflammasome.[30] The clinical response to TNF inhibition in our patient suggests that this is an important cytokine in PAAND, even though TNF was not elevated in the serum of these patients. This may be because at the time of the study, the patient was receiving treatment with immunomodulatory drugs including adalimumab. Alternatively, increased cell death in PAAND (figure 4A) could release damage-associated molecular patterns that trigger local cytokine production in tissues such as the skin. Furthermore, it would be interesting to assess tissue specific cytokines and cell responses as these may reveal pathogenic factors not present in peripheral blood. Regardless, given the difficulty controlling disease activity and the need for multiple therapeutic agents, PAAND is likely to be driven by more than a single cytokine.

The p.E244K pyrin mutation in PAAND patients highlights the importance of the 14-3-3 binding motif in pyrin activation, in addition to the p.S242R mutation described originally. Our study suggests that although PAAND and FMF mutations are located in the same gene, they are distinct diseases clinically, with unique cytokine profiles, cellular responses and 14-3-3 binding.

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OPEN ACCESS

EXTENDED REPORT

B cell OX40L supports T follicular helper cell development and contributes to SLE pathogenesis

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ABSTRACT

Objectives *TNFSF4* (encodes OX40L) is a susceptibility locus for systemic lupus erythematosus (SLE). Risk alleles increase *TNFSF4* expression in cell lines, but the mechanism linking this effect to disease is unclear, and the OX40L-expressing cell types mediating the risk are not clearly established. Blockade of OX40L has been demonstrated to reduce disease severity in several models of autoimmunity, but not in SLE. We sought to investigate its potential therapeutic role in lupus.

Methods We used a conditional knockout mouse system to investigate the function of OX40L on B and T lymphocytes in systemic autoimmunity.

Results Physiologically, OX40L on both B and T cells contributed to the humoral immune response, but B cell OX40L supported the secondary humoral response and antibody affinity maturation. Our data also indicated that loss of B cell OX40L impeded the generation of splenic T follicular helper cells. We further show that in two models of SLE—a spontaneous congenic model and the H2-IA^{bm12} graft-versus-host-induced model—loss of B cell OX40L ameliorates the autoimmune phenotype. This improvement was, in each case, accompanied by a decline in T follicular helper cell numbers. Importantly, the germline knockout did not exhibit a markedly different phenotype from the B cell knockout in these models.

Conclusions These findings contribute to a model in which genetically determined increased OX40L expression promotes human SLE by several mechanisms, contingent on its cellular expression. The improvement in pathology in two models of systemic autoimmunity indicates that OX40L is an excellent therapeutic target in SLE.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterised by autoantibodies against nuclear antigens along with the deposition of immune complexes.[1 2] As with most other autoimmune diseases, environmental and genetics factors contribute to the risk of developing SLE. Genome-wide association studies have revealed over 50 susceptibility loci.[3 4] *TNFSF4* (tumour necrosis factor ligand family, member 4, CD252) is an established susceptibility gene for SLE[4 5] and for several other autoimmune diseases.[6–9] Fine-mapping of this locus in SLE identified two independent association signals upstream of *TNFSF4* in multiple ancestries.[10] These two signals align with separate expression quantitative trait loci, each one associated with elevated expression of *TNFSF4* in Epstein Barr virus (EBV) lymphoblastoid cell lines,[11] suggesting that *TNFSF4* transcription is upregulated in individuals harbouring risk alleles.

TNFSF4 encodes the costimulatory molecule, OX40L, a type II transmembrane protein expressed on several immune cell types on activation, including anitigen presenting cells (APCs), such as dendritic cells (DCs), B cells and macrophages,[12–14] activated T cells,[15 16] and mast cells and vascular endothelial cells.[17] In contrast, its only known receptor, OX40, is expressed mainly on activated CD4+ T cells.[18–21] The OX40L-OX40 signalling pathway is fundamental for effector T cell proliferation and memory T cell development, maintenance of cytokine production by T cells and DCs, increasing Ig production, and promoting plasma cell development.[15 22–27] Nevertheless, how these various functions relate to the cell types expressing OX40L is still unclear. Constitutive expression of OX40L on T cells has been shown to induce spontaneous autoimmunity in C57BL/6 mice.[23] A recent study showed that OX40L expression on a subset of myeloid DCs is implicated in the pathogenesis of SLE.[28] The beneficial effect of blocking the OX40L-OX40 signalling pathway has been shown in several different mouse models of autoimmune diseases,[17] but experimental evidence of its efficacy in SLE is unknown.

We sought to understand the function of OX40L using CD4+ T cell and B cell conditional knockout mice. We investigated the role of OX40L using immunisation and we went on to determine how the loss of OX40L affected the pathology in two different SLE mouse models.

MATERIALS AND METHODS

Mice

A bacterial artificial chromosome (BAC) clone encoding the extracellular domain and 3′-untranslated region of *Tnfsf4* was obtained from a C57BL/6-derived genomic library. The *Tnfsf4* conditional targeting vector was constructed using recombineering,[29] as described in online supplementary figure S1A. The mice (*Tnfsf4*$^{fl/fl}$) were made according to a standard gene targeting approach in A9 embryonic stem cells (ES). We used (129xC57BL/6)F1 ES; therefore, microsatellite analyses were undertaken to confirm that the targeting vector had recombined on the C57BL/6 chromosome. The mice were backcrossed for eight generations on the C57BL/6 background. *Tnfsf4*$^{fl/fl}$ mice were crossed with β-actin-cre, CD4-cre and CD19-cre (Jackson Laboratories) to

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obtain *Tnfsf4*$^{-/-}$, *Tnfsf4*$^{fl/fl}$/CD4-cre and *Tnfsf4*$^{fl/fl}$/CD19-cre, respectively. Before each experiment mice were genotyped by PCR. The primers and expected PCR product size are listed in supplementary table S1. B6.*Sle16* mice were bred in-house and B6.*Sle16*.*Tnfsf4*$^{-/-}$ were generated by crossing them with *Tnfsf4*$^{-/-}$ mice. B6-H2^{bm12} mice were purchased from the Jackson Laboratories (B6(C).*H2-Ab1*^{bm12}/KhEgJ; strain no. 001162, https://www.jax.org/strain/001162). The mice used were female, 8–12 weeks old and housed in specific pathogen-free conditions. All animal procedures were performed in accordance with institutional guidelines and approved by the UK Home Office.

In vitro analysis of OX40L expression

To assess OX40L expression in vitro, different cell subsets were purified from mouse spleen using LS Columns and MACS Technology (Miltenyi Biotec) and stimulated as described before.[16 24] Briefly, single cell suspensions were obtained from collagenase-treated spleens, and B cells, DCs and T cells were then purified incubating the splenic cell suspension with anti-CD43(Ly-48) microbeads, anti-CD11c microbeads or CD4+ T cell isolation kit, respectively, following the manufacturers' protocols. The purity of each subpopulation was tested routinely by fluorescence-activated cell sorting (FACS) and a value >95% was measured for each purification. Purified B cells and DCs were stimulated for 72 hours with anti-CD40 (Clone3/23 at 2.5 µg/mL) plus F(ab')2 anti-mouse IgM (10 µg/mL) or anti-CD40 alone, respectively. T cells were stimulated with anti-CD3 (0.005 µg/mL), IL-2 (100 U/mL) and IL-12 (10 ng/mL) for 7 days. After stimulation, the cells were harvested and analysed by FACS for OX40L expression.

Flow cytometry

Flow cytometry was performed using a five-colour or six-colour staining protocol and analysed with a BD FACSVerse (BD Biosciences, San Jose, California, USA). The following Abs were used: anti-CD4 (GK.5), anti-CXCR5 (L138D7), anti-PD1(29F.1a12), anti-CD62L (MEL-14), anti-CD44 (IM7), anti-B220 (RA3-6B2), anti-GL7 (GL7), anti-CD138 (281-2) and anti-IgD (11–26 c.2a). Abs were purchased from BioLegend (San Diego, California, USA). Staining was performed in the presence of a saturating concentration of 2.4G2 mAb (anti-FcγRII/III). Data were analysed using FlowJo V.9 (Tree Star, Ashland, Oregon, USA).

Immunisation and ELISA

Mice were immunised subcutaneously with 50 µg 4-hydroxy-3-nitrophenylacetyl-chicken gamma globulin (NP-CGG) in complete Freund's adjuvant. For the analysis of the secondary response mice were reimmunised with 50 µg of NP-CGG in incomplete Freund's adjuvant 35 days after receiving the first immunisation. Serum was collected on days 7, 14, 28 and 42, and titres of isotype-specific low-affinity and high-affinity antibodies to NP were measured by ELISA in plates coated with either NP25-BSA or NP4-BSA (4-hydroxy-3-nitrophenylacetyl hapten conjugated to bovine serum albumin), respectively.[30] Briefly NUNC plates were coated with the antigen at 5 µg/mL in borate buffered saline (BBS) overnight at 4°C. Plates were washed with phosphate buffered saline (PBS) and then blocked for 1 hour at room temperature with 0.5% BSA in PBS. Samples were diluted in dilution buffer (PBS 2%, bovine serum albumin (BSA) 0.05% Tween-20) and added, in duplicate, to the plates for 3 hours at 37°C. Plates were washed and incubated with alkaline phosphatase (AP)-conjugated secondary antibody specific for the different Ig isotype (SouthernBiotech) for 3 hours at room temperature. Plates were developed with p-nitrophenylphosphate (Sigma). A standard serum was generated from a pool of reactive serum of immunised wild-type mice. Absorbance was read at 405 nm and data were expressed as arbitrary ELISA unit (AEU) in reference to a standard curve obtained by serial dilution of the standard serum.

cGvHD mouse model and autoantibody assays

Knockout and control mice were injected intraperitoneally with 5×10^7 splenocytes from B6.*H2*^{bm12} mice. Briefly, splenocytes were obtained as a single cell suspension by mashing the spleen collected through 70 µm cell strainers using the plunger from a syringe. After lysis of the red blood cells, splenocytes were counted and resuspended at 5×10^8 cells/mL in PBS and 100 µL was injected in each mouse. Serum was collected on days 14, 28 and 42, and titres of IgG antibodies to double-stranded deoxyribonucleic acid (dsDNA) were measured by ELISA using dsDNA (100 µg/mL) or single-stranded deoxyribonucleic acid (ssDNA) (10 µg/mL) in BBS buffer as coating antigen. Bound Abs were detected with AP-conjugated goat anti-mouse IgG (-chain specific) (Sigma-Aldrich) or IgM (Southern Biotechnology Associates). The results were expressed as AEU relative to a standard positive sample derived from an MRL/Mp$^{lpr/lpr}$ mice pool.

Total serum IgG and IgM levels

Total serum IgM and IgG levels were assayed by capture ELISA as previously described.[31]

IgG, IgM and C3 kidney deposition

Fluorescein (FITC)-conjugated goat Abs against mouse total IgG (1/400 dilution; Sigma-Aldrich), mouse total IgM (1/200 dilution, eBioscience) and against mouse C3 (1/50 dilution; ICN Pharmaceuticals) were used on snap-frozen kidney sections. The staining with FITC-conjugated Abs was quantified as previously described[31] and expressed as arbitrary fluorescence units.

Statistical analysis

Where appropriate either the Student's t-test, two-way analysis of variance (ANOVA) or one-way ANOVA followed by Fisher's least significant difference (LSD) multiple comparison test was performed using GraphPad Prism V.6.00 for Windows (GraphPad Software, La Jolla, California, USA).

RESULTS

Generation of *Tnfsf4* conditional knockout strains

We generated a floxed *Tnfsf4* mouse (*Tnfsf4*$^{fl/fl}$) on the C57BL/6 genetic background (see online supplementary figure S1A,B) to avoid the confounding effects caused by epistatic interactions between 129 and C57BL/6 genes that promote an autoimmune phenotype.[32] Germline knockout (KO) mice were obtained by crossing *Tnfsf4*$^{fl/fl}$ with the β-actin *Actb-cre* mouse strain. Conditional T cell *Tnfsf4*$^{fl/fl}$*(CD4)*$^{-/-}$ and B cell *Tnfsf4*$^{fl/fl}$*(CD19)*$^{-/-}$ specific knockout mice were created by crossing with CD4-*cre*[33] and CD19-*cre* mice,[34] respectively. Lack of OX40L was observed in all cell types from *Tnfsf4*$^{-/-}$ mice, while a cell-specific deletion was confirmed in the conditional knockouts (see online supplementary figure S1C).

B cell OX40L promotes antibody affinity maturation

Conflicting data have been reported on the importance of the OX40L-OX40 pathway in controlling T-dependent antibody responses.[24 26 35] Thus, we explored this response by immunising

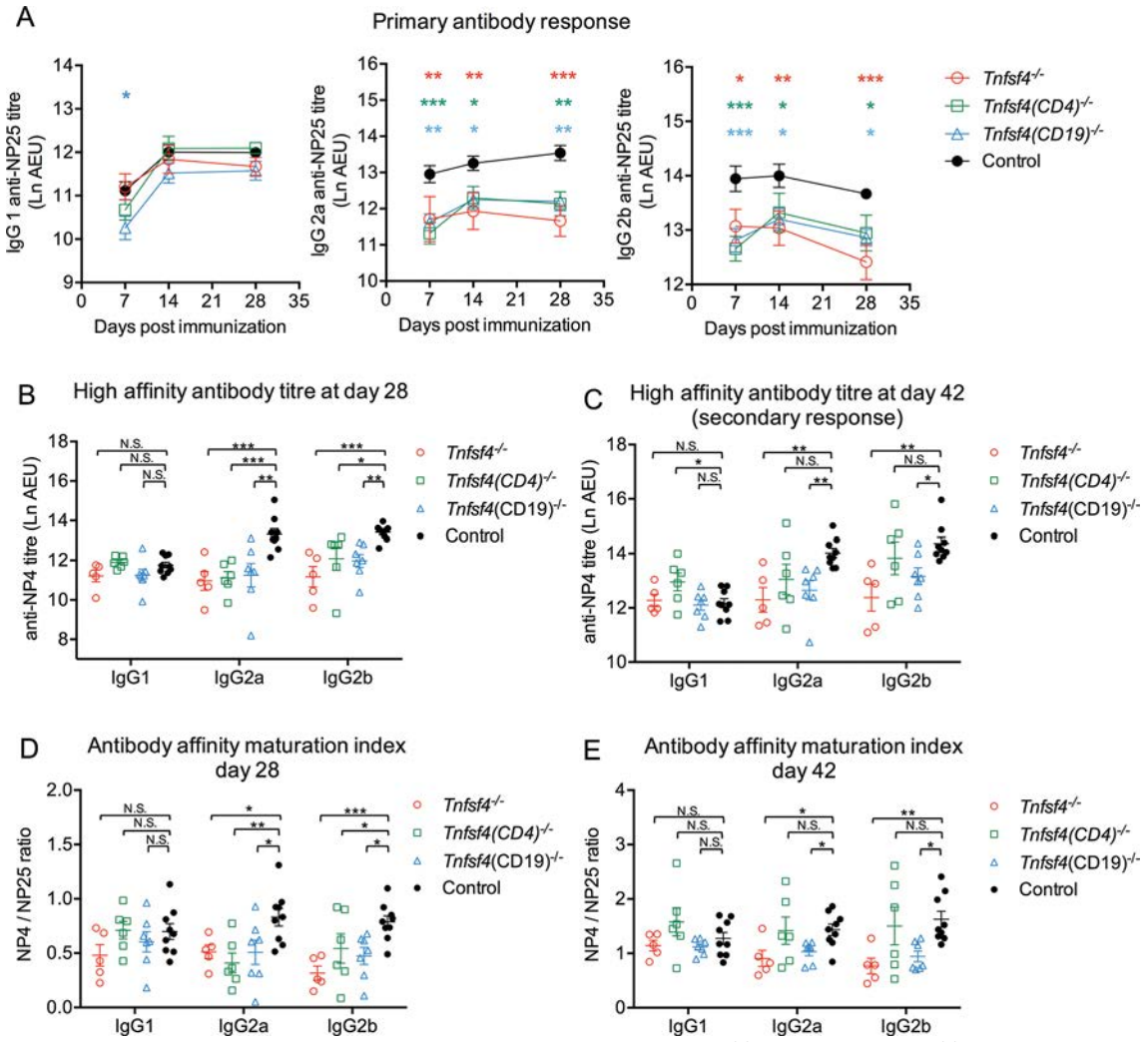

Figure 1 OX40L in T cell-dependent humoral response. Wild-type controls, *Tnfsf4*$^{-/-}$, *Tnfsf4*$^{fl/fl}$(CD19)$^{-/-}$ and *Tnfsf4*$^{fl/fl}$(CD4)$^{-/-}$ mice were immunised with NP-CGG in CFA and reimmunised on day 35 with NP-CGG in IFA. Sera were collected on days 7, 14 and 28 for the primary response and on day 42 for the secondary response. (A) Titres of NP-specific low-affinity antibody measured with NP25-BSA. (B) Titres of NP-specific high-affinity antibodies on day 28 measured with NP4-BSA. (C) Titres of NP-specific high-affinity antibodies on day 42 measured with NP4-BSA. (D) Affinity maturation index calculated as ratio of the titres of IgG detected with NP4-BSA to those with NP25-BSA on day 28. (E) Affinity maturation index calculated as the ratio of the titres of IgG detected with NP4-BSA to those with NP25-BSA on day 42. Each symbol represents an individual mouse; dots in (A) and bars in (B–E) indicate the mean titre, each being shown mean±SEM. AEU is arbitrary ELISA unit; N.S. is not significant; *p<0.05, **p<0.01 and ***p<0.001 (A, two-way ANOVA; B–E, one-way ANOVA). ANOVA, analysis of variance; CFA, complete Freund's adjuvant; IFA, incomplete Freund's adjuvant; NP-CGG, nitrophenylacetyl-chicken gamma globulin; NP25-BSA, NP4-BSA, 4-Hydroxy-3-nitrophenylacetyl hapten conjugated to bovine serum albumin.

the three KO strains and a control group with NP-CGG, a well-studied T cell-dependent antigen. All three strains showed significantly lower titres of low-affinity IgG2a and IgG2b antibodies against NP25-BSA compared with wild-type mice (figure 1A). In contrast, the IgG1 response was hardly affected by the lack of OX40L. Affinity maturation during the primary response was also assessed by measuring antibody against NP4-BSA on day 28 and by calculating the affinity maturation index (ratio of high-affinity to low-affinity antibody responses). All three knockout strains displayed lower titres of high-affinity IgG2a and IgG2b (figure 1B,C) compared with wild-type animals and a lower affinity maturation index (figure 1D), which suggested OX40L contribution in the antibody affinity maturation process. To investigate the role of OX40L in the secondary immune response, mice were then boosted with NP-CGG on day 35, and the high-affinity antibody response was measured 1 week later. *Tnfsf4*$^{-/-}$ and *Tnfsf4*$^{fl/fl}$*(CD19)*$^{-/-}$ mice both showed a significantly impaired IgG2a and IgG2b memory response compared with control mice, associated with a lower affinity maturation index (figure 1C,E). In contrast, the memory response in the *Tnfsf4*$^{fl/fl}$*(CD4)*$^{-/-}$ mice was normal (figure 1C). These results indicate a role for both B and T cell OX40L in the primary immune response, with a distinct role for B cell OX40L in the affinity maturation of the secondary humoral immune response.

OX40L is essential for T cell activation

As the impaired humoral response could be a consequence of defective T cell activation, we decided to investigate the splenic T cell composition (see online supplementary figure S2A) of immunised mice on days 14 and 42 (figure 2). By day 14, *Tnfsf4*$^{-/-}$ and *Tnfsf4*$^{fl/fl}$*(CD4)*$^{-/-}$ had a markedly lower proportion of effector T and effector/memory CD4+ T cells. In contrast, *Tnfsf4*$^{fl/fl}$*(CD19)*$^{-/-}$ mice showed only a reduction in the proportion of effector/memory T cells, indicating that B cell OX40L may not play a major role in priming naïve T

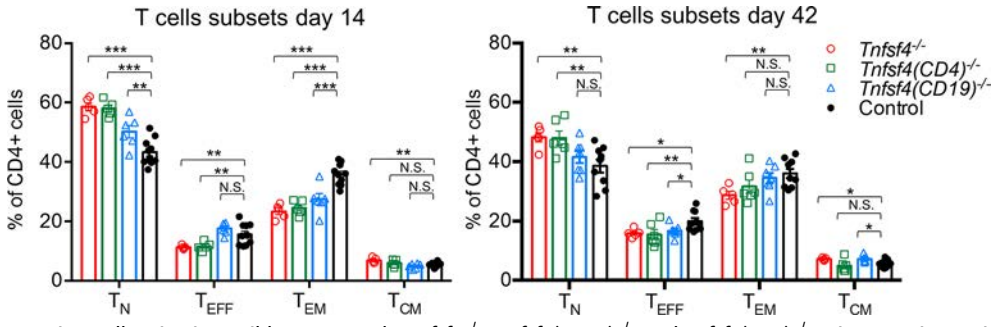

Figure 2 Role of OX40L in T cell activation. Wild-type controls, *Tnfsf4*$^{-/-}$, *Tnfsf4*(CD19)$^{-/-}$ and *Tnfsf4*(CD4)$^{-/-}$ mice were immunised with NP-CGG in CFA and reimmunised on day 35 with NP-CGG in IFA. Spleens were taken and analysed by FACS either on day 14 or day 42. Quantification of naïve (CD4+, CD62L+, CD44^{low}) T cells, effector (CD4+, CD62L^{low}, CD44^{low}) T cells, effector/memory (CD4+, CD62L$^{low/neg}$, CD44^{hi}) T cells and central/memory (CD4+, CD62L+, CD44^{hi}) T cells on day 14 (left) and day 42 (right). Each symbol represents an individual mouse. Bars indicate the mean±SEM. N.S., not significant; *p<0.05, **p<0.01 and ***p<0.001 (one-way analysis of variance). CFA, complete Freund's adjuvant; FACS, fluorescence-activated cell sorting; IFA, incomplete Freund's adjuvant; NP-CGG, nitrophenylacetyl-chicken gamma globulin.

cells. On day 42, all three knockout strains had fewer T effector cells than control mice (figure 2). Interestingly, *Tnfsf4*$^{-/-}$ and *Tnfsf4*$^{fl/fl}$*(CD19)*$^{-/-}$ mice also showed a small, but statistically significant, increment in the frequency of central memory T cells, suggesting that OX40L may regulate the balance between effector and central memory T cells during the recall response (figure 2). Our data confirmed the previous reported role of OX40-OX40L signalling in T cell activation and development of T effector memory cells.[18 22 23 36] The explanation for the difference in the secondary humoral response between *Tnfsf4*$^{fl/fl}$*(CD4)*$^{-/-}$ and *Tnfsf4*$^{fl/fl}$*(CD19)*$^{-/-}$ mice was not evident. We therefore decide to investigate further analysing the extent of the germinal centre (GC) reaction in the immunised mice.

OX40L on B cells supports plasma cell development

All three groups of immunised KO mice showed no difference in the GC B cell population (see online supplementary figure S2B) on day 14 (figure 3A), although during the secondary response, 1 week after the rechallenge, *Tnfsf4*$^{-/-}$ mice showed a smaller proportion of GC B cells (figure 3A). Similarly, no differences were detected in the plasma cell frequency on day 14, but *Tnfsf4*$^{-/-}$ *and Tnfsf4*$^{fl/fl}$*(CD19)*$^{-/-}$ mice showed a significantly lower percentage of plasma cells on day 42 (figure 3B).

B cell OX40L is essential for T_{FH} maturation

Having demonstrated the importance of OX40L in T cell activation and in plasma cell development, we investigated its possible role in T follicular helper cell (T_{FH}) maturation. We identified the GC T_{FH} population as a subset of CD4+ T cells expressing CXCR5 and high levels of PD-1 (CXCR5+PD-1^{hi}) (figure 3C,F), and in figure 3G the frequencies of splenic GC T_{FH} cells (as a proportion of CD4+ T cells) following immunisation are illustrated. There were fewer GC T_{FH} cells in the spleens of *Tnfsf4*$^{-/-}$ *and Tnfsf4*$^{fl/fl}$*(CD19)*$^{-/-}$ compared with wild-type mice during both the primary and secondary responses. In contrast, no differences were observed between controls and *Tnfsf4*$^{fl/fl}$*(CD4)*$^{-/-}$ mice. Both *Tnfsf4*$^{-/-}$ *and Tnfsf4*$^{fl/fl}$*(CD19)*$^{-/-}$ mice showed a reduction in the expression levels of PD1 at both time points, and importantly displayed a greater frequency of CXCR5+ PD1^{low} cells (T_{FH} precursors) in the CD4+ population compared with control mice on day 42 (figure 3D,F). Interestingly, *Tnfsf4*$^{-/-}$ and *Tnfsf4*$^{fl/fl}$*(CD19)*$^{-/-}$ mice also revealed a reduced number of CXCR5+ cells during the primary (day 14) but not the secondary response (day 42) (figure 3E).

Lack of OX40L reduces T_{FH} number and ameliorates the lupus phenotype

In view of the genetic association of *TNFSF4* and SLE and the functional results outlined above, we investigated the effect of loss of OX40L in SLE using two different mouse models: a congenic model and a graft-versus-host model.

Tnfsf4$^{-/-}$ mice were crossed with B6.*Sle16* lupus-prone mice, which are characterised by development of humoral autoimmunity associated with splenomegaly, high level of total IgG and IgM, autoantibodies production and glomerulonephritis linked to Ig and C3 deposition in the kidney.[31 32] The resultant B6.*Sle16*.*Tnfsf4*$^{-/-}$ female animals were monitored for 9 months (figure 4). The absence of OX40L was associated with a marked reduction in splenomegaly (figure 4A,B) and a lower serum level of total IgG and IgM (figure 4C). No detectable levels of IgG anti-DNA were observed either in the knockout or the B6.*Sle16* control group. However, when we analysed IgM anti-ssDNA autoantibodies, a significant lower titre was observed in mice lacking OX40L compared with the B6.*Sle16* group (figure 4D). To investigate the effect of loss of OX40L on target organs, we quantified glomerular IgG, IgM and complement C3. As expected fluorescent quantification revealed significantly lower amount of IgG and IgM deposition in the glomerular in the absence of OX40L; in contrast a similar level of C3 deposition was observed (see online supplementary figure S3). Mice lacking OX40L had less T cell activation and higher proportions of central memory and naïve T cells (CD62L+ CD44^{hi} cells) (figure 4E). Consistent with the immune response data (figure 3), the *B6.Sle16.Tnfsf4*$^{-/-}$ showed a fivefold reduction, relative to the *B6.Sle16* mice, in the proportion of CD4+ T_{FH} cells (figure 4F), along with a dramatic reduction of PD-1 expression on CD4+ cells (figure 4G). Furthermore, the percentage of plasma cells and GC B cells (B220+ IgD GL7+) was also significantly lower in the absence of OX40L (figure 4H,I).

We then used the I-A^{bm12} chronic graft-versus-host-disease (cGvHD) mouse model, in which an allogeneic interaction of T and B cells expressing different major histocompatibility complex (MHC) class II (I-A) induces an SLE-like phenotype.[37 38] As shown in figure 5A, *Tnfsf4*$^{-/-}$ and *Tnfsf4*$^{fl/fl}$*(CD19)*$^{-/-}$ mice injected with B6.*H2-Ab1*^{bm12} splenocytes developed a lower titre of IgG anti-dsDNA compared with controls. In addition, both knockout groups showed a lower percentage of effector/memory T and T_{FH} cells (figure 5B,C). A trend towards a lower percentage of plasma

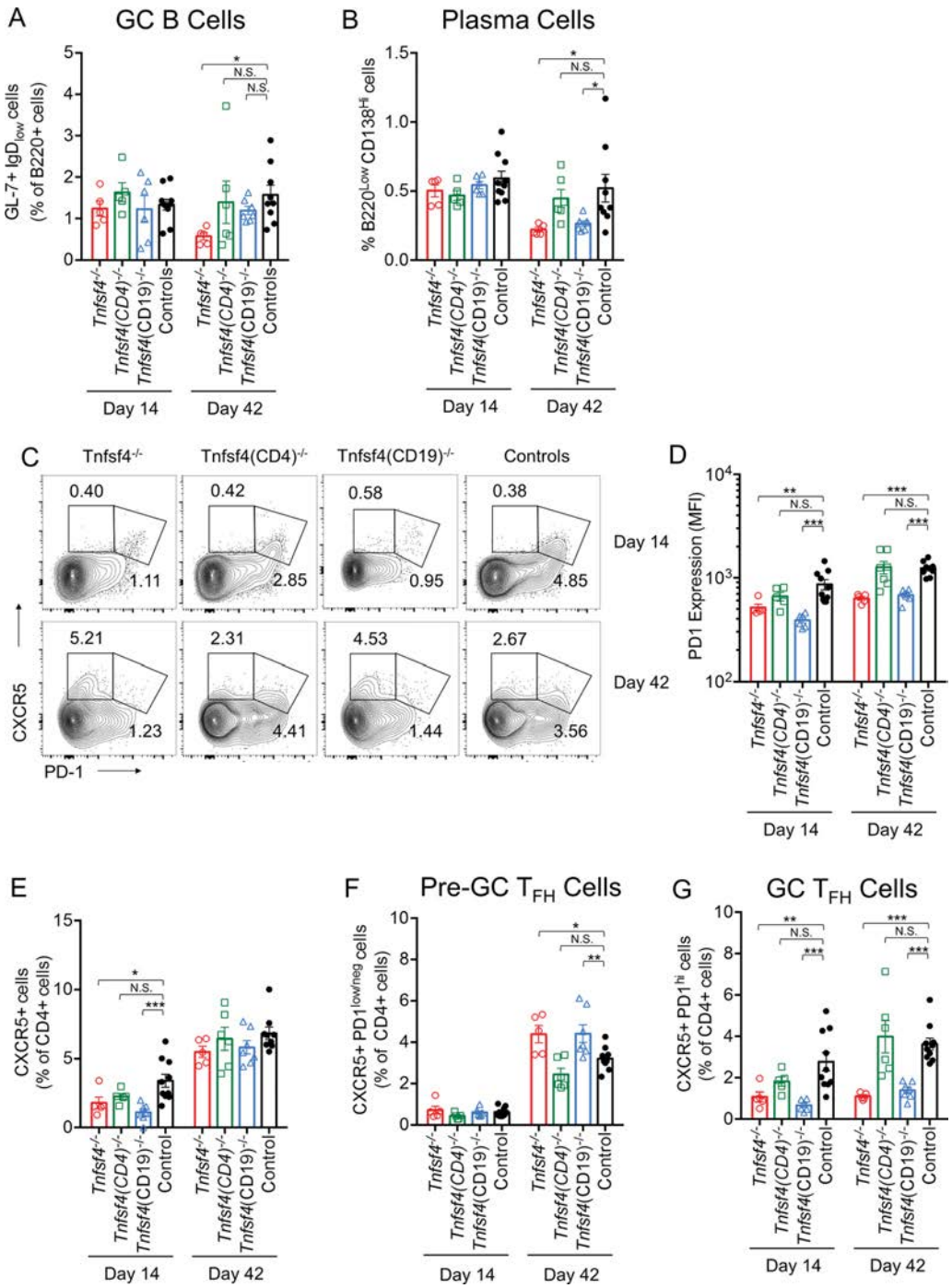

Figure 3 OX40L function in GC reaction. Wild-type controls, *Tnfsf4*$^{-/-}$, *Tnfsf4*(CD19)$^{-/-}$ and *Tnfsf4*(CD4)$^{-/-}$ mice were immunised with NP-CGG in CFA and reimmunised on day 35 with NP-CGG in IFA. Spleens were taken and analysed by FACS either on day 14 or day 42. (A) Frequency of GC B cell (B220+, GL7+, IgD–) presented as frequency among the B220+ population. (B) Percentage of plasma cells (B220^{low}, CD138^{hi}). (C) Gating of T follicular helper (T$_{FH}$) (CD4+, CXCR5+, PD-1^{hi}) and pre-T follicular helper (pre-T$_{FH}$) (CD4+, CXCR5+, PD-1$^{Low/neg}$) cells. (D) PD-1 expression level in CD4+ cells assessed by FACS. (E) Frequency of CXCR5+ cells presented as frequency among the CD4+ population. (F) Quantification of pre-GC T$_{FH}$ and (G) GC-T$_{FH}$ cells as gated in (C) presented as frequency among the CD4+ population. Each symbol represents an individual mouse. Bars indicate the mean±SEM, N.S., not significant; *p<0.05, **p<0.01 and ***p<0.001 (one-way analysis of variance). CFA, complete Freund's adjuvant; FACS, fluorescence-activated cell sorting; GC, germinal centre; IFA, incomplete Freund's adjuvant; NP-CGG, nitrophenylacetyl-chicken gamma globulin.

and GC B cells was observed in both OX40L-deficient groups (figure 5D,E). Of note, no differences were seen between *Tnfsf4*$^{-/-}$ and *Tnfsf4*$^{fl/fl}$*(CD19)*$^{-/-}$ mice, indicating that the observed differences are primarily due to the lack of OX40L on B cells.

DISCUSSION

The *TNFSF4* locus (that encodes OX40L) shows association with several autoimmune diseases; it has one of the most consistent and strongest genetic risk factors in SLE. OX40L has a well-established role in the activation and maintenance of T cell-mediated

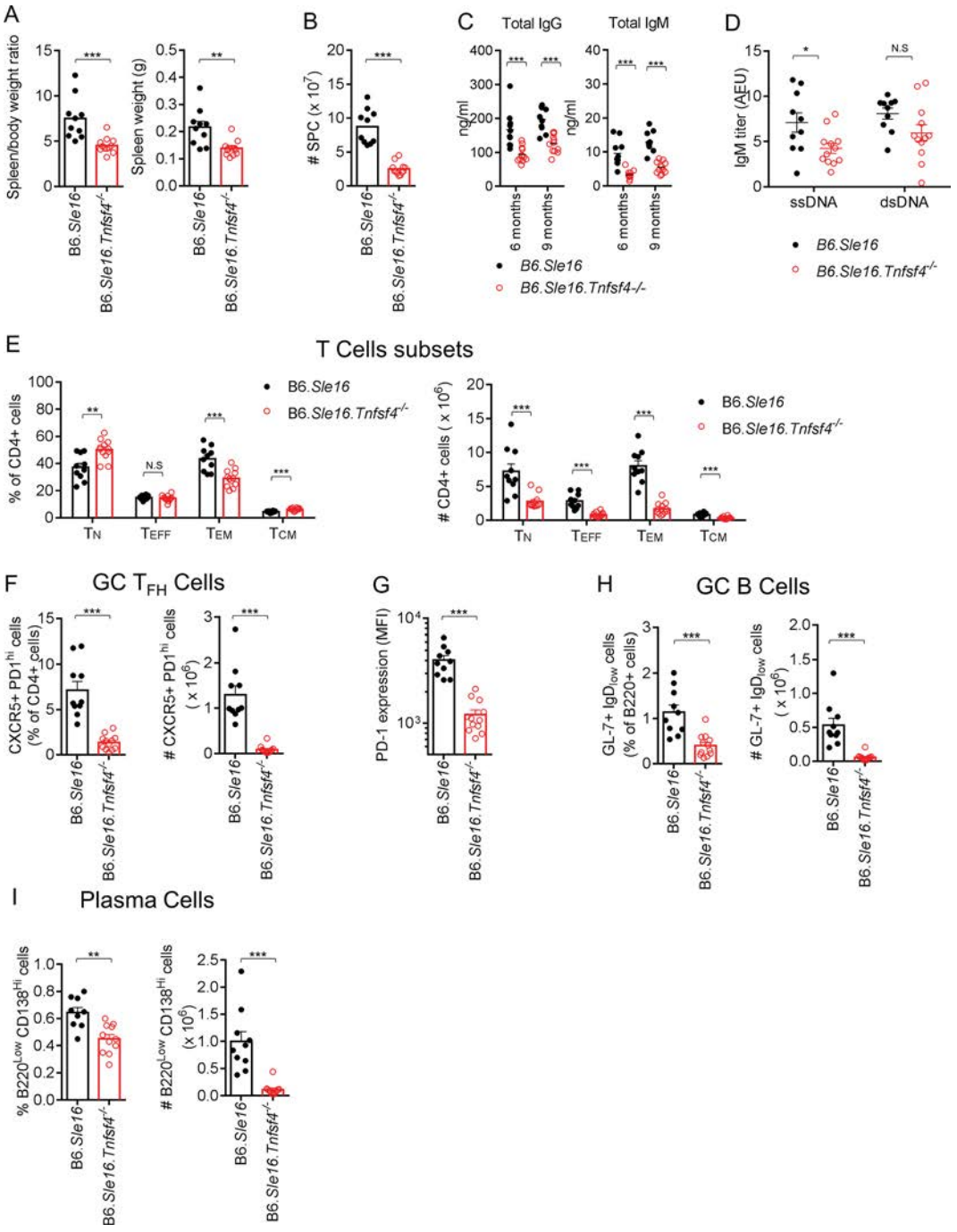

Figure 4 OX40L deficiency ameliorates the phenotype of B6.*Sle16* lupus-prone mice. Comparison between female B6.*Sle16* and *B6.Sle16.Tnfsf4*$^{-/-}$ female mice at 9 months of age. (A) Quantitation of spleen/body weight ratio and spleen weight. (B) Absolute number of cells per spleen. (C) Serum level of IgG and IgM at 6 and 9 months. (D) Titre of IgM anti-dsDNA and anti-ssDNA. (E) Quantitation of naïve (T_N) (CD4+, CD62L+, CD44^{low}), (T_{EFF}) effector (CD4+, CD62L^{low}, CD44^{low}), T_{EM} effector/memory (CD4+, CD62L$^{low/neg}$, CD44^{hi}) and T_{CM} central/memory (CD4+, CD62L+, CD44^{hi}) T cells expressed as a percentage of CD4+ cells and absolute number. (F) GC T_{FH} cells (CD4+, CXCR5+, PD-1^{hi}) presented as frequency among the CD4+ population and absolute number. (G) PD-1 expression level in CD4+ cells assessed by FACS. (H) GC B cell (B220+, GL7+, IgD–) presented as frequency among the B220+ population and absolute number. (I) Percentage and absolute number of plasma cells (B220^{low}, CD138^{hi}). Each symbol represents an individual mouse. Bars indicate the mean±SEM N.S., not significant; *p<0.05, **p<0.01 and ***p<0.001 (t-test). dsDNA, double-stranded deoxyribonucleic acid; FACS, fluorescence-activated cell sorting; GC, germinal centre; ssDNA, single-stranded deoxyribonucleic acid.

immune responses. However, the diversity of cells that express OX40L is such that a pathogenic mechanism relating the genetic findings to disease has not been clearly established. In this study, we generated B and CD4+ T cell OX40L conditional knockout mice, alongside a complete OX40L knockout, to explore and compare the function of OX40L on these cells.

Although a role for OX40L in the T-dependent antibody response has been suggested, conflicting results using different OX40L-deficient mice have been reported.[24 35] These contradictory results may be partly explained by variability in genetic background.[39] Our conditional knockout mice were on a pure C57BL/6 background and, in accord with the one study,[24]

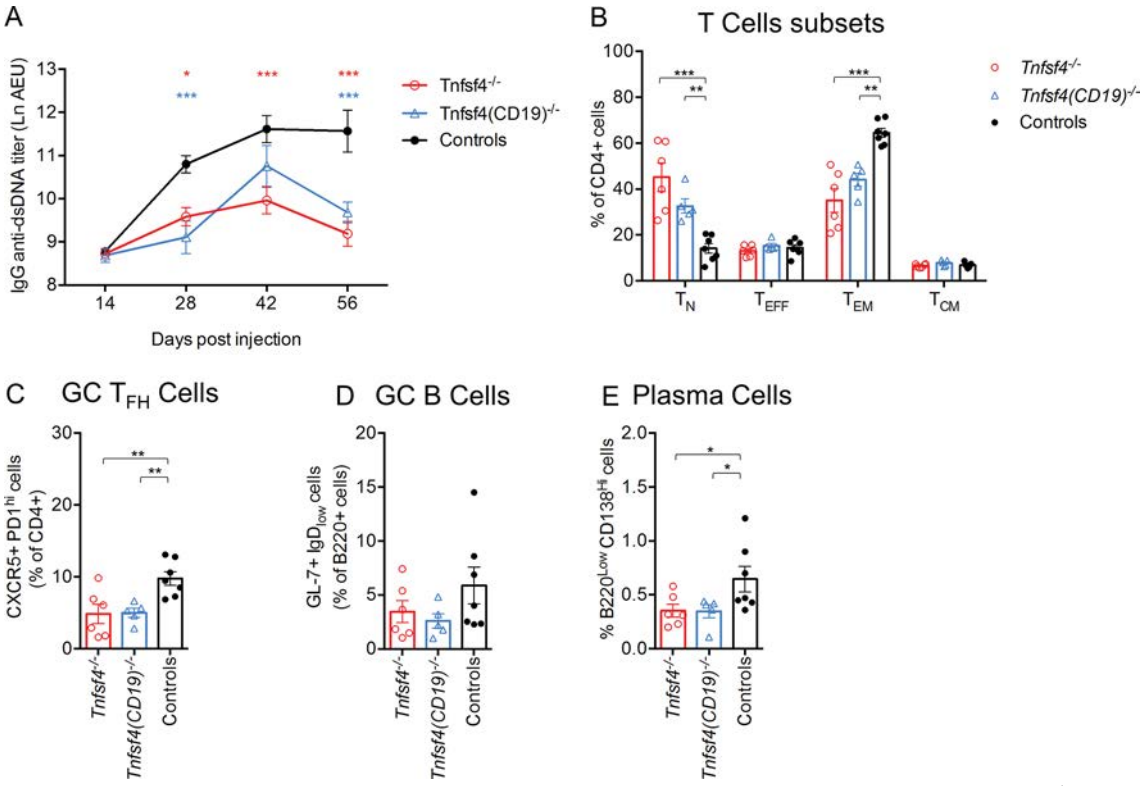

Figure 5 OX40L deficiency diminishes anti-dsDNA antibody production in the cGvHD model. Female wild-type controls, *Tnfsf4*$^{-/-}$ and *Tnfsf4*(CD19)$^{-/-}$ mice were injected intraperitoneally with 5×10^7 splenocytes from B6.*H2*^{bm12} female mice. Sera were collected on days 14, 28, 42 and 56. On day 56, spleens were collected and analysed by FACS. (A) Titre of IgG anti-dsDNA in the sera of injected mice at different time points. (B) Quantification of naïve (T_N) (CD4+, CD62L+, CD44^{low}), (T_{EFF}) effector (CD4+, CD62L^{low}, CD44^{low}), (T_{EM}) effector/memory (CD4+, CD62L$^{low/neg}$, CD44^{hi}) and (T_{CM}) central/memory (CD4+, CD62L+, CD44^{hi}) T cells expressed as percentage of CD4+ cells. (C) Quantification of GC T_{FH} cells (CD4+, CXCR5+, PD-1^{hi}) presented as frequency among the CD4+ population. (D) Frequency of GC B cells (B220+,GL7+, IgD–) presented as frequency among the B220+ population. (E) Percentage of plasma cells (B220^{low}, CD138^{hi}). Each symbol represents data from an individual mouse. Bars indicate the mean±SEM. N.S., not significant; *p<0.05, **p<0.01 and ***p<0.001 (one-way analysis of variance). cGvHD, chronic graft-versus-host-disease; dsDNA, double-stranded deoxyribonucleic acid; FACS, fluorescence-activated cell sorting; GC, germinal centre.

our *Tnfsf4*$^{-/-}$ mice showed a reduced primary and secondary antibody response. However, while the *Tnfsf4*$^{fl/fl}$*(CD19)*$^{-/-}$ mice showed the same phenotype as the *Tnfsf4*$^{-/-}$ mice, the *Tnfsf4*$^{fl/fl}$*(CD4)*$^{-/-}$ mice had a normal secondary response, indicating that only OX40L expression by B cells is essential for the generation of an effective secondary humoral response and by implication B cell memory. We then investigated whether this defective humoral response was due to impaired T cell activation; as expected, *Tnfsf4*$^{-/-}$ mice showed lower percentage of T effector and T effector memory cells (figure 2). The same defect, although at a lower extent, was also shown by both conditional knockouts, despite the normal secondary response in *Tnfsf4*$^{fl/fl}$*(CD4)*$^{-/-}$ mice. These results suggest that B cell OX40L may be involved in biological processes that promote memory responses that are independent of T cell activation.

T cell-dependent B cell immune response involves both an extrafollicular response, which generates short-lived plasma cells and an early wave of low-affinity antibody production, and a GC response, which gives rise to long-lived plasma or memory cells and a later wave of high-affinity antibodies. OX40L has been previously suggested to be essential for the development of high-affinity Ig-producing plasma cells[26]; however, no further evidence has been subsequently reported. In our study, alongside an impaired memory response (figure 1C), there were fewer plasma cells on day 42 in *Tnfsf4*$^{-/-}$ and *Tnfsf4*$^{fl/fl}$*(CD19)*$^{-/-}$ mice (figure 3B), which suggests that B cell OX40L contributes to an effective GC reaction.

We show that *Tnfsf4*$^{-/-}$ and *Tnfsf4*$^{fl/fl}$*(CD19)*$^{-/-}$ mice have a lower percentage of GC T_{FH}, one of the main contributors to the GC reaction. The development of mature GC T_{FH}, which characteristically expresses CXCR5, along with high levels of the surface receptors ICOS, CD40 ligand (CD40L), PD-1 and importantly OX40,[40 41] includes two stages: after activation, a fraction of CD4+ T cells migrate towards B cell follicles by upregulating the chemokine receptor CXCR5, and these T_{FH} precursors then interact with antigen-presenting B cells at the border of the B cell follicle and T cell zone and fully maturate into functional GC T_{FH} cells.[41] In particular OX40L has been shown to be essential for the expression of CXCR5 and the consequent migration of T cells at the T/B border of B cell follicles,[22 42 43] providing the first evidence of the role of OX40L in this process. Our results corroborate this finding; we found that fewer CXCR5+ T cells were generated during the primary response in *Tnfsf4*$^{-/-}$ and *Tnfsf4*$^{fl/fl}$*(CD19)*$^{-/-}$ mice (figure 3E). Whether OX40L-OX40 signal is responsible for the induction, maturation or maintenance of T_{FH} cells and which cell types expressing OX40L are necessary is still unclear; however, a recent work by Tahiliani and colleagues[44] shows a markedly diminished humoral response and production of fewer T_{FH} cells in OX40 KO mice following immunisation with vaccinia virus. In particular the authors show a direct association between OX40+ T_{FH} cells and OX40L-expressing DCs and B cells at the T/B borders and GC providing supportive evidence to how a sustained OX40L-OX40 signal on T_{FH} cells is necessary for the induction of T_{FH} cells and their

maturation to maintain a proper GC reaction. In our study, the reduced numbers of T_{FH} cells in *Tnfsf4*$^{-/-}$ and *Tnfsf4*$^{fl/fl}$*(CD19)*$^{-/-}$ mice were accompanied by an increase in CXCR5+ PD1^{low} cells during the secondary response (figure 3F,G). Since low levels of cell-surface PD1 have been shown to characterise T_{FH} precursor cells,[45] our data suggest a novel role for OX40L on B cells: after activation by DCs, immature T_{FH} cells migrate towards the T/B borders of the B cells follicles, where activated antigen presenting B cells induced their maturation into the GC T_{FH} resident state and their maintenance by sustaining OX40L-OX40 signalling.

TNFSF4 has been reproducibly associated with SLE.[4 5] A recent important study from Jacquemin and colleagues[28] demonstrated that stimulation through OX40 induced T cells to express T_{FH} cells-specific genes such as Bcl6 and CXCR5. They also observed a positive correlation between disease activity, percentage of blood T_{FH} cells and frequency of OX40L+myeloid APC, suggesting OX40L-OX40 axis as a contributor factor in the aberrant T_{FH} response observed in SLE.[46 47] However, the ability to study tissue T_{FH} in humans is limited. In our study, although in a murine model, the generation of T_{FH} cells in the spleen is similarly impeded in the B cell conditional knockout and in the germline *Tnfsf4* knockout, indicating the importance of B cell OX40L. In the human study,[28] there was no correlation between blood B cells expressing OX40L and T_{FH} cells. However, this lack of correlation could be a consequence of the compartmentalisation of activated B cells expressing OX40L in the secondary lymphoid organs rather than an evidence of their lack of involvement in the development of pathogenic T_{FH} cells in SLE.

In our study, to elucidate the role of OX40L in SLE, we used two different SLE mouse models, and in particular the GvHD model was chosen to investigate the role of OX40L on B cells during the B–T cell interaction. In both models of systemic autoimmunity, the lack of OX40L-OX40 signalling was associated with amelioration of the disease phenotype, as shown by a reduced production of anti-dsDNA autoantibodies and Ig kidney deposition together with reduced numbers of GC T_{FH} (figures 4F and 5C) and plasma cells (figures 4I and 5E). These data suggest that OX40L supports the expression of the disease phenotype as well as autoantibody production. This conclusion is further strengthened by the observation that blockade of OX40L reduces degree of proteinuria associated with glomerulonephritis in an accelerated murine model.[48]

The results presented in this paper support a mechanism by which genetically determined elevated expression of OX40L predisposes to SLE via increased B cell expression, which in turn supports T_{FH} development. In light of the argument that genetic factors augment the likelihood of success with a drug target,[49] our data strongly support exploration of this therapeutic strategy. It is potentially important for optimal treatment to know which OX40L-expressing cell types should be targeted, and the defined risk alleles at *TNFSF4* further raise the possibility that genetic screening may identify individuals most likely to benefit from OX40L inhibition.

Correction notice This article has been corrected since it published Online First. The fourth author's name has been corrected to Deborah S Cunninghame Graham.

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Contributors AC designed, performed and analysed experiments and wrote the manuscript. UE performed experiments, helped with the statistical analysis and discussed the data. THM performed experiments. DSCG discussed the data and edited the manuscript. MB and TJV designed experiments, discussed the data and wrote the manuscript.

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Competing interests None declared.

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Data sharing statement There are no additional unpublished data. The mouse model described in this study is available to other researchers on request and has already been shared with other investigators.

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Erratum: *Dietary intake of fibre and risk of knee osteoarthritis in two US prospective cohorts*

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EXTENDED REPORT

Identification of a transitional fibroblast function in very early rheumatoid arthritis

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ABSTRACT

Objectives Synovial fibroblasts actively regulate the inflammatory infiltrate by communicating with neighbouring endothelial cells (EC). Surprisingly, little is known about how the development of rheumatoid arthritis (RA) alters these immunomodulatory properties. We examined the effects of phase of RA and disease outcome (resolving vs persistence) on fibroblast crosstalk with EC and regulation of lymphocyte recruitment.

Methods Fibroblasts were isolated from patients without synovitis, with resolving arthritis, very early RA (VeRA; symptom ≤12 weeks) and established RA undergoing joint replacement (JRep) surgery. Endothelial-fibroblast cocultures were formed on opposite sides of porous filters. Lymphocyte adhesion from flow, secretion of soluble mediators and interleukin 6 (IL-6) signalling were assessed.

Results Fibroblasts from non-inflamed and resolving arthritis were immunosuppressive, inhibiting lymphocyte recruitment to cytokine-treated endothelium. This effect was lost very early in the development of RA, such that fibroblasts no longer suppressed recruitment. Changes in IL-6 and transforming growth factor beta 1 (TGF-β_1) signalling appeared critical for the loss of the immunosuppressive phenotype. In the absence of exogenous cytokines, JRep, but not VeRA, fibroblasts activated endothelium to support lymphocyte.

Conclusions In RA, fibroblasts undergo two distinct changes in function: first a loss of immunosuppressive responses early in disease development, followed by the later acquisition of a stimulatory phenotype. Fibroblasts exhibit a transitional functional phenotype during the first 3 months of symptoms that contributes to the accumulation of persistent infiltrates. Finally, the role of IL-6 and TGF-β_1 changes from immunosuppressive in resolving arthritis to stimulatory very early in the development of RA. Early interventions targeting 'pathogenic' fibroblasts may be required in order to restore protective regulatory processes.

INTRODUCTION

Fibroblasts are a type of mesenchymal stromal cell with immunomodulatory capabilities.[1] They display distinct spatial identities[2 3] that govern their behaviour and allow them to establish tissue-specific 'address-codes'.[4] It is these address codes that actively regulate the recruitment of leucocytes to inflamed sites and their subsequent behaviour.[1] Fibroblasts achieve these effects in part by conversing with neighbouring vascular endothelial cells (EC) to regulate leucocyte adhesion.[1] We have previously reported that dermal fibroblasts potently downregulate the responsiveness of EC to cytokines, suppressing lymphocyte recruitment in an interleukin 6 (IL-6) and transforming growth factor beta 1 (TGF-β_1) dependent manner.[5] Consequently, each inflammatory response is contextual, defined by the phenotype of the local fibroblast population.

In rheumatoid arthritis (RA), the stable reprogramming of synovial fibroblasts disrupts their protective regulatory processes, promoting their survival and enhancing their production of proinflammatory agents and proteases for example.[6] Additionally, rheumatoid synovial fibroblasts invade human cartilage in an severe combined immunodeficiency (SCID) model of arthritis[7 8] and appear to display tropism for damaged tissue, migrating to distant cell-free cartilage in vivo, potentially 'spreading' disease.[9] This pathogenic phenotype causes RA fibroblasts to bypass many of the regulatory checkpoints that coordinate the successful resolution of an inflammatory episode. Indeed, we have shown that rheumatoid synovial fibroblasts activate endothelium to inappropriately recruit leucocytes,[5 10] while simultaneously blocking leucocyte apoptosis.[11] Thus, rheumatoid synovial fibroblasts are capable of generating and supporting persistent leucocyte infiltrates.

Fibroblasts are endogenous regulators of inflammation, and in our hands demonstrate a spectrum of responses, ranging from suppression of cytokine-induced responses to stimulation of a persistent leucocyte influx.[5] This suggests that at some stage during the development and progression of RA,[12] immunomodulatory capability is lost, and a proinflammatory phenotype is acquired in synovial fibroblasts. However, it remains unclear when these events occur. Here, we show for the first time that fibroblast–EC interactions evolve with disease progression and that fibroblasts at the earliest phase of RA exhibit a transitional functional phenotype that contributes to the accumulation of persistent infiltrates.

MATERIALS AND METHODS

Isolation of human fibroblasts, ECs and lymphocytes

Synovial tissue samples were obtained by ultrasound-guided biopsy[13] from treatment-naive patients with a new onset of clinically apparent arthritis and a symptom duration of ≤12 weeks, who at follow-up had either a resolving arthritis (Res) or fulfilled RA classification criteria (very early RA; VeRA).[14] Patients

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were classified as having resolving arthritis if there was no clinical evidence of synovial swelling at any peripheral joint (out of a swollen joint count of 66 joints) on final examination at least 1 year after initial presentation, in the absence of disease-modifying antirheumatic drugs (DMARD) or glucocorticoid therapy for at least the previous 3 months.[15] In addition, synovial tissue samples were collected from subjects (A) with established, treated RA undergoing joint replacement (JRep) surgery; or (B) undergoing exploratory arthroscopy for unexplained joint pain with no macro or microscopic evidence of inflammation (non-inflamed—NI). RA was classified according to 2010 American College of Rheumatology criteria.[16] Prior to biopsy, the extent of greyscale synovitis and power Doppler enhancement within the synovium of the biopsied joint was systematically graded using a 0–3 scale.[14] Fibroblasts were isolated as previously described[17] and used between passages 4 and 6[5].

Human umbilical vein EC were isolated from umbilical cords using collagenase as previously described.[5] Peripheral blood lymphocytes from healthy individuals were isolated by centrifugation on Histopaque 1077 (Sigma-Aldrich, Poole, UK) followed by panning on plastic.[5] Lymphocytes were washed, counted and adjusted to a final concentration of 2×10^6/mL in M199 supplemented with 0.15% bovine serum albumin (BSA; Sigma) and 35 µg/mL gentamycin (M199BSA).

All human samples were obtained with written, informed consent and approval from the Human Biomaterial Resource Centre (Birmingham, UK), West Midlands and Black Country Research Ethics Committee, North East Tyne and West South Research Ethics Committee, or University of Birmingham Local Ethical Review Committee in compliance with the Declaration of Helsinki.

Lymphocyte recruitment to cocultures from flow

Endothelial-fibroblast cocultures were established on opposite sides of 0.4 µm pore Transwell filter inserts (BD Pharmingen, Cowley, UK) for 48 hours prior to treatment with or without 100 U/mL tumour necrosis factor alpha (TNFα; R&D Systems, Abingdon, UK) and 10 ng/mL interferon gamma (IFNγ; Peprotech, London, UK) for a further 24 hours as previously described.[5] In some experiments, neutralising antibodies against IL-6 (clone 6708) or TGF-β_1 (clone 9016; both 10 µg/mL; R&D Systems) were added alone or in combination when cocultures were established.[5 18 19] Neutralising antibodies were present throughout the coculture and cytokine stimulation. A flow-based adhesion assay[5] (see online supplementary methods) was used to analyse lymphocyte recruitment from flow.

Gene expression analysis

Isolated EC mRNA[5] (RIN≥7.80) was analysed by qPCR using Taqman Universal PCR Master Mix[20] and Assay on Demand primer kits according to manufacturer's instructions (Applied Biosystems, Warrington, UK). Samples were analysed using 7900HT Real-Time PCR machine and SDS 2.4 (Applied Biosystems), and expressed as $2^{-\Delta CT}$ relative to 18S.

Flow cytometry

Expression of intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) on cytokine-stimulated EC mono and cocultures were analysed by flow cytometry (see online supplementary methods). Data are expressed as median fluorescent intensity.

Quantification of soluble mediators

Soluble agents in culture supernatants were quantified using IL-6 DuoSet ELISA, sIL-6R Quantikine ELISA Kit or VersaMAP Luminex according to manufacturer's instructions (R&D Systems).

Statistical analysis

Multivariant data were analysed using analysis of variance with Dunnett post-test or Kruskal-Wallis test with Dunn post-test.

Table 1 Demographic and baseline p characteristics

	NI (n=11)	Resolving (n=14)	VeRA (n=11)	JRep (n=13)
Age (years)†	42 (34–47)	40 (32–66)	49 (48–60)	59 (39–62)
Female, n (%)	5 (45)	4 (29)	5 (45)	9 (69)
Symptom duration (weeks)†	‡	6 (4–7)	6 (4–9)	1040 (780–1098)**, *****
DAS28 ESR at baseline§	‡	3.8±1.3	4.7±1.5	5.4±1.2*
ESR (mm/hour)†	‡	9.5 (5–27)	25 (10–58)	37 (19–59)*
CRP (mg/L)†	‡	8.5 (0–14)	26 (0–45)	32 (15–56)*
RF positive (%)	‡	0 (0)	5 (45)	11 (85)**
ACPA positive (%)	‡	0 (0)***	7 (64)	–
SJC28†	‡	3 (2–6)	4 (3–9)	9 (4–14)
TJC28†	‡	3 (1–6)	6 (3–13)	7 (2–12)
VAS†	‡	41 (28–79)	46 (16–70)	64 (42–86)
US GS†	‡	2 (1–2)	2 (2–3)****	‡
US PD†	‡	1 (0–1)	2 (0–2)	‡
NSAID (%)	‡	9 (64)	7 (64)	8 (62)

Kruskal-Wallis test showed a significant effect of outcome group on DAS28 baseline, ESR, CRP ($p<0.05$), symptom duration and RF positive ($p<0.001$).
*$p<0.05$ and **$p<0.01$ compared with the resolving cohort by Dunn's post-test; ***$p<0.01$ compared with the VeRA by Wilcoxon signed-rank test; ****$p<0.01$ compared with the resolving by Mann-Whitney U test; *****$p<0.01$ compared with the VeRA cohort by Dunn's post-test.
†Median (IQR).
‡Data not obtained from patients at time of presentation.
§Mean±SD.
ACPA, anticitrullinated protein antibody; CRP, C-reactive protein; DAS28, Disease Activity Score 28; ESR, erythrocyte sedimentation rate; JRep, joint replacement; NI, non-inflamed; NSAID, non-steroidal anti-inflammatory drugs; RF, rheumatoid factor; SJC28, 28 swollen joint counts; TJC28, 28 tender joint counts; US GS, ultrasound greyscale grade at the biopsied joint; US PD, ultrasound power Doppler grade at the biopsied joint; VAS, visual analogue scale; VeRA, very early RA.

Alternatively, Mann-Whitney U test, Wilcoxon signed-rank test or unpaired t-test was performed. $p<0.05$ was considered as statistically significant.

RESULTS

Demographic and baseline clinical characteristics of patients

The characteristics of the patients are shown in table 1. There was no significant difference in age, gender, 28 swollen joint counts, 28 tender joint counts, patient global visual analogue scale score, non-steroidal anti-inflammatory drug usage and ultrasound power Doppler score at the biopsied joint[14] between clinical outcome groups. As expected, patients with RA undergoing joint replacement surgery had experienced symptoms for significantly longer than those with resolving synovitis and very early RA. However, there was no difference in symptom duration between patients with resolving synovitis or very early RA. Patients with resolving disease had significantly lower DAS28 (Disease Activity Score 28) erythrocyte sedimentation rate (ESR)

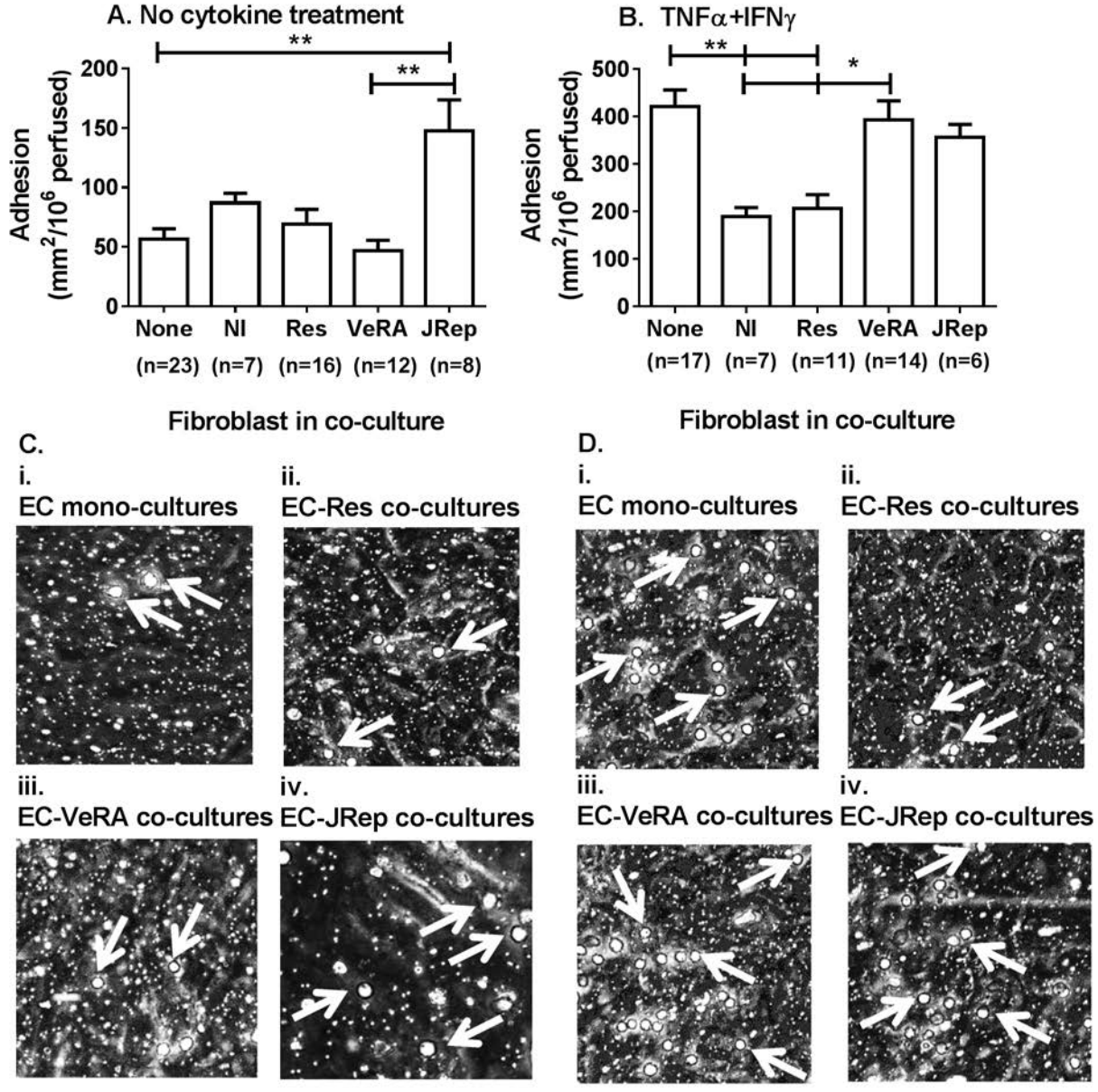

Figure 1 Fibroblasts from patients with resolving and persistent arthritis differentially modulate lymphocyte recruitment from flow. Cocultures were established by culturing endothelial cells and fibroblasts on opposite sides of a porous insert, prior to treatment (A) without or (B) with TNFα+IFNγ for 24 hours. Endothelial monolayers without fibroblasts (none) were used as controls. Lymphocytes were perfused and their interactions with endothelial cells were assessed by digital microscopy. (C, D) Micrograph images showing lymphocyte adhesion to (i) endothelial cells cultured alone, with fibroblasts from (ii) resolving, (iii) VeRA or (iv) JRep patients (C) in the absence of cytokine treatment and (D) in response to TNFα+IFNγ treatment. White arrow indicates an adherent lymphocyte. In A and B, Kruskal-Wallis test shows a significant effect of fibroblasts on lymphocyte adhesion ($p<0.01$). Data are the mean±SEM for n experiments; each incorporated a different donor for all three cell types. *$p<0.05$ and **$p<0.01$ by Dunn post-test. EC, endothelial cells; IFNγ, interferon gamma; JRep, joint replacement; NI, non-inflamed; Res, resolving; TNFα, tumour necrosis factor alpha; VeRA, very early RA.

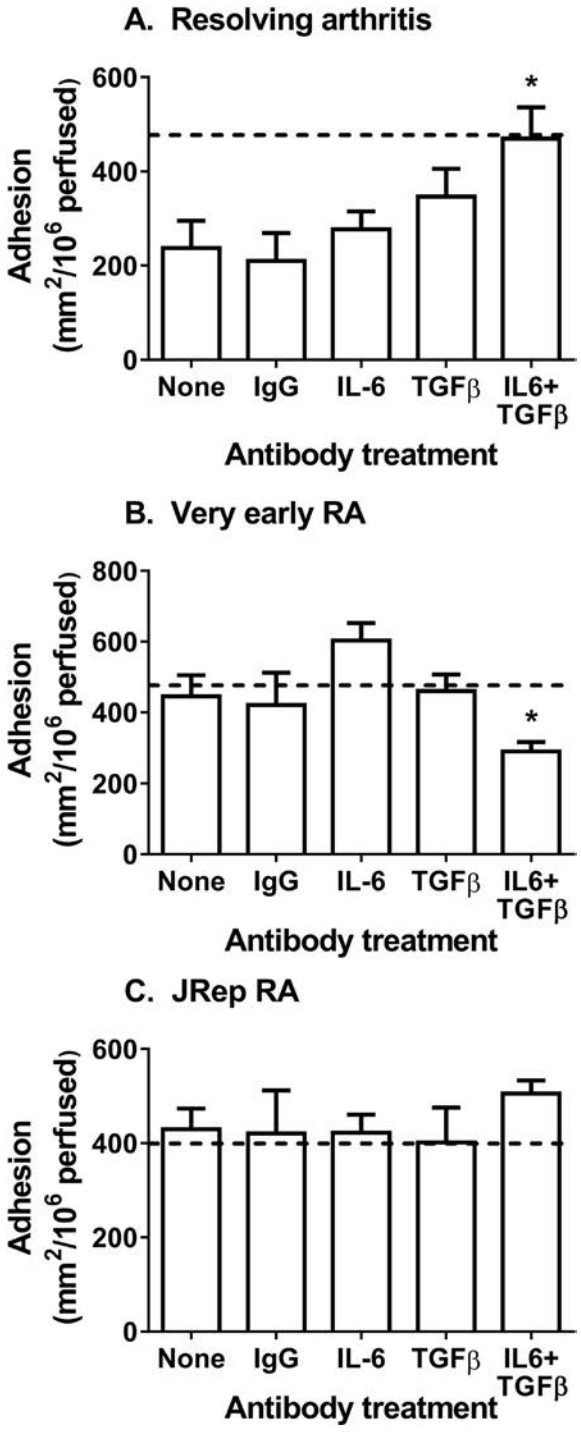

Figure 2 Resolving fibroblasts mediated immunosuppressive effect through IL-6 and TGF-β_1. Actions of IL-6 or TGF-β_1 were neutralised, alone or in combination, in TNFα+IFNγ-treated cocultures incorporating fibroblasts from patients with (A) resolving synovitis, (B) very early RA or (C) joint replacement RA (JRep). Dotted line (-----) represents adhesion to TNFα+IFNγ-treated endothelial monocultures for paired experiments. IgG represents cocultures incubated with isotype control antibodies. In A and B, ANOVA shows a significant effect of antibody treatment on lymphocyte adhesion ($p<0.01$). Data are the mean±SEM from three to five independent experiments each incorporating a different donor for all cell types. *$p<0.05$ compared with None (untreated cocultures) by Dunnett post-test. ANOVA, analysis of variance; IFNγ, interferon gamma; IL-6, interleukin 6; JRep, joint replacement; RA, rheumatoid arthritis; TGF-β, transforming growth factor beta; TNFα, tumour necrosis factor alpha.

at baseline, ESR and C-reactive protein when compared with patients undergoing joint replacement, but not those with very early RA. Patients with very early RA had a significantly higher ultrasound greyscale grade at the biopsied joint when compared with patients with resolving synovitis. Patients with resolving arthritis were diagnosed as having unclassified arthritis (n=6), parvovirus (n=3), reactive arthritis (n=2), pseudogout (n=1) and RA (n=2) according to established criteria. Of note, the two patients diagnosed with resolving RA had no evidence of joint-related soft tissue swelling on final examination. In both patients, synovitis resolved rapidly after briefly fulfilling criteria at presentation and no DMARDs were used in their treatment. All individuals with resolving arthritis were negative for rheumatoid factor and anticitrullinated protein antibody.

Fibroblasts from VeRA lose an immunosuppressive phenotype before becoming proinflammatory

We have previously reported that fibroblasts from joints of patients with advanced RA directly induce leucocyte recruitment in the absence of exogenous cytokines.[5 10] In this model, fibroblasts from patients with RA undergoing joint replacement, but not very early RA, significantly increased lymphocyte adhesion when compared with untreated EC monocultures (figure 1A). Moreover, in the absence of exogenous cytokines, similar levels of binding were observed when fibroblasts from non-inflamed, resolving or very early RA tissue were incorporated into coculture (figure 1A).

Using a model of inflammation where cultures were stimulated with inflammatory cytokines, we examined the ability of synovial fibroblasts from different outcome groups to influence the cytokine-induced endothelial recruitment of lymphocytes. Fibroblasts from non-inflamed joints and resolving synovitis were immunosuppressive, inhibiting lymphocyte recruitment to TNFα+IFNγ-treated endothelium (figure 1B). By contrast, this effect was not observed when fibroblasts from patients with RA (either very early or longer duration disease) were incorporated into coculture. These fibroblasts no longer suppressed recruitment but rather supported lymphocyte adhesion at similar levels to those observed on cytokine-treated EC monocultures (figure 1B).

Collectively, these data indicate that fibroblasts from patients with very early RA are functionally distinct from both resolving synovitis and long-established disease, existing in a transitional state.

Unless otherwise stated, all future experiments were performed using cytokine-treated cocultures incorporating resolving or very early RA fibroblasts.

Role of IL-6 and TGF-β_1 in effects of resolving and very early RA fibroblasts in coculture

The immunosuppressive response of mesenchymal stromal cells from healthy tissues is facilitated by common bioactive mediators, IL-6 and TGF-β_1.[5 18 19] It is possible that such endogenous pathways are corrupted early in the pathogenesis of RA. Neutralisation of both IL-6 and TGF-β_1 significantly blocked the inhibitory effects of resolving fibroblasts in coculture (figure 2A). In contrast, neutralisation of IL-6 and TGF-β_1 in very early RA cocultures significantly reduced lymphocyte adhesion (figure 2B), restoring immunoprotective functions to those of resolving cocultures. Interestingly, blockade of IL-6 and TGF-β_1 had no effect on lymphocyte recruitment to cocultures incorporating RA fibroblasts from joint replacement patients (figure 2C). In all conditions, single antibody blockade or the presence of isotype

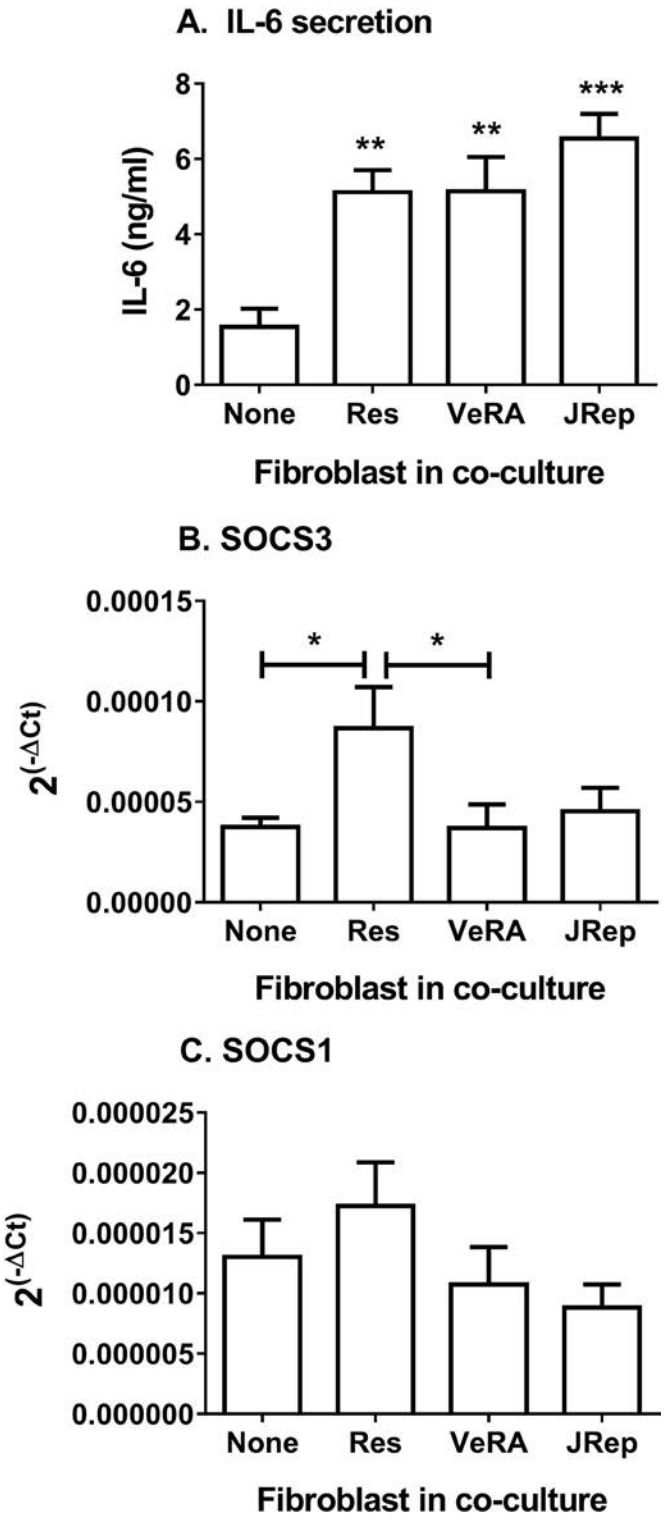

Figure 3 Secretion and signalling of IL-6 in cocultures. (A) IL-6 release during TNFα+IFNγ-treated cocultures. ANOVA shows a significant effect of culture conditions on the secretion of IL-6 ($p<0.001$). (B) SOCS3 and (C) SOCS1 gene expression analysed by qPCR. Data are expressed as $2^{-\Delta CT}$ relative to 18S expression. Data are the mean±SEM from three to five independent experiments each incorporating a different donor for all cell types. *$p<0.05$, **$p<0.01$ and ***$p<0.001$ compared with None (endothelial monoculture) by Dunnett post-test, unless otherwise indicated. ANOVA, analysis of variance; IFNγ, interferon gamma; IL-6, interleukin 6; JRep, joint replacement; Res, resolving; SOCS, suppressor of cytokine signalling; TNFα, tumour necrosis factor alpha; VeRA, very early RA.

antibodies had no effect on adhesion (figure 2A–C). Thus, IL-6 and TGF-β_1 had essentially opposite effects on cytokine-treated cocultures with either resolving or very early RA fibroblasts.

Exploring this further, we detected significantly more IL-6 in supernatants from cocultures compared with endothelial monocultures following cytokine treatment (figure 3A). However, there was no difference between the clinical outcome groups (figure 3A). Of note, resting fibroblasts from different disease stages release comparable levels of IL-6 in culture (online supplementary figure 1A). Soluble IL-6R transcripts in EC were also similar between all culture conditions tested (online supplementary figure 1B); however, we were unable to detect measurable levels of sIL-6R released by these cultures. Suppressor of cytokine signalling 3 (SOCS3) and SOCS1 regulate signal transducer and activator of transcription (STAT) activation in response to IL-6.[21] Here, expression of SOCS3 was upregulated in EC from resolving cocultures, but not in EC from very early RA cocultures (figure 3B). In contrast, SOCS1 expression in EC remained unchanged upon coculture (figure 3C). Downstream signalling from IL-6 differed between the two forms of cytokine-activated cocultures.

Profile of secretome released by resolving and VeRA fibroblasts in coculture

We also wondered whether very early RA fibroblasts altered the secretome generated during coculture, such that it was no longer immunosuppressive. Using multiplex analysis, we detected significantly higher levels of the chemokines CXCL10 and IL-8, and a tendency for higher CXCL5 in the very early RA coculture supernatants when compared with the resolving cocultures (figure 4A–C). However, expression of the chemokines CXCL1, CCL5 and CCL2 was comparable between both coculture conditions (figure 4D–F), while IL-4, IL-10 and IL-1α were undetectable. We observed no significant difference in the concentration of these chemokines released by resolving and very early RA monocultures, although overall levels were lower than that found in cocultures (online supplementary figure 2). Therefore, the composition, and potentially the bioactivity, of the secretome appeared to differ between resolving and very early RA cocultures.

Analysis of gene expression in EC upon coculture

To further investigate the loss of suppression of lymphocyte adhesion, we analysed the expression of adhesion molecules and chemokines by inflamed EC upon coculture. Comparing EC from resolving and very early RA cocultures, we detected no difference in the expression of ICAM-1 or VCAM-1 (online supplementary figure 3), or CXCR3 ligand transcripts (data not shown). While coculture significantly reduced expression of E-selectin mRNA compared with cytokine-treated EC alone (data not shown), this effect was similar for each disease outcome. Thus, changes in the expression of the genes analysed showed no clear differences between disease outcomes or correlation with the functional differences in recruitment observed with resolving and very early RA cocultures.

DISCUSSION

Little is known about how the development of RA alters the immunomodulatory properties of synovial fibroblasts. We examined for the first time the effects of phase of disease and disease outcome on synovial fibroblast regulation of the inflammatory infiltrate through crosstalk with EC. Synovial fibroblasts show outcome-specific and stage-specific effects. Upon coculture with

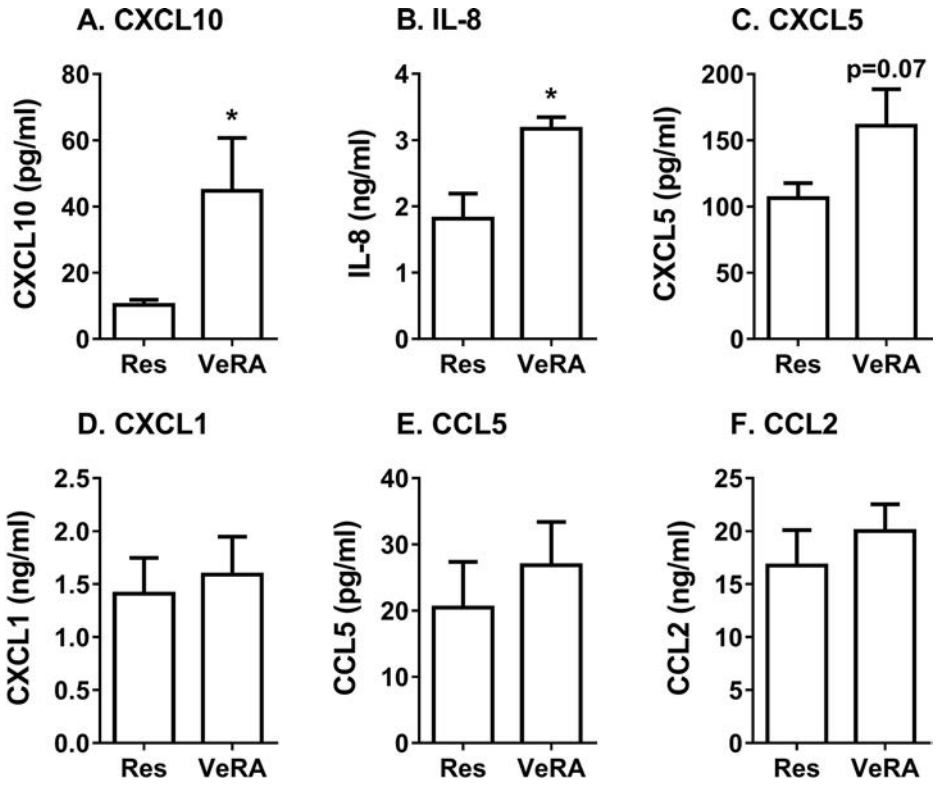

Figure 4 Secretome from resolving and very early RA cocultures. Conditioned media from resolving or very early RA fibroblast cocultures were measured by multiplex analysis. (A) CXCL10, (B) IL-8, (C) CXCL5, (D) CXCL1, (E) CCL5 and (F) CCL2 expression. Data are the mean±SEM from five to nine independent experiments each incorporating a different donor for all cell types. *p<0.05 by unpaired t-test. IL-8, interleukin 8, also known as CXCL8; Res, resolving; VeRA, very early RA.

EC, fibroblasts from resolving synovitis suppressed lymphocyte adhesion in response to cytokines. This immunoprotective effect was lost in fibroblasts from very early RA, allowing increased lymphocyte recruitment. Hence, fibroblasts cultured from tissues with divergent disease outcomes (resolving vs persistence) are functionally distinct. Moreover, fibroblast–EC interactions evolve with RA progression. In contrast to established disease, fibroblasts from very early RA have not yet acquired the ability to autonomously activate EC in the absence of exogenous cytokines. Thus, we have shown for the first time that synovial fibroblasts undergo two distinct functional changes as RA evolves: first the early loss of immunosuppressive capability, and second the slower acquisition of an intrinsically stimulatory phenotype during disease progression.

IL-6 and TGF-β are pleiotropic cytokines, each able to induce divergent proinflammatory or anti-inflammatory effects depending on the inflammatory context or cell type (reviewed by ref 1). Moreover, emerging evidence reveals complex and intricate crosstalk between IL-6 and TGF-β_1 signalling pathways, in which each cytokine can positively[22–24] or negatively[25–27] regulate the expression or activity of the other depending on the inflammatory context. Using T cell biology as an example, TGF-β_1 inhibited the production of the IL-6 inhibitor SOCS3, thus prolonging IL-6 signalling to initiate Th17 differentiation.[28] Conversely, IL-6 augmented expression of the TGF-β signalling inhibitor SMAD7, preventing TGF-β_1-induced T_{Reg} differentiation.[26] In the context of leucocyte recruitment, treatment with recombinant IL-6 or TGF-β_1 or both suppressed neutrophil infiltration into lipopolysaccharide (LPS)-inflamed lungs.[29] Such apparently divergent, contextually determined roles are seen in our study. Here, IL-6 and TGF-β_1 were identified as the bioactive agents required for the inhibitory effects on recruitment of cocultured resolving fibroblasts. Similar findings have been reported for stromal cells from non-inflamed tissues,[5 18] suggesting the existence of shared stromal immunoprotective mechanisms. In contrast, neutralisation of IL-6 and TGF-β_1 inhibited the prorecruitment effect of cocultured very early RA fibroblasts. This suggests that in VeRA, IL-6 and TGF-β_1 have not simply lost efficacy, but trigger stimulatory rather than inhibitory downstream events.

Synovial fibroblasts are a major source of IL-6 in RA,[30] which we also observed in our EC-fibroblast cocultures. IL-6 can signal through its membrane-bound (CD126; IL-6R) or soluble receptor (sIL-6R) (reviewed by ref 31). Indeed, synovial fibroblasts induce STAT3 phosphorylation and activation in response to sIL-6R engagement.[32–34] The absence of detectable sIL-6R in our supernatants (both measured here and previously[10]) strongly indicates that IL-6 released during coculture signals through CD126 expressed by EC,[5] but not fibroblasts. Given that fibroblasts cannot respond to IL-6 generated during coculture, distinct fibroblast–EC interactions must regulate EC responses to IL-6 and produce the discrete patterns of lymphocyte recruitment that we observed. Here, we observed two different patterns of expression of the negative regulator, SOCS3, in EC from resolving and very early RA cocultures. We hypothesise that high SOCS3 expression (ie, negative regulation of STAT activation), as seen in the EC from resolving cocultures, triggers an immunoprotective IL-6 response. Conversely, failure to induce SOCS3 was associated with loss of immunosuppressive responses in the EC from very early RA cocultures. Such a situation has been observed in adjuvant-induced arthritis, where low endothelial SOCS3 levels, and therefore negative regulation of IL-6 signalling, has been linked with more severe arthritis and elevated neutrophil influx into the joint.[35] Collectively, these data reveal

two distinct IL-6 signalling pathways in EC from cocultures, which are induced in a disease outcome-specific manner and elicit different functional consequences in EC.

Our data clearly show that IL-6 acts synergistically with TGF-β_1 to mediate the differential effects on lymphocyte adhesion to inflamed EC in coculture. TGF-β_1 is secreted in its bioactive form by a variety of cell types, including EC and fibroblasts. However, the ELISA kits available during this study only measured total TGF-β_1 after acid activation, rather than bioactive TGF-β_1. Therefore, it is not possible to distinguish which cell type was secreting bioactive TGF-β_1. EC in all conditions expressed similar transcript levels of the three TGF-β receptors (data not shown), indicating that EC were potentially capable of responding to TGF-β_1 produced during coculture. The requirement to understand the inflammatory context of TGF-β_1 production is once again emphasised by conflicting findings on the impact (suppressive[36] vs stimulatory[37]) of TGF-β_1 on in vivo models of arthritis, where exogenous TGF-β_1 treatment either exacerbated,[38 39] alleviated[40] or had no effect[41] on disease severity.

Biological therapies that target IL-6 and its receptor (eg, tocilizumab) are efficacious in RA including for those individuals who do not respond to anti-TNFα treatment.[42 43] Although we detected similar concentrations of IL-6 in cocultures, we did observe a different profile of soluble mediators released by resolving and very early RA fibroblast cocultures. This raises the intriguing possibility that the bioactivity of the secretome is different between the two cocultures, where soluble agents exclusive to the very early RA cocultures alter IL-6 and TGF-β_1 responses to generate a stimulatory effect. Moreover, difference in the secretomes by resolving and very early RA cocultures could influence the presentation of chemokines by the endothelium and therefore might account for the altered lymphocyte adhesion profiles observed here. For example, fibroblast-induced production of proteases during coculture can adversely affect lymphocyte binding.[44] Further work is required to identify the exact soluble mediator(s) responsible for these changes, as they are likely to offer novel targets for early therapeutic intervention in RA.

Failure to suppress recruitment may represent a manifestation of the transition the stroma undergoes as the disease progresses. It is unlikely that such changes in very early RA are due to the fibroblasts passively responding to local inflammatory responses. Instead, fibroblasts with a transitional functional phenotype will actively contribute to disease development and persistence, further fuelling the evolution of their phenotype towards so-called 'imprinted aggressors' (eg, ref 45). Emerging evidence strongly indicates such reprogramming is due to accumulated epigenetic modifications,[46 47] which may directly alter the production of proinflammatory mediators or modify the balance of microRNAs (eg, mir155, 146, 203) within the fibroblast.[(e.g, ref 48)] This could explain how the regulation of IL-6 mRNA stability becomes altered in rheumatoid synovial fibroblasts, where the negative regulator Zc3h12a (RNA-binding protein) switches its activity to positively stabilise IL-6 mRNA.[49] Epigenetic modifications in the earliest phases of disease could also account for the differential effects seen in this study by resolving and VeRA fibroblasts in coculture.

Our study is not the first to indicate that Vvery early RA is subtly different from resolving arthritis or established RA. Patients with very early disease have a distinct serum metabolomic profile and synovial fluid cytokine profile when compared with patients with established RA.[15 50] Moreover, fibroblasts from very early RA showed increased dickkopf-related protein 1 (DKK-1) expression with the potential to adversely alter bone remodelling; a feature not apparent in fibroblasts from patients with resolving synovitis.[51] Collectively, these data strongly support the transitional nature of synovial pathology during the earliest stages of disease development. Early interventions targeting 'pathogenic' fibroblasts may therefore be required in order to restore protective regulatory processes.

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Contributors HMM contributed to the conception, design, organisation, conduct, and acquired funding for the study. HMM, LSCW, SK, HM and RR carried out the experimental investigations. HMM and CSD have analysed and interpreted data. CDB, KR and AF recruited and diagnosed patients and acquired the clinical data. HMM, CDB, GBN and AF conceived the study, interpreted data and drafted the manuscript. All authors contributed to the analysis and discussion of the data, along with editing of the manuscript.

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Competing interests HMM has received research funding from Pfizer. All other authors declare that they have no conflicts of interest.

Ethics approval All human samples were obtained with written, informed consent and approval from the Human Biomaterial Resource Centre (Birmingham, UK), West Midlands and Black Country Research Ethics Committee, North East Tyne and West South Research Ethics Committee, or University of Birmingham Local Ethical Review Committee in compliance with the Declaration of Helsinki.

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Alternative interpretation of data for recommendations on how to manage rheumatoid arthritis

This letter is inspired by the 'EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2016 update'.[1]

There is no question that we are in full agreement with the overarching principles of the EULAR recommendations. What goes against our principles, however, is the tone of the bullet points. Research that has formed the basis of a robust evidence base practised for years in various countries across the globe has been sidetracked and overlooked.[2–4]

As an alternative interpretation of the existing data and hands-on guidance on cost-effective rheumatology care, we refer to the current care guidelines for the management of rheumatoid arthritis formulated by the Finnish Medical Society Duodecim and the Finnish Rheumatology Society, published in 2015 and now available in English (or rather Finglish).[5]

The Finnish Medical Society Duodecim has created a system for the production of current care guidelines on the most prevalent diseases. These guidelines provide the basis for evidence-based treatment of about 100 common health problems. They are based on a rigorous evaluation of all available evidence on the issue. The formal level of evidence is provided. The major difference between the current guidelines and most other guidelines are the short reviews of the literature presenting the evidence supporting the claim for a certain level of evidence and these review documents being publicly available.

With regard to comparison and further interpretation of the EULAR recommendations[1] and the current care guidelines,[5] we wish to leave that to the reader.

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Response to: 'The time has come to revisit alternative interpretations of data underlying the EULAR management recommendations for rheumatoid arthritis' by Pirilä *et al*

The views presented by Pirilä *et al*,[1] which focus primarily on the FINnish Rheumatoid Arthritis Combination therapy (FIN-RACo) trial, have been taken into serious consideration by the task force. Indeed, as extensively explained in the text accompanying the updated European League Against Rheumatism (EULAR) rheumatoid arthritis (RA) management recommendations,[2] several committee members referred to the references provided by Pirilä *et al* as well as other publications on this topic. The discussion also included comments that in some countries local societies recommended combination therapy with conventional synthetic disease-modifying antirheumatic drugs (DMARDs) (csDMARDs), and this is also mentioned in the publication. Nevertheless, after a long debate regarding csDMARD combinations, the task force arrived at the decision to recommend primarily the use of methotrexate (MTX) monotherapy plus short-term glucocorticoids (GC). The decision to delete combinations of csDMARDs as a major recommendation was based on the evidence provided by the respective systematic literature reviews (SLR).[3] Indeed, several new data revealed that MTX+GC was as efficacious as csDMARD combinations+GC, but significantly safer.[4–6] Moreover, MTX+GC is as efficacious as MTX+antitumour necrosis factor.[7] The debate on the usefulness of csDMARD combinations exists for long[8] and it has also been pointed out that in the FIN-RACo trial the use of GC favoured the csDMARD combination group.[9] Indeed, the authors themselves mentioned once that the FIN-RACo trial studied 'combination therapy including corticosteroids in early rheumatoid arthritis' versus 'single DMARD treatment strategy … with or without prednisolone',[10] revealing differences in the use of GCs between the two treatment arms. Thus, the Finnish data have neither been 'side-tracked' nor 'overlooked', but very thoroughly discussed, although not found to be convincing in light of other evidence. Also, when MTX monotherapy (+GC) has failed and bad prognostic factors are absent, the 2016 version of the EULAR recommendations continues to advocate switching to or adding another csDMARD and thus even explicitly includes csDMARD combination therapy.[2]

Three additional points should be considered when addressing the comments of Pirilä *et al*. First, despite strongly advocated opinions in favour of csDMARD combination therapy, the task force arrived at a 71% majority in favour of recommending MTX monotherapy and the level of agreement with this recommendations was 9.8 on a scale of 0–10.[2] Second, also the American College of Rheumatology, which had previously always recommended csDMARD combination treatment,[10] has now favoured MTX monotherapy over csDMARD combination therapy in its most recent guideline,[11] stating that 'there is no evidence in favour of triple therapy' and 'DMARD monotherapy is generally better tolerated than combination DMARD therapy'. And third, the EULAR recommendations clearly state the following: 'it should be mentioned that the simple fact that csDMARD combination therapy is not included in the bullet point anymore does not preclude using it. This is obviously at the discretion of the physician and the patient in light of all pros and cons that had been discussed ('shared decision')'.[2] This may have escaped the attention of Pirilä *et al*.[1]

In summary, based on information from SLRs[3 11 12] and a thorough discussion process that included the aspects raised by Pirilä *et al*,[1] the large task force arrived at a big majority decision with an extremely high level of agreement in favour of the current recommendation. Thus, the Finnish Society may wish to revisit its recommendation.

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HLA-A 31:01 is not associated with the development of methotrexate pneumonitis in the UK population: results from a genome-wide association study

We read with interest the article by Furukawa *et al*[1] suggesting an association between HLA-A 31:01 and methotrexate (MTX)-induced interstitial lung disease (ILD) in Japanese patients with rheumatoid arthritis (RA). MTX-ILD or MTX-pneumonitis (MTX-P) is an idiosyncratic hypersensitivity reaction to MTX that usually occurs within the first year of MTX therapy, inducing inflammation, cytokine release and the activation of CD4+ T-lymphocytes within the lung parenchyma,[2–4] with a reported prevalence of 1% of the Caucasian RA population prescribed MTX.[5]

To investigate this association further, we conducted a genome-wide association study. Rheumatologists working within the National Health Service in the UK identified Caucasian patients with RA, who developed clinician diagnosed MTX-P (n=65). Caucasian controls, matched for age and gender, were identified from a prospective observational cohort study of patients starting MTX (n=195). In order to be eligible, controls were required to have 1 year of continuous MTX therapy without the development of MTX-P. Assuming HLA-A 31:01 prevalence of 3.6% in the European Caucasian population,[6] this provided 80% power to detect an OR of 3.0. Genotyping was performed using the Illumina Infinium HumanCoreExome 12 BeadChip genome-wide array (Illumina, San Diego, USA); HLA-A 31:01 was imputed using SNP2HLA[7] and a subset of samples (n=24) were directly genotyped for the allele using an established wet-lab technique described previously.[8]

Following quality control, data for 62 cases and 175 controls remained. HLA-A 31:01 was not associated with MTX-P in this cohort (p=0.21). Wet-lab genotyping of a subset of samples confirmed concordance with in silico imputation (κ=1.00). One locus, rs6593803 mapping to an intergenic region between the *GJA5* and *ACP6* genes, was associated with MTX-P, however, the results did not reach genome-wide significance thresholds for claims of confirmed association ($p=1.85\times10^{-7}$, OR=3.13).[9] Nonetheless, rs6593803 is known to affect the expression of *GJA5*.[10] *GJA5* is a member of the connexin gene family and the resulting protein is connexin 40. The connexin 40 protein is a component of gap junctions that act at sites of cell–cell contact allowing diffusion of signalling molecules between cells.[11] Transgenic mice deficient in connexin 40 and 43 ($cx40^{-/-}/cx43^{-/-}$) have a reduced life span due to lung abnormalities including pulmonary fibrosis, alveolar wall thickening and increased lung fibroblasts,[12] histopathological findings similar to MTX-P.[13]

In summary, we have found no evidence of association between HLA-A 31:01 and MTX-P in a European population. Three loci reached suggestive evidence for association with MTX-P (rs6593803 ($p=1.85\times10^{-7}$, OR=3.13), rs9299346 ($p=1.76\times10^{-6}$, OR=2.76) and rs1624005 ($p=6.54\times10^{-6}$, OR=2.59)), but further studies with larger numbers of patients with this rare disease are required to confirm these non-HLA associations with MTX-P.

James Bluett,[1] Sally-Ann Owen,[1] Jonathan Massey,[1] Ana Alfirevic,[2] Munir Pirmohamed,[2] Darren Plant,[3] Suzanne M M Verstappen,[4] Anne Barton,[1,3] on behalf of The Pneumonitis Study Consortium

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Contributors JB recruited patients, NHS sites, co-conducted the GWAS and analysis. S-AO applied to the ethics committee, recruited patients and NHS sites. JM co-conducted the GWAS and analysis. AA co-genotyped the HLA 31:01. MP co-wrote the article. SMMV is PI of the control cohort. AB is the PI of the cases cohort.

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Response to: '*HLA-A* 31:01* is not associated with the development of methotrexate pneumonitis in the UK population: results from a genome wide association study' by Bluett *et al*

We appreciate the comments by Bluett *et al*[1] on our report of an association of *HLA-A*31:01* with methotrexate-induced interstitial lung disease (ILD) in patients with rheumatoid arthritis (RA).[2] They have tried to reveal the genetic factors associated with methotrexate-induced ILD, but found no significant association except three suggestive loci in chromosome 1, 9 and 14. This study did not show the association of *A*31:01* with methotrexate-induced ILD, though the p value of the analysis was still 0.21 in spite of the small sample size with 62 cases and 175 controls. The meta-analysis with our previous study[2] or other forthcoming *HLA* association studies on methotrexate-induced ILD may reveal more conclusive results in the future.

Genetic factors would be involved in the pathogenesis of methotrexate-induced ILD, because the susceptibility of methotrexate-induced ILD in Japanese patients with RA are thought to be higher than other ethnic groups or patients with other autoimmune diseases.[3 4] However, there are few reports of genome-wide association study of drug-induced ILD.[5] Since the prevalence of drug-induced ILD is low and the clinical conditions of the patients with drug-induced ILD are various,[6] genetic analyses of drug-induced ILD are difficult. The incidence of methotrexate-induced ILD was reported to be 3.8 per 1000 Japanese patients with RA.[4] The maximum weekly dosage of methotrexate for adult RA had been officially limited to 8 mg in Japan until February 2011.[7] The number of the reports on the development of ILD in patients with RA under the treatment with biological disease-modifying antirheumatic drugs has been increased,[8] and methotrexate are frequently administered with biological disease-modifying antirheumatic drugs in recent treatments for patients with RA. In addition, the risk of *Pneumocystis* pneumonia has been increased in patients with RA[9] and it is quite difficult to distinguish *Pneumocystis* pneumonia, RA-associated ILD and drug-induced ILD.[10 11] Thus, the clinical conditions of patients with RA vary at different periods and regions.

The association between *HLA* alleles and adverse drug reactions has been reported, but the susceptible alleles are occasionally different between ethnic populations.[12 13] Other culprit genes in linkage disequilibrium with *HLA* loci could cause adverse drug reactions; this hypothesis might explain the different susceptible alleles in different ethnic groups. The stratified analyses should be performed in further large-scale studies to clarify the actual genetic association with methotrexate-induced ILD. The pathogenesis of methotrexate-induced ILD would be heterogeneous and the genetic association analyses of this rare disease should be continued to reveal the true aetiology.

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EULAR recommendations underplay importance of severe anxiety and depression in fibromyalgia treatment

The European League Against Rheumatism recently published updated recommendations on the management of fibromyalgia (FM), including pharmacological and non-pharmacological measures.[1] While non-pharmacological measures are now the first approach, there is no stratification according to severity of anxiety or depression prior to selecting treatment options.

We are concerned that these recommendations seriously underplay the role of the need of expert psychological and psychiatric assessment prior to the selection of treatment strategies. Severe anxiety and/or depression may prevent the ability to comply with non-pharmacological therapy such as exercise or cognitive behavioural therapy, and the interactions with chronic pain and fatigue can become cyclical and self-perpetuating. Additionally it is known in FM that a negative mood can lead to a poor perception of one's physical health but does not affect clinical and experimental pain, suggesting that neural processing of experimental pain and negative affect are mediated by two separate mechanisms.[2]

We sought to quantify this effect and propose an alternative pathway for the assessment of FM sufferers.

We assessed the psychological needs of patients with FM in our dedicated multidisciplinary FM clinic by looking for an association between the level of anxiety and depression in FM and symptom severity, functional status, and social or demographic factors. One hundred and fifty-five consecutive patients (92% female) were recruited, all of whom fulfilled the 2010 American College of Rheumatology diagnostic criteria. For each patient we recorded demographic data, Widespread Pain Index (WPI), Symptom Severity Score (SSS), Visual Analogue Scale (VAS) pain and VAS fatigue, Revised Fibromyalgia Impact Questionnaire (FIQR), and the Hospital Anxiety and Depression Scale (HADS). We used cross-tabs and χ^2 analysis to study the associations between anxiety/depression and social and demographics, and logistic regression analysis to identify whether WPI, SSS, FIQR, VAS pain and VAS fatigue were predictors of severe anxiety and depression.

In our study, over 30% of patients with FM had severe undiagnosed depression and 60% severe anxiety. The mean HADS-A was 11.8 (SD: 4.13) and mean HADS-D was 9.1 (SD: 3.8). The mean FIQR was 60.3 (SD: 17.6). While SSS was the single best predictor for anxiety (p=0.001), disease duration (p=0.01), SSS (p=0.02) and FIQR (0.04) predicted depression. We found no association with fatigue, age or social factors including occupation, marital status, level of education and family support.

We feel that a psychiatrist and psychotherapist are essential members of the multidisciplinary team to ensure that anxiety and depression are addressed prior to further interventions.

To ascertain whether baseline psychological state could influence treatment options, we additionally studied the determinants influencing patients' decisions to start pharmacological treatment in FM. Predictors to start treatment were fatigue VAS (p=0.045), increasing age (p=0.001), current employment (p=0.005) and a lack of family support (p=0.023). Unsurprisingly, the main predictor for declining treatment was a high HADS-D (p=0.007). Satisfaction with healthcare professional support, pain, number of good days per week, education and marital status were not predictive.

This adds further evidence to the need to address severe anxiety and depression prior to considering any other treatment options. We propose a pathway for assessment and management of FM sufferers (figure 1).

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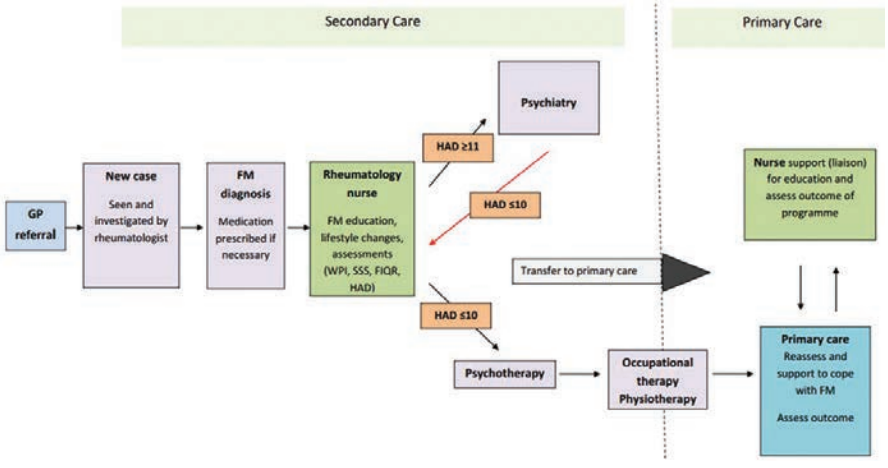

Figure 1 Multidisciplinary fibromyalgia pathway. FIQR, Revised Fibromyalgia Impact Questionnaire; FM, fibromyalgia; GP, general practitioner; HAD, Hospital Anxiety and Depression; SSS, Symptom Severity Score; WPI, Widespread Pain Index.

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EULAR recommendations for management of fibromyalgia

The EULAR recommendations for the management of fibromyalgia are based on more than 100 reviews and meta-analyses of individual therapies and medicines.[1] Thus, the quality of the evidence in making recommendations on effectiveness and efficacy is generally very high. In contrast, there is little published research evaluating models of care for patients with fibromyalgia, and thus this aspect of the recommendations is based on expert opinion of the working group, which was drawn from throughout Europe and across specialties.

Mercieca and Borg[2] provide an alternative model of care based on the practice within their own hospital. Their study and evaluation has not been published, and therefore there is not sufficient detail provided for us to evaluate it. However, there are features of the pathway outlined that are unappealing and directly contradict the EULAR recommendations. Their first-line approach is prescription of medication (stated as 'if required'), and this happens even before the patient is educated about the condition. Many clinicians and patients would find this unacceptable. Their local pathway and the EULAR recommendations do agree on the important role of stratified care including for psychological comorbidities.

Nevertheless, it does highlight the need for more research around models of care for fibromyalgia patients, so that we can identify which deliver better outcomes at an affordable cost and are acceptable to patients.

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