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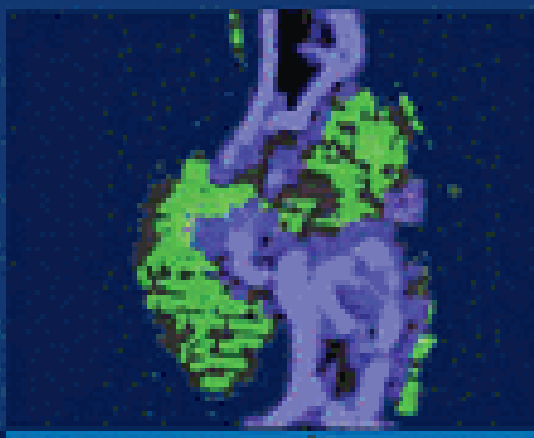

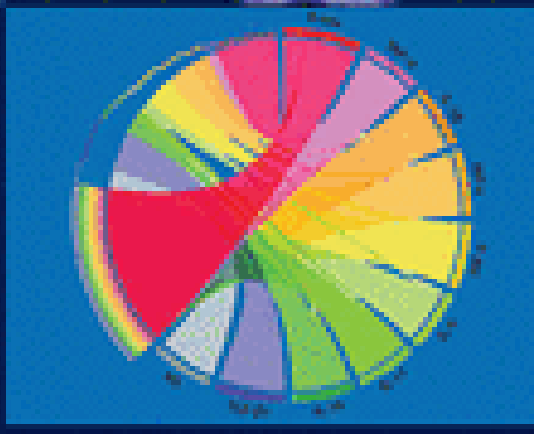

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BMJ Journals, BMA House, Tavistock Square
London WCIH 9JR,UK
E: **ard@bmj.com**

Production Editor
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EULAR
EULAR Executive Secretariat
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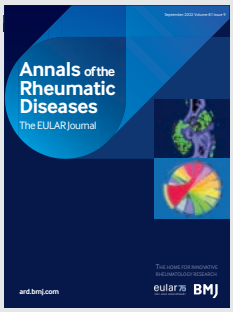

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Annals of the Rheumatic Diseases
BMJ Publishing Group Ltd
BMA House
Tavistock Square
London WCIH 9JR,UK
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MORE CONTENTS ►

Developing guidelines for ultrarare rheumatic disorders: a bumpy ride

David Piskin ,[1,2,3] Micol Romano,[3,4] Daniel Aletaha ,[5] Brian M Feldman ,[6] Raphaela Goldbach-Mansky,[7] Loreto Carmona ,[8] Erkan Demirkaya[2,3,4]

Handling editor Gerd-Rüdiger R Burmester

[1]Lawson Health Research Institute, London, Ontario, Canada
[2]Department of Epidemiology and Biostatistics, Western University, London, Ontario, Canada
[3]Canadian Behcet and Autoinflammatory Disease Center (CAN-BE-AID), Western University, London, Ontario, Canada
[4]Division of Pediatric Rheumatology, Department of Pediatrics, Schulich School of Medicine & Dentistry, Western University, London, Ontario, Canada
[5]Division of Rheumatology, Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria
[6]Division of Rheumatology, Hospital for Sick Children, Toronto, Ontario, Canada
[7]National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, USA
[8]Instituto de Salud Musculoesquelética (INMUSC), Madrid, Spain

Correspondence to
Dr David Piskin, Lawson Health Research Institute, London, ON N6C 2R5, Canada; david.piskin@lhsc.on.ca

DP and MR contributed equally.

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ABSTRACT

Clinical practice guidelines are useful tools for both patients and physicians. Several standardised operating procedures are in existence to describe tasks step by step to develop guidelines/recommendations. The end product consists of data synthesis from the systematic literature search and patient/physician's inputs. For the prevalent diseases, the process for developing guidelines is straightforward; it is based on physicians'/patients' experiences and abundance of the literature. When it comes to the realm of ultrarare diseases, there are few physicians who are familiar with a disease, and there is a scarcity of literature. In this viewpoint, we describe challenges from the methodological perspectives that occurred during the process of developing recommendations for autoinflammatory disorders with the goal of finding solutions that facilitate the development of guidelines for ultrarare diseases in the future.

INTRODUCTION

Clinical practice guidelines can be defined as 'statements that include recommendations intended to optimise patient care that are informed by a systematic review of evidence and an assessment of the benefits and harms of alternative care options'.[1] Guidelines—the production of which brings policy makers, practitioners and patients under the same roof—are useful for improving quality of care, standardising clinical practice, preventing accusations of malpractice, reducing the cost of the treatment and reducing adverse events.[2] To provide guidance for the scientific approach to the development of these guidelines, European Alliance of Associations for Rheumatology (EULAR) published its first standardised operating procedures (SOPs) in 2004, with the main aim of 'improving outcome of patient with rheumatic disorders'' the SOPs were updated in 2014.[3 4] EULAR is revising their SOPs, which will be available soon as living document at the EULAR website and more accessible than a journal publication.

Recently, the joint EULAR and American College of Rheumatology (ACR) panel endorsed consensus guidelines that were developed by a group of experts for ultrarare, genetically defined autoinflammatory diseases, including the type I interferonopathies,[5] and the interleukin (IL)-1-mediated autoinflammatory diseases.[6] The interferonopathies are characterised by the presence of a chronically elevated type I interferon signature in peripheral blood and the IL-1-mediated diseases by increased release or signalling of the proinflammatory cytokine IL-1. The estimated point prevalence for each of the diseases is less than one in a million; some diseases, that is, deficiency of IL-1 receptor antagonist (DIRA), may have a worldwide prevalence of less than 100 patients. Their prevalence is significantly lower than the definitions for rare diseases (<1 per 2000) and ultrarare diseases (<1 per 50 000) as described by the National Institute for Health and Care Excellence.[7] As there is no agreed-on term for disorders with a prevalence less than one in a million, the term 'ultrarare' is used in this 'viewpoint' for the aforementioned disorders.

The EULAR/ACR committee suggested the term 'recommendations' and/or 'points to consider' instead of 'guidelines' is reserved for conditions with a larger body of pubications including randomised controlled clinical trials, a bar too high to pass, considering the paucity of literature that exists for these ultrarare diseases.[3] The steps for developing recommendations, as opposed to guidelines, are also clearly defined in the EULAR SOPs.[3] The process starts with defining the research questions and by performing a systematic literature review (SLR) to address these questions. The next step is to assess the quality and validity of the retrieved papers using a validated tool. After that, the recommendations are formulated and graded based on the evidence from the SLR. For the grading of the evidence, it is suggested to use the Oxford Centre for Evidence-based Medicine (CEBM) 'levels of evidence', which define the level of evidence based on the rigour of the study design used.[8]

The process of developing recommendations, from start to end, appears to be straightforward and clearly defined, but each step can be challenging when trying to apply these rules to ultrarare diseases. We list stumbling blocks that we encountered, during the process of developing recommendations for the listed autoinflammatory diseases, with the goal of finding solutions that facilitate the development of guidelines for ultrarare diseases in the future.

CHALLENGES OF WORKING WITH ULTRARARE DISEASES

As the evidence-based medicine (EBM) approach has become widely accepted, it is thought that not all evidence is of the same quality.[9] Evidence pyramids sum up a so-called hierarchy of the evidence; the most reliable and rigorous evidence is derived from systematic reviews and meta-analysis of randomised controlled trials (RCTs), which is placed on top of the level-of-evidence pyramid, followed by individual RCTs, cohort studies, case–control studies, case series and finally expert opinion.[8]

For questions regarding treatment, RCTs are the preferred standard. However, for ultrarare autoinflammatory diseases, satisfactory evidence from RCTs is usually not available,[10] and large-scale multicentre trials that would be required for the RCTs to meet the standard but are not feasible due to the overall small number of patients and the difficulties in accruing adequate sample sizes. Commercial enthusiasm is dampened by the logistical hurdles of adding multiple centres (that each can contribute only few patients).[11 12] Thus, in ultrarare conditions such as the autoinflammatory diseases we examined, the majority of evidence regarding treatment comes from case series and individual case reports.[13–15] Therefore, the level of evidence remained low and resulted in the guidance documents being assigned points to consider instead of the recommendation label.

In that context, an observational open-label design not only facilitates recruitment but also accommodates the need to treat these often severely affected patients, which makes a randomised study that includes placebo or an inferiority treatment unethical. Other acceptable and ethical design options include self-controlled observational study designs (which are similar to crossover designs but do not involve random assignment), case–control designs, modular adaptive designs and prospective inception cohorts.[16]

Currently, there is no cure for type I interferonopathies, and treatment options aim at preventing development or progression of end organ damage, and to improve quality of life and disease outcomes. In the study by Sanchez *et al*, 10 patients with chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (CANDLE), 4 patients with stimulator of interferon genes-associated vasculopathy with onset in infancy (SAVI) and 4 patients with other interferonopathies were enrolled to evaluate the treatment effects of the Janus kinase (JAK) inhibitor baricitinib.[17] All 10 out of 10 patients with CANDLE and 3 of 4 patients with SAVI seemed to benefit from the treatment.[17] This was an open-label study and, despite what appeared to be clear benefits, recommendations for baricitinib for treatment of CANDLE and SAVI can only be graded as 2b using the CEBM system (individual cohort study (including low quality RCT), eg, <80% follow-up).[8]

The current standard of care for patients with cryopyrin-associated periodic syndrome (CAPS), tumour necrosis factor receptor-associated periodic syndrome (TRAPS), mevalonate kinase deficiency (MKD) and DIRA is subcutaneous IL-1-targeted biological therapy. While there is strong evidence supporting this treatment for CAPS, TRAPS and MKD, for DIRA, there are only 25 diagnosed patients worldwide; hence, for DIRA, the recommendation can only be based on limited case series.

The other obstacle is the quality and validity assessment of the selected papers. The EULAR SOP mentions Cochrane Risk of Bias (RoB) tool, the Quality Assessment of Diagnostic Accuracy Studies II and the Quality in Prognosis Studies for relevant studies.[3] There are several RoB assessment tools for different type of studies.[18] The Cochrane RoB tool is the most commonly used for RCTs.[19] For observational studies, the typical RoB tool is the Newcastle-Ottawa Scale,[20] among several other options.[18] Unlike the aforementioned study types, there is a scarcity of RoB tools for case series and case reports.[21–23] As for the each self-assessment-based evaluation, these tools have their own advantages and disadvantages. They are focusing, the most common problems related with the observational design, namely, confounding, information bias and selection bias. The performance of the tools is open to discussion, and they are still not widely used. On the other hand, case series and case reports for treatment purposes are categorised as level 4 at Oxford Level of Evidence,[8] and there is no point to critically appraise them for the guideline purposes; hence, the level of evidence is almost lowest.

Last but not the least, there is an obstacle in combining the data from different diseases. Per SOP, the recommendations should be based on the SLR, as a principle. Due to the rarity of these conditions, still many aspects of the disease remained unknown at the time of writing recommendations. Given, for example, the IL-1-mediated autoinflammatory diseases, all four diseases described in the respective EULAR/ACR points to consider, respond to IL-1 blockade which allows to combine them under the same term of 'IL-1-mediated diseases', all four diseases have their own clinical characteristics and patterns of organ manifestations. Some well-known biomarkers like C reactive protein, erythrocyte sedimentation rate and S100 protein that are used to assess inflammation equally respond to IL-1 blocking therapy; on the other hand, clinical features are heterogeneous even in a disease such as CAPS that is caused by autosomal dominant gain-of-function mutations in the same gene, NLRP3, which results in calling them NLRP3-associated autoinflammatory diseases. However, CAPS presents as a disease spectrum that includes three different phenotypes with varying severities: familial cold autoinflammatory syndrome (FCAS; a mild phenotype presenting with fever and rash), Muckle-Wells syndrome (MWS; a moderate phenotype) and neonatal-onset multisystem inflammatory disease (NOMID; a severe phenotype that includes also aseptic meningitis and bone inflammation), which require different clinical features that need to be followed and different doses of IL-1 blocking treatments to be controlled.[24] Because of these nuances, it is challenging to even combine data from studies in FCAS, MWS and NOMID to formulate recommendations, leaving us with small numbers despite impressive treatment results in all three disease groups.

Another important point is the implications such recommendations have for governmental authorities such as the US Food and Drug Administration and the European Medicines Agency, which have a role in authorising patients' access to diagnostic tools and treatment options and who are also end users of these guidelines. Their decision-making process is based on meaningful outcomes that are defined by the physician community and their validation. However, in ultrarare diseases, generating meaningful outcomes is no easy task. These guidelines serve to raise awareness of challenges of working with ultrarare autoinflammatory diseases and can hopefully generate a basis for working with regulatory authorities in accelerating and facilitating approval of treatments for patients with ultrarare diseases.

CONCLUSION

Evidence-based medicine, according to Masic *et al*, 'is the conscientious, explicit, judicious and reasonable use of modern, best evidence in making decisions about the care of individual patients. EBM integrates clinical experience and patient values with the best available research information'.[25] For ultrarare diseases, the *best* evidence may come from case reports and case series. Clinical experience, as such, must be given great weight when RCTs are not feasible or are simply not done. It would be incredibly helpful if rare and ultrarare disease methodologies were considered as part of SOPs for guideline (or recommendation) development, as researchers in the field of rare diseases otherwise will always have to bend the rules, not by choice but by circumstances. Meaningful adaptations of the SOPs for ultrarare diseases would include the consideration of a higher level of evidence for case reports and case series that includes hard

outcomes such as inflammatory remission and open-label withdrawal studies that report both objective clinical endpoints and important biomarkers; these sources, while typically excluded from activities in the major diseases area for development of rare disease recommendations, are the key bits and pieces of information.

Twitter Loreto Carmona @carmona_loreto

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ORCID iDs
David Piskin http://orcid.org/0000-0002-2315-514X
Daniel Aletaha http://orcid.org/0000-0003-2108-0030
Brian M Feldman http://orcid.org/0000-0002-7813-9665
Loreto Carmona http://orcid.org/0000-0002-4401-2551

REFERENCES

1 Steinberg E, Greenfield S, Wolman DM. *Clinical practice guidelines we can trust: national academies press*, 2011.
2 Kredo T, Bernhardsson S, Machingaidze S, *et al*. Guide to clinical practice guidelines: the current state of play. *Int J Qual Health Care* 2016;28:122–8.
3 van der Heijde D, Aletaha D, Carmona L, *et al*. 2014 update of the EULAR standardised operating procedures for EULAR-endorsed recommendations. *Ann Rheum Dis* 2015;74:8–13.
4 Dougados M, Betteridge N, Burmester GR, *et al*. EULAR standardised operating procedures for the elaboration, evaluation, Dissemination, and implementation of recommendations endorsed by the EULAR standing committees. *Ann Rheum Dis* 2004;63:1172–6.
5 Cetin Gedik K, Lamot L, Romano M, *et al*. The 2021 European alliance of associations for Rheumatology/American College of rheumatology points to consider for diagnosis and management of autoinflammatory type I interferonopathies: CANDLE/PRAAS, SAVI and AGS. *Ann Rheum Dis* 2022;81:601–13.
6 Romano M, Arici ZS, Piskin D, *et al*. The 2021 EULAR/American College of rheumatology points to consider for diagnosis, management and monitoring of the interleukin-1 mediated autoinflammatory diseases: cryopyrin-associated periodic syndromes, tumour necrosis factor receptor-associated periodic syndrome, mevalonate kinase deficiency, and deficiency of the interleukin-1 receptor antagonist. *Ann Rheum Dis* 2022;81:907–21.
7 Harari S, Humbert M. Ultra-rare disease: an European perspective. *Eur Respir Rev* 2020;29:200195.
8 OCEBM Levels of Evidence Working Group. Oxford centre for evidence-based medicine – levels of evidence (March 2009). Available: https://www. cebm. net/2009/06/ oxford- centre- evidence- based- medicine- levels- evidence- march- 2009/ [Accessed 18 Mar 2021].
9 Guyatt G, Rennie D, Meade M. *Users' guides to the medical literature: a manual for evidence-based clinical practice: AMA press Chicago*, 2002.
10 Clarke JTR, Coyle D, Evans G, *et al*. Toward a functional definition of a "rare disease" for regulatory authorities and funding agencies. *Value Health* 2014;17:757–61.
11 Rath A, Salamon V, Peixoto S, *et al*. A systematic literature review of evidence-based clinical practice for rare diseases: what are the perceived and real barriers for improving the evidence and how can they be overcome? *Trials* 2017;18:1–11.
12 De Benedetti F, Gattorno M, Anton J, *et al*. Canakinumab for the treatment of autoinflammatory recurrent fever syndromes. *N Engl J Med* 2018;378:1908–19.
13 Patel PN, Hunt R, Pettigrew ZJ, *et al*. Successful treatment of chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (candle) syndrome with tofacitinib. *Pediatr Dermatol* 2021;38:528–9.
14 Volpi S, Insalaco A, Caorsi R, *et al*. Efficacy and adverse events during Janus kinase inhibitor treatment of SAVI syndrome. *J Clin Immunol* 2019;39:476–85.
15 Frémond M-L, Rodero MP, Jeremiah N, *et al*. Efficacy of the Janus kinase 1/2 inhibitor ruxolitinib in the treatment of vasculopathy associated with TMEM173-activating mutations in 3 children. *J Allergy Clin Immunol* 2016;138:1752–5.
16 Whicher D, Philbin S, Aronson N. An overview of the impact of rare disease characteristics on research methodology. *Orphanet J Rare Dis* 2018;13:14.
17 Sanchez GAM, Reinhardt A, Ramsey S, *et al*. JAK1/2 inhibition with baricitinib in the treatment of autoinflammatory interferonopathies. *J Clin Invest* 2018;128:3041–52.
18 Ma L-L, Wang Y-Y, Yang Z-H, *et al*. Methodological quality (risk of bias) assessment tools for primary and secondary medical studies: what are they and which is better? *Mil Med Res* 2020;7:7.
19 Higgins JPT, Altman DG, Gøtzsche PC, *et al*. The Cochrane collaboration's tool for assessing risk of bias in randomised trials. *BMJ* 2011;343:d5928.
20 Wells GA, Shea B, O'Connell D. *The Newcastle-Ottawa scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses: Oxford*, 2000.
21 Institute JB. *JBI critical appraisal checklist for case reports. Joanna Briggs Institute*, 2017.
22 Guo B, Moga C, Harstall C, *et al*. A principal component analysis is conducted for a case series quality appraisal checklist. *J Clin Epidemiol* 2016;69:e2:199–207.
23 Murad MH, Sultan S, Haffar S, *et al*. Methodological quality and synthesis of case series and case reports. *BMJ Evid Based Med* 2018;23:60–3.
24 Romano M, Arici ZS, Piskin D, *et al*. The 2021 EULAR/American College of rheumatology points to consider for diagnosis, management and monitoring of the interleukin-1 mediated autoinflammatory diseases: cryopyrin-associated periodic syndromes, tumour necrosis factor receptor-associated periodic syndrome, mevalonate kinase deficiency, and deficiency of the interleukin-1 receptor antagonist. *Ann Rheum Dis* 2022;81:907–21.
25 Masic I, Miokovic M, Muhamedagic B. Evidence based medicine - new approaches and challenges. *Acta Inform Med* 2008;16:219–25.

CLINICAL SCIENCE

Inflammatory correlates of the Patient Global Assessment of Disease Activity vary in relation to disease duration and autoantibody status in patients with rheumatoid arthritis

Serena Bugati ,[1,2] Ludovico De Stefano,[1,2] Bernardo D'Onofrio,[1,2] Andrea Nicrosini,[1,2] Eleonora Mauric,[1,2] Michele di Lernia,[1,2] Garifallia Sakellariou,[2,3] Ennio Giulio Favalli ,[4,5] Antonio Manzo,[1,2] Roberto Caporali,[4,5] Carlomaurizio Montecucco [1,2]

Handling editor Josef S Smolen

[1]Division of Rheumatology, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy
[2]Department of Internal Medicine and Therapeutics, Università di Pavia, Pavia, Italy
[3]Maugeri Clinical Research Institutes IRCCS Pavia, Pavia, Italy
[4]Department of Rheumatology, Gaetano Pini-CTO, Milano, Italy
[5]Department of Clinical Sciences and Community Health, University of Milan, Milano, Italy

Correspondence to
Professor Serena Bugati, Division of Rheumatology, Fondazione IRCCS Policlinico San Matteo, 27100 Pavia, Italy; serena.bugati@unipv.it

SB and LDS contributed equally.

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ABSTRACT

Objective To investigate the associations between the Patient Global Assessment (PGA) and measures of disease activity in patients with rheumatoid arthritis (RA) in relation to disease duration and autoantibody status.

Methods 1412 patients from three independent cohorts were studied: a prospective cohort of 810 patients with early RA followed up for 24 months; a cross-sectional cohort of 210 patients with established RA in low disease activity; a cross-sectional cohort of 401 patients with established RA in moderate-to-high disease activity. Correlations of the PGA were analysed by Pearson's coefficients and multivariable linear regression at baseline and at months 6, 12 and 24 in the overall populations and after stratification for autoantibody subgroup and remission status (Boolean remission, PGA near remission and non-remission).

Results In patients with early RA in non-remission, swollen joints correlated independently with the PGA; the correlation became progressively weaker but persisted at all time points in autoantibody-positive patients (adjusted r=0.30–0.12) but lost significance after month 12 in autoantibody-negative patients. Swollen joints independently correlated with the PGA also in near remission until month 12 (adjusted r=0.18–0.16) in autoantibody-positive patients. No independent correlations of inflammatory variables were instead found in patients with established RA irrespective of disease activity and autoantibody status.

Conclusions In the early phases of RA, particularly in autoantibody-positive patients, inflammatory variables directly correlate with the PGA across different disease activity states. The optimal cut-off values of the PGA capable of identifying absence of disease should be better explored in relation to disease duration and autoantibody status.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The Patient Global Assessment (PGA) is included in disease activity indices and remission criteria in rheumatoid arthritis (RA). However, the low correlation with measures of inflammation in established RA raises controversies on the interpretation of the PGA as a marker of disease activity.

WHAT THIS STUDY ADDS

⇒ This study is the first to analyse the inflammatory correlates of the PGA in patients with early RA. In autoantibody-positive, but not in autoantibody-negative patients, swollen joints consistently correlate with the PGA across different disease activity states, including near remission.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE AND/OR POLICY

⇒ Interpretation of the PGA as a measure of disease activity RA should take into account important variables such as disease duration and autoantibody status. Optimal cut-off values of the PGA might even differ in different phases of the disease and autoantibody subgroups.

Patient-reported outcomes (PROs) such as the Patient Global Assessment (PGA) integrate components of disease activity that are not captured by other core variables, discriminate active treatment from placebo, and predict physical function, well-being and work productivity in patients with rheumatoid arthritis (RA).[1–3] As such, PROs are included in the definition of disease activity and remission in RA and are part of the primary outcome measures required by regulatory agencies to approve new drugs.[4]

The inclusion of the PGA as a treatment target and driver of intensification of immunosuppressive therapy, at least at its current threshold of ≤1, is, however, at the centre of intense debate.[5–8] In patients with established RA, major determinants of the PGA are pain severity, functional limitations and fatigue, with psychological factors, comorbidities and background culture playing additional roles.[9–15] In contrast, the correlation with more objective measures of inflammation is weaker and disappears at low levels of disease activity.[10 12–14] Accordingly, outcomes strictly related to inflammatory variables, such as radiographic progression,[16 17] do not appear to be significantly affected if the PGA

is either omitted or relaxed in the definition of disease remission.[2 18] Although increasing thresholds of the PGA do influence worse functional outcomes,[2 18] the intricate relationship between inflammation and physical function[19] complicates the interpretation of the PGA as a true measure of disease activity. Based on these lines of evidence, amended definitions of disease remission and treatment targets in RA are being discussed, ranging from more permissive PGA cut-offs to exclusion of the patient perspective from the drivers of immunosuppressive intervention.[2 5]

Controversies over the significance of the PGA, however, mainly arise from studies in established RA. In the early stages of the disease, when structural joint damage and non-nociceptive pain processing mechanisms have not accumulated yet,[20 21] the PGA might in theory more strictly reflect the patient's perception of inflammation. Accordingly, little evidence available thus far in patients with early RA indicates numerically higher rates of radiographic progression[22] and greater functional decline[23] associated with the omission or relaxation of PGA thresholds in comparison with established RA. Adding further complexity, other sources of variation might be intrinsic to certain disease characteristics, including the autoantibody status, known to be differently associated with nociplastic pain and fibromyalgianess.[24]

These and other gaps of knowledge underline the need to better understand the meaning of the PGA in different contexts before introducing substantial revisions in its interpretation. The aim of our study was therefore to investigate the associations of the PGA with measures of impact and inflammation in relation to disease duration and autoantibody status in patients with RA.

METHODS

Patients

The study used data from a total of 1412 patients with RA from three independent cohorts:

- A prospective cohort of 801 consecutive patients with early RA referred to the Early Arthritis Clinic of the Division of Rheumatology of the Policlinico San Matteo in Pavia[25–27] in the years 2005–2017, and followed up longitudinally from enrolment to 24 months.
- A cross-sectional cohort of 210 consecutive patients with established RA (>5 years) extracted from the outpatient clinic of the division of rheumatology of the Policlinico San Matteo in Pavia based on the fulfilment of at least low disease activity according to the 28-joints disease activity score (Disease Activity Score on 28 Joints (DAS28) <3.2).
- A cross-sectional cohort of 401 consecutive patients with established RA (>5 years) extracted from the Department of Rheumatology of Gaetano Pini in Milan in at least moderate disease activity (DAS28 >3.2).

Prospective early RA cohort

The prospective early RA cohort served to explore the associations between the PGA and different domains of disease activity in patients with short disease duration and to test whether such associations varied with time.

Patients had a symptom duration of <12 months at inclusion, fulfilled either the 1987 American College of Rheumatology (ACR)[25] and/or the 2010 ACR/European League Against Rheumatism (EULAR)[26] classification criteria for RA, and were naïve to glucocorticoids and disease-modifying antirheumatic drugs. On diagnosis, patients were seen every 2 months in the first semester and 3 monthly afterwards, with an overall follow-up available for this study of 24 months. The treatment protocol has been described previously.[27–29] Briefly, patients were treated to achieve low disease activity (LDA). Before October 2010, patients classified as RA based on the 1987 criteria were treated with methotrexate (MTX) from 10 mg/wk and were randomised to receive low-dose prednisone.[27] According to our treatment protocol for undifferentiated arthritis,[28] patients retrospectively fulfilling the 2010 criteria (1987 undifferentiated arthritis) were treated with hydroxychloroquine 200 mg two times per day for 2 months and 200 mg/day afterwards, increased to MTX in case of failure to achieve LDA. After October 2010, patients classified as RA based on the 2010 criteria received MTX from 15 mg/week plus low-dose prednisone. To increase consistency of the results, patients starting biological disease-modifying antirheumatic drugs (bDMARDs) or targeted synthetic disease-modifying antirheumatic drugs (tsDMARDs) were excluded from this study.

Cross-sectional cohorts of established RA

The two cross-sectional cohorts of patients with established RA were used to explore the associations of the PGA in a more chronic phase of the disease and across a broader range of disease activity.

In both cohorts, only patients with RA of >5 years' duration who had never received treatment with bDMARDs/tsDMARDs were selected. The cohort of non-active RA consisted of consecutive patients from the division of rheumatology of Pavia who, at the time of consultation of the electronic records (January–June 2017), had a Disease Activity Score on 28 Joints (DAS28) of <3.2. The cohort of active RA was extracted from the repository of the Gaetano Pini of Milan and included consecutive patients (2014–2017) candidate to escalate treatment because of DAS28 of >3.2.

Assessments

In both the prospective early RA and in the cross-sectional established RA cohorts, data collection at baseline included demographic characteristics, symptoms duration, the Tender Joint Count on 28 Joints (TJC28) and Swollen Joint Count on 28 Joints (SJC28), the PGA and physician's assessment of disease activity on 0–100 mm Visual Analogue Scale (VAS), VAS for general health and pain score (0–100 mm), the Health Assessment Questionnaire (HAQ), the erythrocyte sedimentation rate and C reactive protein (CRP) levels. In the early RA cohort, the same clinical characteristics were collected at each follow-up visit. The PGA was systematically assessed using the formulation of the ACR/EULAR definition of remission[30]: 'considering all the ways your arthritis has affected you, how do you feel your arthritis is today?' Rheumatoid factor (RF) and anticitrullinated protein antibodies (ACPAs) were evaluated on centrally analysed baseline sera in patients with early RA and on patient's records in the two cohorts of established RA. Patients were classified as autoantibody-positive if RF and ACPA were above the reference cut-off values and autoantibody-negative if RF and ACPA were both negative.

Definitions of remission

In the prospective early RA cohort, the achievement of different remission states was assessed after 6, 12 and 24 months and classified as follows: (1) Boolean-based remission (TJC28, SJC28, CRP (mg/dL) and PGA, all ≤1)[30]; (2) remission solely missed because of PGA 1 (PGA near remission: TJC28, SJC28 and CRP (mg/dL) ≤1; PGA >1)[31]; (3) non-remission (TJC28 or SJC28 or CRP (mg/dL) >1).

Statistical analysis

Quantitative data were expressed as means and SDs and categorical data as frequencies. There was no imputation of missing data. In the prospective early RA cohort, the data set included baseline and follow-up visits at months 6, 12 and 24. Correlations of the PGA with disease impact measures (VAS pain and HAQ scores) and inflammatory variables (SJC28 and CRP) were first analysed by univariate analysis using Pearson's correlation coefficients and were categorised as strong (r≥0.60), moderate (r=0.40–0.59), low (r=0.20–0.39) and little (r<0.20).[32] Variables with p values of ≤0.20 were included in multivariable linear regression models (backward method) with PGA as a dependent variable after adjustment for confounders (age, gender, symptoms duration, calendar year—quartiles and, from month 6 onwards, treatment with conventional synthetic disease-modifying antirheumatic drugs (csDMARDs) and glucocorticoids). To prevent multicollinearity, possible explanatory variables were assessed in bivariate correlations prior to inclusion in the multivariable models, with r<0.80 as threshold for inclusion; none of the variables was excluded based on this criterion. Analyses were performed in the overall populations and after stratification for remission and autoantibody status. Results were presented with no correction for multiple testing. However, the false discovery rate of the variables of interest was controlled through the Benjamini-Hochberg procedure.[33] All analyses were conducted using MedCalc V.12.7.0.0, and the significance level was set at 0.05.

The patients enrolled in the present analysis were part of a larger ongoing observational study, and formal determination of the sample size was not performed. However, we estimated the theoretical sample size needed to determine whether a correlation coefficient differs from zero, in case of an expected correlation coefficient of 0.2, with an alpha of 0.05 and a 1−beta of 0.8. Under these circumstances, a sample of 194 individuals would be needed. Our population and most of the subpopulations exceeded this size, providing an adequate sample for the main analyses.

RESULTS

Prospective cohort of patients with early RA

The prospective cohort consisted of 801 consecutive patients with early RA, all fulfilling the 2010 ACR/EULAR (89%) and/or the 1987 ACR (74.7%) criteria, and who were treatment-naïve at inclusion. Baseline characteristics are summarised in table 1. As expected, autoantibody-negative patients had higher numbers of involved joints and worst PROs (data not shown).

During the observation period of 24 months, 172 patients (21.4%) were lost to follow-up, and 75 (9.4%) started bDMARDs/tsDMARDs, resulting in 747, 681 and 554 patients on csDMARDs assessable at 6, 12 and 24 months, respectively. At 6 months, 102 patients (13.6%) were in Boolean remission, 79 (10.6%) in near remission and the remaining 566 (75.8%) in non-remission. Rates of remission, near remission and non-remission were 20%, 12.3% and 67.7% at 12 months and 27.4%, 16.1% and 56.5% at 24 months.

Association of the PGA with disease impact measures and inflammatory variables over time in patients with early RA

The correlations of the PGA were analysed from enrolment to month 24. At univariate analysis (figure 1), the PGA showed a strong correlation (r≥0.60) with VAS pain score at all time points and a moderate correlation with the HAQ. The correlation with the number of swollen joints was low (r<0.40) but remained statistically significant over time, while the correlation with CRP levels was little and lost significance at month 24. At multivariable analysis (table 2), VAS pain score and the HAQ independently explained changes in the PGA at all time points, whereas joint swelling was independently associated only until month 12. Such associations were confirmed also after correction for remission status and autoantibody positivity.

Table 1 Demographic and clinical characteristics of the study populations

	Early RA n=801	Established RA non-active* n=210	Established RA active* n=401
Age (years)	59 (14.6)	65.4 (14)	52.4 (12.8)
Female gender, n (%)	586 (73.2)	143 (68.1)	326 (81.3)
Symptom duration			
Weeks	23 (20.1)	483.6 (161.2)	509.6 (442)
Years	0.4 (0.4)	9.3 (3.1)	9.8 (8.5)
1987 criteria fulfilled, n (%)	598 (74.7)	n.a.	n.a.
2010 criteria fulfilled, n (%)	713 (89)	n.a.	n.a.
SJC28	7.2 (4.9)	0.2 (0.5)	6.4 (4.9)
TJC28	7.7 (6.3)	0.5 (1.1)	7.4 (5.1)
DAS28	4.81 (1.20)	2.19 (0.71)	5.33 (1.19)
VAS pain score (0–100)	56.8 (26.7)	26.9 (25.3)	64.6 (19.1)
VAS PGA score (0–100)	56.9 (26.4)	22.9 (23)	62.3 (18.3)
VAS physician score (0–100)	41.7 (20.4)	3.4 (7.4)	51.5 (17.8)
HAQ score (0–3)	1.1 (0.7)	0.31 (0.49)	1.36 (0.57)
ESR (mm/1 hour)	29.1 (23.4)	16 (13.9)	40.4 (24.8)
CRP (mg/dL)	1.8 (3)	0.5 (0.9)	2.5 (3.4)
RF and/or ACPA positive, n (%)	359 (44.8)	101 (48.1)	309 (77.1)
SHS erosion ≥1, n (%)	284 (35.5)	n.a.	n.a.
PDN, n (%)†	557 (69.5)	54 (25.7)	328 (81.8)
csDMARD, n (%)†	801 (100)	200 (95.2)	321 (80)
HCQ	180 (22.5)	74 (35.2)	18 (4.5)
MTX	599 (74.8)	136 (64.8)	258 (64.3)
LFN	7 (0.8)	4 (1.9)	38 (9.5)
SSZ	15 (1.9)	4 (1.9)	2 (0.5)
Others	0 (0)	0 (0)	5 (1.2)

Results are expressed as means (SD) if not otherwise stated.
*According to the DAS28: non-active=DAS28 <3.2, active=DAS28 >3.2.
†In patients with early RA, PDN and csDMARDs refer to treatments instituted after baseline visit. Patients were glucocorticoid-naïve and DMARD-naïve at referral (T0).
ACPA, anticitrullinated protein antibody; CRP, C reactive protein; csDMARD, conventional synthetic disease-modifying antirheumatic drug; DAS28, Disease Activity Score on 28 Joints; DMARD, disease-modifying antirheumatic drug; ESR, erythrocyte sedimentation rate; HAQ, Health Assessment Questionnaire; HCQ, hydroxychloroquine; LFN, leflunomide; MTX, methotrexate; n.a., not available; PDN, prednisone; PGA, Patient Global Assessment; RA, rheumatoid arthritis; RF, rheumatoid factor; SDAI, Simplified Disease Activity Index; SHS, Sharp van der Heijde Score; SJC28, Swollen Joint Count on 28 Joints; SSZ, sulfasalazine; T0, time zero; TJC28, Tender Joint Count on 28 Joints; VAS, Visual Analogue Scale.

Association of the PGA with disease impact measures and inflammatory variables over time in relation to the autoantibody status

Longitudinal analyses of the correlates of the PGA were repeated in autoantibody-positive and autoantibody-negative patients separately (figure 1). While the VAS pain score and the HAQ score were independently associated with the PGA at all time points in both groups (table 2), the association of inflammatory variables behaved differently in relation with the autoantibody

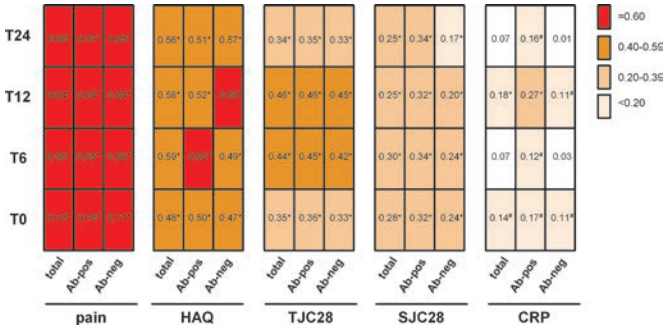

Figure 1 Pearson's correlation coefficients of the PGA with disease impact measures and inflammatory variables in patients with early RA in relation to the autoantibody status. Strength of correlations of the PGA with pain (Visual Analogue Scale 0–100), HAQ score, number of tender and swollen joints on a 28-joints count (TJC28 and SJC28) and CRP levels in patients with early RA at baseline and follow-up (months 6, 12 and 24). Correlations are shown in the overall population and in autoantibody subgroups separately. *P<0.001, †P<0.01. Ab, autoantibody; CRP, C reactive protein; HAQ, Health Assessment Questionnaire; PGA, Patient Global Assessment; RA, rheumatoid arthritis; SJC28, Swollen Joint Count on 28 Joints; TJC28, Tender Joint Count on 28 Joints.

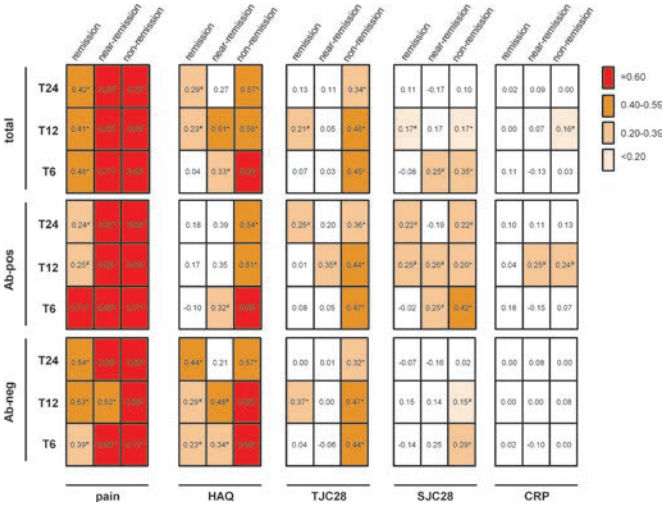

Figure 2 Pearson's correlation coefficients of the PGA with disease impact measures and inflammatory variables in patients with early RA in relation to remission status. Strength of correlations of the PGA with pain score (Visual Analogue Scale 0–100), HAQ score, number of tender and swollen joints on a 28-joints count (TJC28 and SJC28) and CRP levels in patients with early RA at months 6, 12 and 24 stratified for different remission status. Correlations are shown in the overall population and in autoantibody subgroups separately. *P<0.001, †P<0.01. Ab, autoantibody; CRP, C reactive protein; HAQ, Health Assessment Questionnaire; PGA, Patient Global Assessment; RA, rheumatoid arthritis; SJC28, Swollen Joint Count on 28 Joints; TJC28, Tender Joint Count on 28 Joints.

status. In autoantibody-positive patients, swollen joints maintained independent association with the PGA at baseline and at 6 and 12 months, while the association was borderline significant at 24 months (table 2). Multivariable models with swollen joints and autoantibody positivity entered as covariates confirmed the significant association of both factors with the PGA (data not shown). Differently, in autoantibody-negative patients, the number of swollen joints was an independent predictor at baseline and 6 months; the correlation became borderline significant at 12 months and was lost at 24 months (table 2). Corrections for the false discovery rate confirmed the results, with p values being only slightly higher than the critical values at 24 months in autoantibody-positive patients, and at 12 months in autoantibody-negative patients. CRP did not appear to be independently associated at any time point in neither autoantibody-positive nor autoantibody-negative patients.

Table 2 Correlations of the PGA with disease impact measures and inflammatory variables over time in patients with early RA: multivariable analysis*

	T0		T6		T12		T24	
	n=801		n=747		n=681		n=554	
	r part	P value	r part	P value	r part	P value	r part	P value
Total cohort								
Pain	0.66	<0.0001	0.65	<0.0001	0.74	<0.0001	0.76	<0.001
HAQ score	0.19	<0.0001	0.22	<0.0001	0.17	0.0005	0.17	0.001
SJC28	0.10	0.005	0.26	0.001	0.13	0.006	0.10	0.10
TJC28	0.10	0.14	–	–	–	–	–	–
CRP	–	–	–	–	–	–	n.i.	n.i.
Autoantibody-positive								
	n=359		n=339		n=305		n=243	
Pain	0.69	<0.0001	0.64	<0.0001	0.81	<0.0001	0.78	<0.0001
HAQ score	0.14	0.01	0.15	0.04	0.11	0.04	0.13	0.03
SJC28	0.13	0.02	0.30	<0.0001	0.14	0.04	0.16	0.05
TJC28	–	–	–	–	–	–	–	–
CRP	–	–	–	–	–	–	–	–
Autoantibody-negative								
	n=442		n=408		n=376		n=311	
Pain	0.62	<0.0001	0.68	<0.0001	0.73	<0.0001	0.77	<0.0001
HAQ score	0.22	<0.0001	0.31	<0.001	0.18	0.005	0.22	0.002
SJC28	0.10	0.04	0.20	0.003	0.13	0.05	–	–
TJC28	0.10	0.10	–	–	–	–	–	–
CRP	–	–	–	–	–	–	n.i.	n.i.

*Adjusted for age, gender, disease duration (at T0), calendar year and type of treatment, including glucocorticoids. Independent associations were found for younger age and more recent calendar years in autoantibody-negative patients. The inverse correlation between use of prednisone and the PGA was not independent of other covariates. CRP, C reactive protein; HAQ, Health Assessment Questionnaire; n.i., not included; PGA, Patient Global Assessment; RA, rheumatoid arthritis; SJC28, Swollen Joint Count on 28 Joints; T0, time zero; TJC28, Tender Joint Count on 28 Joints.

Association of the PGA with inflammatory variables and disease impact measures over time in relation to remission status

To exclude that the time-related variations in the inflammatory correlates of the PGA could be attributable only to progressive reduction of disease activity in course of treatment, analyses were repeated in different remission status subgroups. As expected, in patients in non-remission, the PGA was associated not only with disease impact measures but also with the tender and swollen joint counts, although the correlation with SJC28 in autoantibody-negative patients became progressively lower over time and was lost at month 24 (figure 2). At multivariable analysis, SJC28 maintained an independent correlation at all time points in autoantibody-positive patients and only until month 12 in autoantibody-negative patients (table 3). In patients in near remission, variations in the PGA in autoantibody-negative subjects were only explained by pain. In contrast, in autoantibody-positive patients, the swollen joint count was also significantly and independently associated with the PGA at 6 and 12 months (figure 2 and table 3). Correction for multiplicity confirmed the statistical significance at 6 months, while

Table 3 Correlations of the PGA with disease impact measures and inflammatory variables over time in patients with early RA based on remission criterion: multivariable analysis

	T6			T12			T24		
	rem	near-rem	non-rem	rem	near-rem	non-rem	rem	near-rem	non-rem
	r part	r part	r part	r part	r part	r part	r part	r part	r part
Total cohort									
	n=102	**n=79**	**n=566**	**n=136**	**n=84**	**n=461**	**n=152**	**n=89**	**n=313**
Pain	0.45*	0.59*	0.58*	0.41*	0.56*	0.77*	0.27*	0.61*	0.75*
HAQ score	n.i.	n.i.	0.27*	–	–	0.22†	0.20†	n.i.	0.23*
SJC28	n.i.	n.i.	0.29*	0.18	n.i.	0.18‡	n.i.	n.i.	n.i.
TJC28	n.i.	n.i.	–	–	n.i.	–	n.i.	n.i.	–
CRP	n.i.	n.i.	n.i.	n.i.	n.i.	–	n.i.	n.i.	n.i.
Autoantibody-positive									
	n=55	**n=37**	**n=247**	**n=65**	**n=37**	**n=203**	**n=80**	**n=36**	**n=127**
Pain	0.70*	0.37*	0.52*	–	0.76*	0.74*	–	0.62*	0.69*
HAQ score	n.i.	–	0.24*	n.i.	0.36†	0.13†	n.i.	n.i.	0.20†
SJC28	n.i.	0.18†	0.31*	0.30†	0.38†	0.16†	0.25†	n.i.	0.12†
TJC28	n.i.	n.i.	0.13†	n.i.	–	–	–	n.i.	–
CRP	n.i.	n.i.	n.i.	n.i.	–	–	n.i.	n.i.	n.i.
Autoantibody-negative									
	n=47	**n=42**	**n=319**	**n=71**	**n=47**	**n=258**	**n=72**	**n=53**	**n=186**
Pain	0.57*	0.78*	0.65*	0.45*	0.37	0.81*	0.57*	0.64*	0.78*
HAQ score	–	–	0.35*	–	–	0.25*	–	n.i.	0.29*
SJC28	n.i.	n.i.	0.32*	n.i.	n.i.	0.22‡	n.i.	n.i.	n.i.
TJC28	n.i.	n.i.	–	–	n.i.	–	n.i.	n.i.	–
CRP	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.

*P<0.001.
†P<0.01.
‡P<0.05.
CRP, C reactive protein; HAQ, Health Assessment Questionnaire; n.i., not included; rem, remission; SJC28, Swollen Joint Count on 28 Joints; TJC28, Tender Joint Count on 28 Joints.

the p value at 12 months marginally exceeded the critical value (0.044>0.04). Accordingly, autoantibody-positive patients in near remission had slightly more swollen joints compared with patients in Boolean remission at both 6 and 12 months (mean SJC28 0.5 (SD 0.5) vs 0.3 (SD 0.4), p=0.02, and 0.5 (SD 0.5) vs 0.2 (SD 0.4), p=0.01), with a trend also at month 24 (0.5 (SD 0.5) vs 0.3 (SD 0.5), p=0.12).

Cohorts of established RA

All analyses on early RA were compared with two independent cross-sectional cohorts of patients with established RA in different disease activity states. The demographic and clinical characteristics of the two cohorts are summarised in table 1. In both cohorts, >80% of the patients were on active treatment with csDMARD (MTX in >60% of the cases), and none was or had been previously treated with bDMARDs/tsDMARDs. Glucocorticoids were taken by >80% of the patients with active disease and ~25% of the patients with LDA. Of the cohort of patients at least with LDA, 69% (n=145) were in remission according to the DAS28, 42.9% (n=90) in Boolean remission and 32.9% (n=69) in near remission.

In both cohorts, irrespective of disease activity, the PGA correlated strongly to moderately with the VAS pain score and the HAQ score on univariate and multivariable analyses. The correlation with inflammatory variables was instead low and did not appear independent of impact measures. The absence of correlation with joint swelling and CRP was striking in autoantibody-negative patients in both cohorts. In these subjects, joint tenderness appeared most strongly related to the PGA compared with swelling, although without statistical independence with respect to generalised pain and function at multivariable analysis (table 4). In contrast, in autoantibody-positive patients, SJC28 maintained a trend towards an independent relation with the PGA at low levels of disease activity (adjusted r=0.21, p=0.09) but not in active disease (table 4). Again, autoantibody-positive patients in near remission tended to have slightly more swollen joints compared with patients in Boolean remission (mean SJC28 0.2 (SD 0.4) vs 0.1 (SD 0.3), p=0.06).

DISCUSSION

The results of our study indicate that, in patients with RA, the significance of the PGA varies in relation to disease duration and autoantibody status. While in established disease the PGA is mostly driven by pain and functional disability, in the early stages of arthritis, the patient's perception is also significantly influenced by levels of inflammation. Importantly, the inflammatory correlates of the PGA are more persistent and pronounced across different disease activity states in autoantibody-positive patients. In contrast, in autoantibody-negative RA, the PGA loses association with inflammation soon after disease onset.

Pain is by far the most important contributor to the PGA in patients with RA,[9–13] and, over the course of the disease, it accumulates multiple causes beyond inflammation, including joint damage, impaired physical function, depression and central pain augmentation.[20 21] In contrast, in cohorts of patients with years of disease duration, disease activity variables such as swollen joints and acute phase reactants have much lower impact on the patient's perception of the disease, and their correlation with the PGA is poor[9 10 34] and mostly indirect even at high levels of inflammation.[10] Confirming the reliability of our measurement of the PGA, we also failed to demonstrate direct associations with inflammatory variables in two independent cohorts of established RA irrespective of disease activity. Yet, absence of overt inflammatory drivers cannot justify straight conclusions

Table 4 Correlations of the PGA with disease impact measures and inflammatory variables in established RA in relation to the Ab status: univariate and multivariable analyses

	Non-active disease, n=210				Active disease, n=401			
	Ab-pos, n=110		Ab-neg, n=100		Ab-pos, n=309		Ab-neg, n=92	
	r	P value	r	P value	r	P value	r	P value
Univariate analysis								
Pain	0.71	<0.0001	0.81	<0.0001	0.82	<0.0001	0.93	<0.0001
HAQ score	0.51	<0.0001	0.57	<0.0001	0.47	<0.0001	0.49	<0.0001
SJC28	0.36	0.003	0.17	0.15	0.27	<0.0001	0.29	0.006
TJC28	0.37	0.002	0.41	0.0002	0.36	<0.0001	0.42	<0.0001
CRP	0.22	0.08	0.21	0.09	0.18	0.002	0.20	0.06
Multivariable analysis*								
Pain	0.75	<0.0001	0.76	<0.0001	0.77	<0.0001	0.90	<0.0001
HAQ score	0.15	0.002	0.23	0.003	0.21	0.0003	0.25	0.03
SJC28	0.21	0.09	–	–	–	–	–	–
TJC28	–	–	0.21	0.09	–	–	0.19	0.10
CRP	–	–	–	–	–	–	–	–

*Adjusted for age, gender, disease duration, type of treatment including glucocorticoids. Independent associations were found for female gender in both Ab-positive and Ab-negative patients, and younger age in Ab-negatives. The correlations between higher treatment (use of prednisone and dose of methotrexate) and the PGA in patients with non-active disease were not independent of other covariates.
Ab, autoantibody; CRP, C reactive protein; HAQ, Health Assessment Questionnaire; PGA, Patient Global Assessment; RA, rheumatoid arthritis; SJC28, Swollen Joint Count on 28 Joints; TJC28, Tender Joint Count on 28 Joints.

on the disease domains that are captured by the PGA in long-standing RA.[6 7] Although PGA outliers undoubtedly reflect health components not susceptible to improve with immunosuppressive intervention,[5] certain intermediate levels may still indicate undetected residual disease activity associated with increased risk of functional deterioration.[2] Accordingly, the amended definition of remission which is currently being proposed only slightly relaxes the PGA cut-off to ≤2.[2 5]

Results of our study, however, add complexity to the interpretation of the PGA by demonstrating that its inflammatory correlates vary significantly in relation to disease duration and autoantibody status. In autoantibody-positive patients, swollen joints continue to explain part of the variability of the PGA at least in the first 2 years from disease onset, although with progressively decreasing strength. From a pathophysiological point of view, this finding fits with the notion that, in the early stages of RA, pain and the patient's perception of the disease may still be conditioned by nociception linked to the stimulation of proinflammatory cytokines.[20 21] In support of this view, it has been recently shown that joint tenderness correlates with ultrasound subclinical inflammation in early but not in established RA.[35] Importantly, the inflammatory correlates of the PGA in the early phases of autoantibody-positive RA span over different disease activity states, including near remission. The slightly higher swollen joint count in near remission compared with Boolean remission found here, together with our previous observation of an increased risk of missing remission because of persistently swollen joints despite a PGA of ≤1,[27] emphasises that, in these patients, the greatest difficulties in the interpretation of the PGA revolve around low levels of disease activity. As a consequence, even small variations in the thresholds used to define remission need to be carefully evaluated because increasing values of the PGA may drag increasing numbers of swollen joints and further increase in the intrinsic risk of bone damage already conferred by autoantibodies.[36 37]

In contrast, in autoantibody-negative patients, the inflammatory correlates of the PGA disappeared earlier and, more importantly, were only observed in the course of active disease. Discrepancies in the perception of disease activity between patients and physicians therefore appear to burden this disease subset since the very beginning, with significant consequences on treatment choices and outcomes.[38] Multiple factors beyond inflammation may affect the PGA in autoantibody-negative patients, including fibromyalgia,[24] age-related degenerative changes and comorbidities. However, autoantibody-negative RA also diverges in its pathogenetic mechanisms,[39] but the specific inflammatory pathways remain largely unexplored. In the absence of better clarification of the health domains relevant to the perspective of autoantibody-negative patients, any proposal on the 'acceptable' cut-off for the PGA risks remains speculative.

Strengths of our study include a large set of data collected longitudinally from patients with early disease, who were mostly disregarded in previous studies. Differently from patients with early RA from clinical trials, our cohort is representative of real-life conditions with short disease duration, moderate disease activity at inclusion and overall favourable disease course. The exclusion of patients switching to bDMARDs/tsDMARDs reduces confounding by factors that might further uncouple inflammation from PROs.[40 41] Clinical assessments and formulation of the PGA have remained mostly consistent over the years at our division, making the longitudinal analysis of our study reliable.

The conclusions of our study, however, need to be considered also in light of potential limitations. Our data refer to a population followed in a semitrial regimen, and the generalisability of our results in other settings needs to be confirmed. Different formulations and scales for the PGA may affect the results,[42] although the intercentre variance in PROs is in fact lower than expected.[43] Variables such fatigue, anxiety, depression and other comorbidities were not systematically recorded, preventing the assessment of all the possible associations of the PGA. However, residual pain at follow-up was relatively low in our cohort (mean VAS pain score 23.2 (SD 24.9) at 24 months), excluding that psychological factors and fibromyalgianess could invalidate the association between joint swelling and the PGA found here. Importantly, objective inflammation explained only a small proportion of the variability of the PGA also in our patients with early RA (5%–10%), similar to the published literature.[12–14] However, the observed differences in relation to disease duration and autoantibody status encourage further reflection on the inflammatory significance of the PGA. The frequency of autoantibody-negative patients in our early

RA cohort is high but in line with the recent trend,[44 45] and the enrichment in this subgroup increases the possibility to highlight differences that are relevant to the clinic. Loss to follow-up reached approximately 20%, in line with other real-life early RA cohorts.[22] Rates of drop-out were, however, comparable between autoantibody-positive and autoantibody-negative patients, with no noticeable imbalance in the disease activity state at the time of the last available assessment (DAS28 >3.2 in approximately 50% of the patients in both subgroups), making it unlikely that missing observations would substantially change the results. Also, although the exclusion of patients in bDMARDs/tsDMARDs may have reduced numbers in LDA states, again treatment escalation occurred at roughly similar rates in autoantibody-positive and autoantibody-negative patients (11.4% vs 7.7%, p=0.10).

Taken together, our data indicate that the interpretation of the PGA as a measure of disease activity in RA should take into account important variables such as disease duration and autoantibody status. Optimal cut-off values of the PGA capable of identifying absence of disease might even differ in different phases and autoantibody subgroups.

Contributors SB, LDS and CM conceived the work, accept full responsibility for the conduct of the study, had access to the data and controlled the decision to publish. SB and LDS contributed to the analysis and interpretation of data and drafted the manuscript. BD'O, AN, MdL, EM and GS contributed to the acquisition and the analysis of data and revised the manuscript critically. EGF, AM, RC and CM contributed to the interpretation of data and revised the manuscript critically for important intellectual content. All the authors provided final approval of the version to be published.

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Patient consent for publication Not applicable.

Ethics approval This study involves human participants and IRCCS Policlinico San Matteo Foundation Ethics Committee (number 20070001302.Milano-2 Ethics Committee) approved the study. The participants gave informed consent to participate in the study before taking part. The study was conducted according to the Declaration of Helsinki.

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ORCID iDs
Serena Bugatti http://orcid.org/0000-0002-5396-7077
Ennio Giulio Favalli http://orcid.org/0000-0003-1471-6467
Carlomaurizio Montecucco http://orcid.org/0000-0001-8263-3925

REFERENCES

1 Felson DT, Smolen JS, Wells G, *et al*. American College of Rheumatology/European League against rheumatism provisional definition of remission in rheumatoid arthritis for clinical trials. *Ann Rheum Dis* 2011;70:404–13.
2 Studenic P, Felson D, de Wit M, *et al*. Testing different thresholds for patient global assessment in defining remission for rheumatoid arthritis: are the current ACR/EULAR Boolean criteria optimal? *Ann Rheum Dis* 2020;79:445–52.
3 Olofsson T, Söderling JK, Gülfe A, *et al*. Patient-Reported outcomes are more important than objective inflammatory markers for sick leave in Biologics-Treated patients with rheumatoid arthritis. *Arthritis Care Res* 2018;70:1712–6.
4 Jones B, Flurey CA, Proudman S, *et al*. Considerations and priorities for incorporating the patient perspective on remission in rheumatoid arthritis: an OMERACT 2020 special interest group report. *Semin Arthritis Rheum* 2021;51:1108–12.
5 Ferreira RJO, Ndosi M, de Wit M, *et al*. Dual target strategy: a proposal to mitigate the risk of overtreatment and enhance patient satisfaction in rheumatoid arthritis. *Ann Rheum Dis* 2019;78:e109.
6 Boers M. Patient global assessment to define remission in rheumatoid arthritis:quo vadis? *Ann Rheum Dis* 2021;80:277–9.
7 Felson D, Lacaille D, LaValley MP, *et al*. Re-Examining remission definitions in rheumatoid arthritis: considering the 28-Joint disease activity score, C-reactive protein level and patient global assessment. *Ann Rheum Dis* 2022;81:4–7.
8 Ferreira RJO, Welsing PMJ, Jacobs JW, *et al*. Correspondence on 'Re-examining remission definitions in rheumatoid arthritis: considering the 28-joint Disease Activity Score, C reactive protein level and patient global assessment' by Felson, et al. *Ann Rheum Dis* 2022. [Epub ahead of print: 17 Feb 2022].
9 Studenic P, Radner H, Smolen JS, *et al*. Discrepancies between patients and physicians in their perceptions of rheumatoid arthritis disease activity. *Arthritis Rheum* 2012;64:2814–23.
10 Ward MM, Guthrie LC, Dasgupta A. Direct and indirect determinants of the patient global assessment in rheumatoid arthritis: differences by level of disease activity. *Arthritis Care Res* 2017;69:323–9.
11 Radner H, Yoshida K, Tedeschi S, *et al*. Different rating of global rheumatoid arthritis disease activity in rheumatoid arthritis patients with multiple morbidities. *Arthritis Rheumatol* 2017;69:720–7.
12 Ferreira RJO, Duarte C, Ndosi M, *et al*. Suppressing inflammation in rheumatoid arthritis: does patient global assessment blur the target? A practice-based call for a paradigm change. *Arthritis Care Res* 2018;70:369–78.
13 Ferreira RJO, Dougados M, Kirwan JR, *et al*. Drivers of patient global assessment in patients with rheumatoid arthritis who are close to remission: an analysis of 1588 patients. *Rheumatology* 2017;56:1573–8.
14 Ferreira RJO, Carvalho PD, Ndosi M, *et al*. Impact of patient's global assessment on achieving remission in patients with rheumatoid arthritis: a multinational study using the Meteor database. *Arthritis Care Res* 2019;71:1317–25.
15 Delcoigne B, Provan SA, Hammer HB. Do patient-reported measures of disease activity in rheumatoid arthritis vary between countries? results from a Nordic collaboration. *Rheumatology* 2022;9:keac081.
16 Bugatti S, Manzo A, Caporali R, *et al*. Assessment of synovitis to predict bone erosions in rheumatoid arthritis. *Ther Adv Musculoskelet Dis* 2012;4:235–44.
17 Navarro-Compán V, Gherghe AM, Smolen JS, *et al*. Relationship between disease activity indices and their individual components and radiographic progression in RA: a systematic literature review. *Rheumatology* 2015;54:994–1007.
18 Ferreira RJO, Welsing PMJ, Jacobs JWG, *et al*. Revisiting the use of remission criteria for rheumatoid arthritis by excluding patient global assessment: an individual meta-analysis of 5792 patients. *Ann Rheum Dis* 2021;80:293–303.
19 Hazes JMW. Determinants of physical function in rheumatoid arthritis: association with the disease process. *Rheumatology* 2003;42 Suppl 2:17ii–21.
20 Walsh DA, McWilliams DF. Mechanisms, impact and management of pain in rheumatoid arthritis. *Nat Rev Rheumatol* 2014;10:581–92.
21 McWilliams DF, Walsh DA. Pain mechanisms in rheumatoid arthritis. *Clin Exp Rheumatol*2017;107:94–101.
22 Ferreira RJO, Fautrel B, Saraux A, *et al*. Patient global assessment of disease activity and radiographic progression in early arthritis: three-year results from the ESPOIR cohort. *Arthritis Care Res* 2021;73:1300–5.
23 Bugatti S, De Stefano L, Favalli EG, *et al*. Increasing the threshold for patient global assessment in defining remission may have a different impact in patients with early and established rheumatoid arthritis. *Ann Rheum Dis* 2022;81:e55.
24 Doss J, Mo H, Carroll RJ, *et al*. Phenome-Wide association study of rheumatoid arthritis subgroups identifies association between seronegative disease and fibromyalgia. *Arthritis Rheumatol* 2017;69:291–300.
25 Arnett FC, Edworthy SM, Bloch DA, *et al*. The American rheumatism association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;31:315–24.
26 Aletaha D, Neogi T, Silman AJ, *et al*. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League against rheumatism collaborative initiative. *Ann Rheum Dis* 2010;69:1580–8.
27 Montecucco C, Todoerti M, Sakellariou G, *et al*. Low-Dose oral prednisone improves clinical and ultrasonographic remission rates in early rheumatoid arthritis: results of a 12-month open-label randomised study. *Arthritis Res Ther* 2012;14:R112.
28 Balduzzi S, Scirè CA, Sakellariou G, *et al*. In early inflammatory polyarthritis more intensive management according to the 2010 ACR/EULAR criteria leads to higher rates of clinical remission: comparison of two cohorts treated according to different treat-to-target protocols. *Clin Exp Rheumatol* 2017;35:401–5.
29 Bugatti S, De Stefano L, Manzo A, *et al*. Limiting factors to Boolean remission differ between autoantibody-positive and -negative patients in early rheumatoid arthritis. *Ther Adv Musculoskelet Dis* 2021;13:1759720X211011826.
30 Felson DT, Smolen JS, Wells G, *et al*. American College of Rheumatology/European League against rheumatism provisional definition of remission in rheumatoid arthritis for clinical trials. *Ann Rheum Dis* 2011;70:404–13.

31 Studenic P, Smolen JS, Aletaha D. Near misses of ACR/EULAR criteria for remission: effects of patient global assessment in Boolean and index-based definitions. *Ann Rheum Dis* 2012;71:1702–5.
32 Munro BH. *Statistical methods for health care research*. 3rd ed. New York: Lippincott Williams & Wilkins, 1997.
33 Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B* 1995;57:289–300.
34 Khan NA, Spencer HJ, Abda E, *et al*. Determinants of discordance in patients' and physicians' rating of rheumatoid arthritis disease activity. *Arthritis Care Res* 2012;64:206–14.
35 Gessl I, Popescu M, Schimpl V, *et al*. Role of joint damage, malalignment and inflammation in articular tenderness in rheumatoid arthritis, psoriatic arthritis and osteoarthritis. *Ann Rheum Dis* 2021;80:884–90.
36 Bugatti S, Bogliolo L, Montecucco C, *et al*. B cell autoimmunity and bone damage in rheumatoid arthritis. *Reumatismo* 2016;68:117–25.
37 Bugatti S, Manzo A, Montecucco C, *et al*. The clinical value of autoantibodies in rheumatoid arthritis. *Front Med* 2018;5:339.
38 Matthijssen XME, Niemantsverdriet E, Huizinga TWJ, *et al*. Enhanced treatment strategies and distinct disease outcomes among autoantibody-positive and -negative rheumatoid arthritis patients over 25 years: a longitudinal cohort study in the Netherlands. *PLoS Med* 2020;17:e1003296.
39 De Stefano L, D'Onofrio B, Manzo A, *et al*. The genetic, environmental, and immunopathological complexity of Autoantibody-Negative rheumatoid arthritis. *Int J Mol Sci* 2021;22:12386.
40 Sergeeva M, Rech J, Schett G, *et al*. Response to peripheral immune stimulation within the brain: magnetic resonance imaging perspective of treatment success. *Arthritis Res Ther* 2015;17:268.
41 Simon LS, Taylor PC, Choy EH, *et al*. The JAK/STAT pathway: a focus on pain in rheumatoid arthritis. *Semin Arthritis Rheum* 2021;51:278–84.
42 Nikiphorou E, Radner H, Chatzidionysiou K, *et al*. Patient global assessment in measuring disease activity in rheumatoid arthritis: a review of the literature. *Arthritis Res Ther* 2016;18:251.
43 Khan NA, Spencer HJ, Nikiphorou E, *et al*. Intercentre variance in patient reported outcomes is lower than objective rheumatoid arthritis activity measures: a cross-sectional study. *Rheumatology* 2017;56:1395–400.
44 Myasoedova E, Davis J, Matteson EL, *et al*. Is the epidemiology of rheumatoid arthritis changing? results from a population-based incidence study, 1985-2014. *Ann Rheum Dis* 2020;79:440–4.
45 Matthijssen XME, Huizinga TWJ, van der Helm-van Mil AHM. Increasing incidence of autoantibody-negative RA is replicated and is partly explained by an aging population. *Ann Rheum Dis* 2022;81:e69.

TRANSLATIONAL SCIENCE

Interferon-α-mediated therapeutic resistance in early rheumatoid arthritis implicates epigenetic reprogramming

Faye A H Cooles ,[1] Jessica Tarn ,[1] Dennis W Lendrem ,[1] Najib Naamane,[1] Chung MA Lin,[1] Ben Millar,[1] Nicola J Maney ,[1] Amy E Anderson,[1] Nishanthi Thalayasingam,[1] Julie Diboll,[1] Vincent Bondet,[2] Darragh Duffy,[2,3] Michael R Barnes,[4] Graham R Smith,[5] Sandra Ng,[4] David Watson,[6] Rafael Henkin,[4] Andrew P Cope,[7] Louise N Reynard,[8] Arthur G Pratt ,[1,9] RA-MAP Consortium, John D Isaacs [1,9]

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For numbered affiliations see end of article.

Correspondence to
Dr Faye A H Cooles, Translational and Clinical Research Institute, Newcastle University, Newcastle upon Tyne, Newcastle upon Tyne, UK; faye.cooles@ncl.ac.uk

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ABSTRACT

Objectives An interferon (IFN) gene signature (IGS) is present in approximately 50% of early, treatment naive rheumatoid arthritis (eRA) patients where it has been shown to negatively impact initial response to treatment. We wished to validate this effect and explore potential mechanisms of action.

Methods In a multicentre inception cohort of eRA patients (n=191), we examined the whole blood IGS (*MxA, IFI44L, OAS1, IFI6, ISG15*) with reference to circulating IFN proteins, clinical outcomes and epigenetic influences on circulating CD19+ B and CD4+ T lymphocytes.

Results We reproduced our previous findings demonstrating a raised baseline IGS. We additionally showed, for the first time, that the IGS in eRA reflects circulating IFN-α protein. Paired longitudinal analysis demonstrated a significant reduction between baseline and 6-month IGS and IFN-α levels (p<0.0001 for both). Despite this fall, a raised baseline IGS predicted worse 6-month clinical outcomes such as increased disease activity score (DAS-28, p=0.025) and lower likelihood of a good EULAR clinical response (p=0.034), which was independent of other conventional predictors of disease activity and clinical response. Molecular analysis of CD4+ T cells and CD19+ B cells demonstrated differentially methylated CPG sites and dysregulated expression of disease relevant genes, including *PARP9, STAT1,* and *EPSTI1*, associated with baseline IGS/IFNα levels. Differentially methylated CPG sites implicated altered transcription factor binding in B cells (GATA3, ETSI, NFATC2, EZH2) and T cells (p300, HIF1α).

Conclusions Our data suggest that, in eRA, IFN-α can cause a sustained, epigenetically mediated, pathogenic increase in lymphocyte activation and proliferation, and that the IGS is, therefore, a robust prognostic biomarker. Its persistent harmful effects provide a rationale for the initial therapeutic targeting of IFN-α in selected patients with eRA.

WHAT IS ALREADY KNOWN ABOUT THIS TOPIC

⇒ Type I interferons (IFNs) and the IFN gene signature (IGS) have received less attention in rheumatoid arthritis (RA) than in other rheumatic diseases such as systemic lupus erythematous.

⇒ Nonetheless, emerging evidence hints at a potentially important role for the IGS in early disease although, until now, it was unknown which IFN class was responsible.

WHAT DOES THIS STUDY ADD?

⇒ We demonstrate that IFN-alpha levels are transiently elevated in some early RA patients and are responsible for generating the IGS.

⇒ We validate the IGS as a robust prognostic biomarker associated with poor 6 month outcomes.

⇒ We also implicate IFN-α/IGS in epigenetic modification of circulating B and T lymphocytes, at genes associated with activation and proliferation, providing a potential mechanism for its persistent harmful effects.

HOW MIGHT THIS IMPACT ON CLINICAL PRACTICE

⇒ Our data provide a strong rationale for the use of therapies that target the IFN-α pathway and the IGS in selected early RA patients.

⇒ Our work also has implications for other conditions with high IFN-α levels, such as COVID-19, and the potential for persistent harmful sequelae.

INTRODUCTION

An interferon gene signatures (IGS) has been reported in multiple autoimmune conditions, including rheumatoid arthritis (RA).[1] It is a composite score of interferon response genes (IRGs) that are classically upregulated in response to type 1 interferons (IFN-I). IFN-I are released on detection of viral/bacterial genetic material by various nucleic acid receptors (NARs),[1 2] but the pathway by which IFN production is triggered in RA is unknown. Furthermore, there is an overlap between downstream signalling pathways for all interferon classes with upregulation of common

IRGs.[3] Historically, the direct measurement of IFN-I has been challenging,[3] creating uncertainty around which IFN class drives the IGS in RA thereby limiting understanding of its pathophysiological relevance.

To date, no association has been reported between the IGS and disease activity in established RA. However, longstanding patients with RA are frequently prescribed additional therapies, which modulate the IGS.[4 5] By contrast, we previously demonstrated in early RA (eRA) patients (naïve for disease-modifying antirheumatic drugs (DMARDs) and glucocorticoids), the IGS positively associates with baseline disease activity and, independent of conventional markers of disease activity, associates with worse clinical outcomes at 6 months.[6] The pathophysiological processes in eRA are distinct from those of established disease and the IGS is more prominent in eRA than in established RA.[6] The role of epigenetics in modifying phenotype is increasingly appreciated in autoimmunity[7] and RA has an early window of therapeutic opportunity. Thus, understanding and predicting heterogeneity to therapeutic response is important for early precision therapeutics.

In a large multicentre eRA cohort, we sought to: (1) confirm the IGS negatively impacts disease outcomes, (2) clarify which IFN classes are responsible for IGS generation, (3) seek evidence that IFN-α exposure contributes to a harmful epigenetic footprint at disease onset, potentially explaining its negative effect on longer-term outcomes.

METHODS

Patient cohorts

DMARD and glucocorticoid naive patients with eRA were recruited from the Newcastle Early Arthritis Clinic (NEAC) as described previously.[6 8] Data and samples relating to the 'Towards A CurE for RA' (TACERA) cohort,[9] an existing additional independent cohort of eRA was obtained from RA-MAP, a multicentre UK industry-academic partnership. All patients with eRA met the 1987[10] or 2010[11] RA classification criteria. TACERA patients were included according to the availability of clinical and transcriptome data (quality controlled) and biological samples. In some analyses, missing data sets, particularly longitudinal clinical data, reduced cohort size.

Clinical parameters including autoantibody titres (anticitrullinated protein/peptide antibody (ACPA) and rheumatoid factor (RF)), disease activity score (DAS-28) and its components were recorded. For TACERA at 6 months, DAS-28 was repeated, drug history recorded and additional biological samples were collected. All patients gave informed consent as described in Clark *et al* and RA-MAP Consortium.[8 9]

Patient and public involvement

This research was done without formal patient and public involvement.

Serum cytokines

Serum IFN-α was measured using the digital Simoa platform as described.[12] Monoclonal antibodies (specifically for all IFN-α subtypes) were isolated from autoimmune polyendocrinopathy candidiasis ecto-dermal dystrophy patients[13] and provided to D. Duffy by Immunoqure under a material transfer agreement. IFN-β, IFN-γ and IFN-λ1/IL29 (referred to hereafter as IFN-λ) were measured by MSD technology (Meso Scale Discovery, MD, USA) as per manufacturers' instructions.

Whole blood and cell-specific transcriptome/methylome

TACERA whole blood analyses used Tempus blood RNA tubes (Applied Biosystems). Peripheral blood mononuclear cells (PBMCs) were isolated using Leucosep separation tubes (Greiner) followed by MagMAX RNA isolation kits (Ambion). For subsequent microarray analysis, amplified RNA was hybridised to beadchips and scanned on an Illumina Beadstation 500 as described further in.[9] Full data are available via Gene Expression Omnibus database (GEO), http://www.ncbi.nlm.nih.gov/geo accession number GSE9747638. Existing paired microarray gene expression and DNA methylation data from CD4+ T cells and CD19+ B cells extracted from NEAC eRA patients was preprocessed as described in Clark *et al*,[8] GEO accession number GSE137634. In brief, this involved positive selection of CD4+ T cells/CD19+ B cells, RNeasy Mini kits or AllPrep DNA/RNA Mini kits (Qiagen) and Illumina Whole Genome 6 V.3/12HT BeadChip or a MethylationEPIC BeadChip for RNA and DNA, respectively. Additional method details included in online supplemental file 1.0.

Whole blood IGS

For both cohorts, the IGS was calculated as an average of whole blood or cell-specific expression of *MX1*, *IFI44L*, *OAS1*, *ISG15* and *IFI6*. IGS scores in the first or fourth quartiles were termed IGS high or low respectively.

Gene expression and DNA methylation analysis of eRA lymphocytes

Analyses included differential gene expression (DEGs) and differential methylation sites (DMSs) between IGS high and low eRA patients, effect of methylation on gene expression, pathway analysis of DEGs and enrichment analysis of DMSs within defined chromatin states. Full methods are provided in online supplemental file 1.0.

Modelling and statistical analysis

GraphPad Prism (V.5.0; GraphPad Software, La Jolla, Calif), JMP Statistical Visualisation (V.14; SAS Institute, Cary, North Carolina) and R Core Team (2020) software was used. Tests included Mann-Whitney U, Wilcoxon matched-pairs signed rank tests, simple linear regression, generalised linear models, multivariable and logistic regression. To adjust for potential confounding variables, tests of a significant association between IGS and 6 month outcomes were performed after adjustment for erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), age, sex, baseline DAS28 and DMARDs, including glucocorticoids. R^2 values are reported as a measure of how well the regression model fits the observed data. Statistical significance when $p<0.05$.

RESULTS

Patient cohorts

The TACERA cohort included 191 seropositive (ACPA and/or RF positive) patients with eRA. A separate NEAC cohort of mixed seropositive and seronegative (ACPA and RF negative) patients with eRA had paired transcriptome and methylome data from circulating B and/or T lymphocytes (n=41 and n=41, respectively, total cohort of n=54) with contemporaneous T and B cell data being available for 28. Patient demographics and clinical characteristics are shown in table 1. The demographic data and relevant methods of an additional smaller validation cohort of patients with NEAC (n=51) who had additional circulating inflammatory cytokines measured in addition to serum-IFN-α and the IGS are shown in online supplemental file 2.0.

Table 1 Early RA cohort demographics

			NEAC	
			NEAC lymphocyte methylome and transcriptome cohorts	
		RA-MAP TACERA cohort	CD4+ T cell	CD19+ B cell
Number (n)		191	41	41
Age, years		55 (20–84)	58 (27–74)	58 (27–74)
Female, n (%)		116 (61%)	26 (63%)	30 (73%)
RF positive, n (%)		155* (90%)	23 (56%)	26 (63%)
ACPA positive, n (%)		147* (85%)	17 (41%)	22 (54%)
DAS-28-CRP		5.27 (2.23–8.14)	4.61 (1.26–6.53)	4.36 (1.26–6.53)
CRP (mg/L)		8.65 (1-136)	9 (5–13)	9.5 (4–53)
Erythrocyte sedimentation rate (mm/h)		28 (2–113)	19 (7–32)	20.5 (2–86)
DMARDS initiated	Number with available data	175		
	MTX	144 (82%)		
	SSZ	10 (6%)		
	HCQ	91 (52%)		
	LFU	0		
	None	0		
	Glucocorticoid	124 (71%)		

*Missing data for 18 patients.
ACPA, anti-citrullinated protein/peptide antibody; CRP, C reactive protein; DAS-28, disease activity score; DMARDS, disease-modifying anti-rheumatic drugs; HCQ, hydroxychloroquine; LFU, leflunomide; MTX, methotrexate; NEAC, Newcastle Early Arthritis Clinic; RA, rheumatoid arthritis; RF, rheumatoid factor; SSZ, sulfasalazine; TACERA, Towards A CurE for RA.

Disease-modifying therapy (DMARD) and glucocorticoid naive patients with eRA were recruited at the time of diagnosis from two independent cohorts, RA-MAP TACERA and NEAC. The clinical characteristics and demographics are displayed. Median values with ranges are displayed for continuous variables. For the NEAC-matched methylation and transcription cohort, the total number of patients was 54 with contemporaneous T and B cell data being available for 28.

Baseline IGS but not baseline IFN-α is associated with 6-month clinical outcomes

We sought to examine the effect of the IGS and IFN-α on initial clinical outcomes. No significant association between baseline IGS and baseline disease activity (DAS-28, p=0.202) was observed among 171 individuals from the TACERA cohort for whom data were available. Nonetheless, in keeping with our previous findings,[6] DAS-28 at 6 months positively associated with the baseline whole blood IGS (n=165, linear regression, p=0.02, R^2=0.245), figure 1A. Furthermore, this effect was independent of sex, age and other known confounding variables, including baseline DAS-28, CRP, ESR, glucocorticoids and DMARDs initiated (n=165, multivariable regression analysis, p=0.017). Crucially the interaction term between baseline IGS and baseline DAS-28 is non-significant (p=0.368), indicating that this effect was independent of baseline DAS-28. This is demonstrated graphically by grouping patients according to baseline disease activity (low DAS-28 <3.1; moderate 3.2–5.1; high ≥5.1), where the relationship between baseline IGS and 6-month outcome is consistent across baseline disease activity groups, figure 1B. Smoking and ACPA status similarly demonstrated no significant impact on 6-month outcomes (p=0.399 and p=0.555, respectively) when included in the regression model. In summary, higher baseline IGS scores predicted smaller reductions in DAS-28 (and, therefore, reduced clinical improvements) at 6 months.

When classifying/scoring 6-month disease activity into EULAR response outcomes (good, moderate and none) patients with higher baseline IGS scores were less likely to achieve a good EULAR response at 6 months (p=0.034, logistic regression), figure 1C. This was again independent of the above variables.

Baseline IFN-α significantly positively associated with both baseline DAS28 (p=0.018) and ESR (p<0.0001), but not CRP (p=0.053) with similar but less marked associations seen at 6 months (DAS-28 p=0.048, ESR p=0.049, CRP p=0.146), demonstrating that IFN-α levels correlate with disease activity (online supplemental file 3). However, unlike the IGS, baseline IFN-α did not associate with 6-month DAS-28 (p=0.557, linear regression), figure 1D, nor when corrected for the above variables (p=0.57, multivariable regression analysis). IFN-β, -γ or -λ levels did not associate with disease activity at any time point or predict any clinical outcomes (p>0.2 for all, data not shown).

Circulating IFN-α drives the IGS in eRA

To elucidate which class of IFN is directly responsible for the IGS in eRA, circulating IFN-α, IFN-β, IFN-γ and IFN-λ were examined in relation to the IGS (TACERA cohort).

There was a strong positive association between the IGS and circulating IFN-α (n=164, R^2=0.417, p<0.0001, linear regression). Most IFN-β measurements were below the detection threshold but, where detectable, there was no significant association with the IGS (p=0.817, n=53). There was no association between the IGS and IFN-λ (p=0.345, n=117) nor with IFN-γ (p=0.065, n=158), figure 2A. An additional NEAC cohort (n=51) validated the significant association between IFN-α and the IGS (p=0.004), online supplemental file 2.0. In addition, there was no association between the IGS and either TNF-α, IFN-γ, IL6, IL-10, IL12-p70 and IL1β (p>0.1 for all) nor was there any association between IFN-α and any of the above cytokines (p>0.1 for all), online supplemental file 2.0.

As shown previously,[6] the IGS significantly fell between baseline and 6 months (n=165, p<0.0001, Wilcoxon signed rank test), figure 2B. Longitudinal serum IFN-α values mirrored the IGS, with a significant fall over 6 months (n=161, p<0.0001). IFN-β, IFN-γ or IFN-λ levels remained static over this period

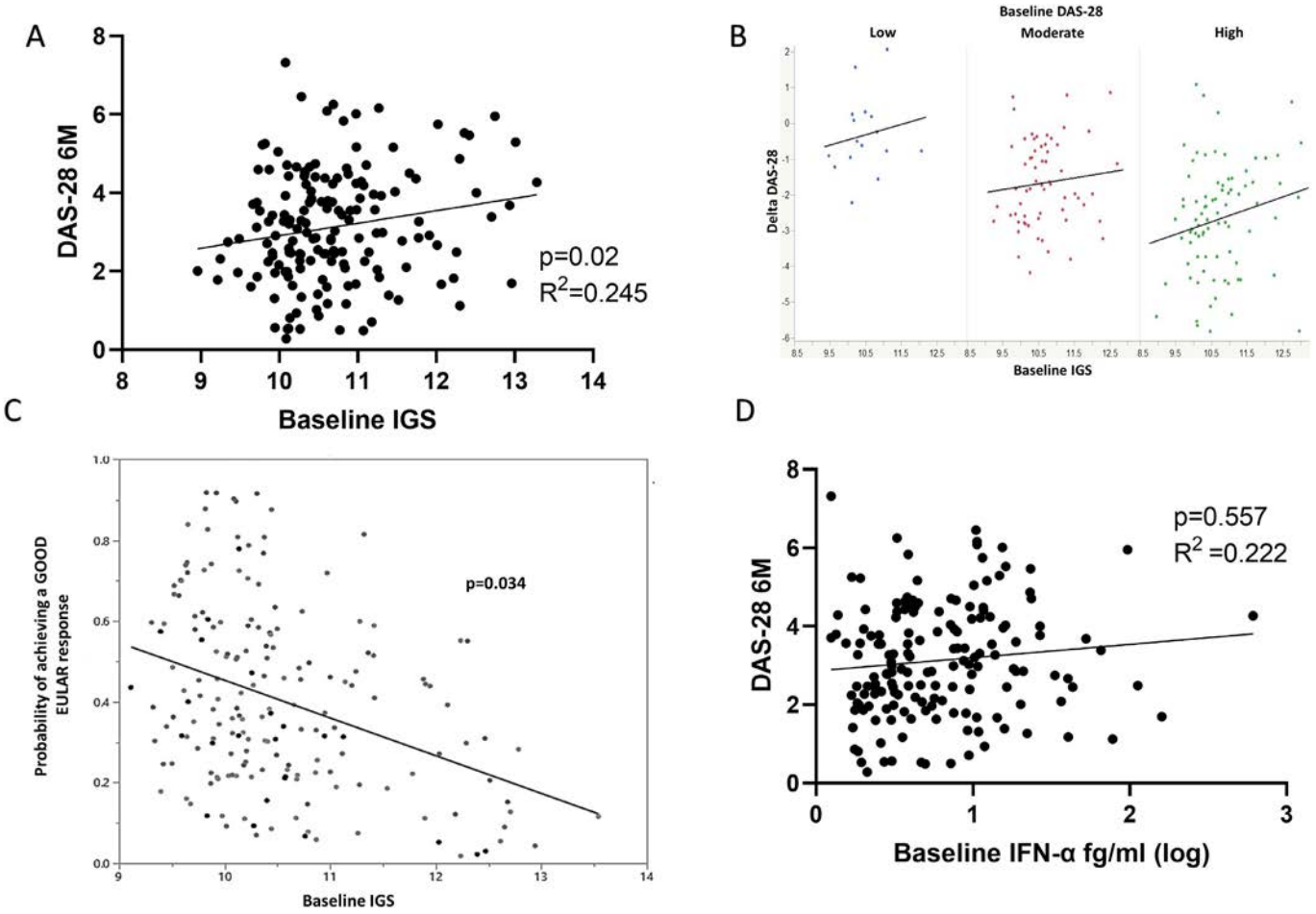

Figure 1 The IGS, circulating IFN-α and clinical outcomes. Early drug naïve RA patients (eRA, n=165) had their IGS calculated from whole blood microarray expression of *IFI6, OAS1, MxA, ISG15, IFI44L* and the impact of this baseline IGS on 6 month clinical outcomes sought. (A) Linear regression between baseline IGS and DAS-28 at 6 months (6M), p=0.02, R^2=0.245. (B) Graphical depiction of baseline IGS consistently impacting on change in DAS-28 at 6 months (Delta DAS-28) regardless of baseline DAS-28 (p=0.017, multivariable regression). Blue dots represent patients with baseline low DAS-28(<3.1), red moderate DAS-28 (3.2–5.1) and green high DAS-28 (>5.1). A negative Delta DAS-28 (Y axis) denotes a fall in DAS-28 and therefore response to therapy. (C) Relationship between the probability of achieving a good EULAR response at 6 months and baseline IGS. Nominal logistic regression, age, sex, DMARD, baseline DAS-28 and glucocorticoid administration corrected, p=0.034. (D) Linear regression between baseline IFN-α and DAS-28 at 6 months (6M), p=0.557, R^2=0.222. DAS-28, disease activity score; DMARDS, disease-modifying antirheumatic drugs; IFN, interferon; IGS, gene signature; RA, rheumatoid arthritis.

(p=0.275, p=0.819 and p=0.453, respectively), figure 2C. Furthermore, changes in circulating IFN-α correlated with the IGS (p<0.0001, multivariate analysis), but this was not seen for IFN-β, -λ or –γ (p>0.5 for all), online supplemental file 4. Finally, circulating IFN-α itself did not correlate with any other IFN (-β, -γ or –λ) measured at baseline (p>0.7 for all, data not shown). These data, in toto, suggest that the IGS is driven by circulating IFN-α in eRA.

To compare IFN-α levels in blood and target tissue, IFN-α was measured in matched synovial fluid and serum samples from five patients with RA. Full demographic and descriptive information (not represented in table 1) are shown in online supplemental file 5. There was no significant difference (p=0.8) between serum and synovial fluid IFN-α levels (median 3.93 fg/mL and 4.54 fg/mL, respectively), figure 2D.

IFN-α/IGS signalling pathways and effect on circulating haematological parameters

IFN-α production is triggered by NAR ligation. Whole blood mRNA expression of key NARs or their signalling proteins, TLR9, TLR7, TMEM173 (STING) and DDX58 (RIG-1) was, therefore, examined between IGS high and low eRA in the TACERA cohort. Expression of RNA sensing NARs RIG-1 and TLR7 was significantly increased in the IGS high patients (p<0.0001 for both). This was not seen for DNA sensing NARs nor their signalling components, TLR9 and TMEM173 (STING) (p=0.424 and p=0.609, respectively), figure 3A. Furthermore, circulating IFN-α and the IGS positively associated with whole blood expression of TLR7 (p=0.0002, R^2=0.191 and p<0.0001 R^2=0.216, respectively, linear regression) and RIG-1 (p<0.0001, R^2=0.216 and p<0.0001 R^2=0.458, respectively). Again this was unique to RNA sensing NARs with no significant association observed between either the IFN-α or the IGS and expression of TLR9 (p=0.926 and p=0.431) or TMEM173 (p=0.835 and p=0.738), figure 3B. TLR7 overexpression, particularly in relation to TLR9 expression, has been linked to autoimmunity and there was a significant positive association between ratio of whole blood TLR7:TLR9 and the IGS (p=0.0003, R^2=0.075) as well as with IFN-α (p=0.037, R^2=0.027) figure 3C. A similar pattern was observed in circulating PBMCs, online supplemental file 6.

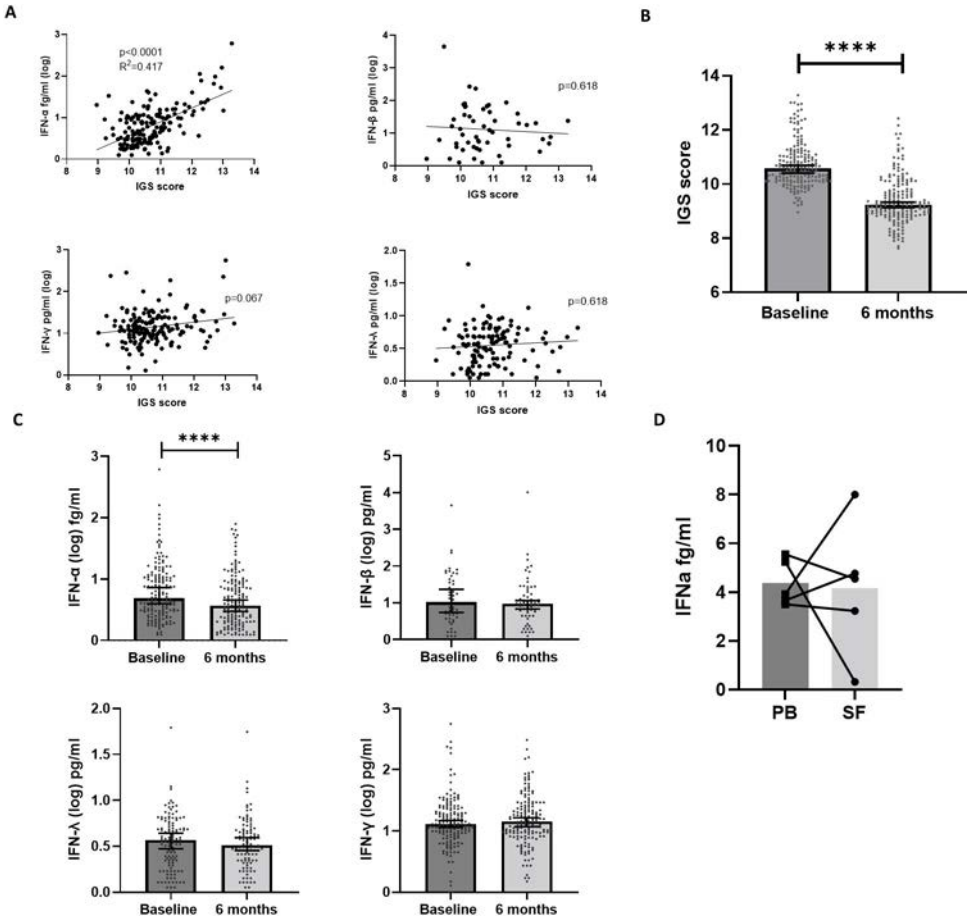

Figure 2 Circulating IFN-I, -II, –III, the IGS and longitudinal expression. (A) Linear regression was performed between the IGS and circulating IFN-α (n=163, p<0.0001, R^2=0.29), IFN-β (n=53, p=0.817, R^2=0.001), IFN-γ (n=164, p=0.067, R^2=0.034), IFN-λ (n=117, p=0.345, R^2=0.007). (B) Paired IGS scores between baseline and 6 months in eRA (n=165). Median values are depicted with 95% CIs and statistical analysis using Wilcoxon signed rank test performed on the differences between baseline and 6 months. (C) Comparisons between baseline and 6 month circulating levels of IFN-α, IFN-β, IFN-γ and IFN-λ. Median values are depicted with 95% CIs and statistical analysis using Wilcoxon signed rank test performed on the differences between baseline and 6 months. (D) Comparison of circulating peripheral blood (PB) IFN-α and synovial fluid (SF) IFN-α from five patients with RA, four of whom had established RA and one who had early RA, Wilcoxon signed rank test demonstrated no significant difference. Median values are depicted with paired samples demonstrated. ****p<0.0001. IFN, interferon; IGS, gene signature; RA, rheumatoid arthritis.

IFN-α is known to affect B cell function so associations with autoantibody titres were sought. RF titre strongly positively correlated with baseline IFN-α (p<0.0001, R^2=0.183) but not with baseline IGS (p=0.091). There was no association between RF titre and IFN-β (p=0.379) nor with IFNγ (p=0.230), but there was a weak positive association with IFN-λ (p=0.005, R^2=0.069), figure 3D and online supplemental file 7. ACPA titres did not correlate with either the IGS nor any interferon examined (p>0.1 for all), figure 3D and online supplemental file 5.

IGS correlates with site-specific DNA methylation in B and T cells

Since both IFN-I levels and IGS fall at 6 months in the context of a continued apparent influence on disease activity, we hypothesised that IFN-I-mediated/associated epigenetic alterations may be a plausible mechanism, whereby gene expression programmes in lymphocytes become persistently dysregulated in eRA in response to IFN signalling. We, thus, examined genome-wide transcriptional and methylation data from CD4+ T and CD19+ B lymphocytes isolated from an independent cohort of NEAC patients with eRA (table 1).

Of 330 CpGs were differentially methylated between IGS high and low CD4+ T cells (57.2% hypomethylated in IGS high, online supplemental file 8) and 287 in CD19+ B cells (58.1% hypomethylated in IGS high, online supplemental file 8). Of the 287 DMSs in CD19+ B cells, 17 (5.9%) showed similar changes in CD4+ T cells, with 16 being hypomethylated in IGS high for both (figure 4A). In addition, 65 DEGs were identified between IGS high and IGS low in CD4+ T cells and 40 in CD19+ B cells. Twelve of these genes were increased for both T and B cells in IGS high patients (figure 4A), online supplemental file 9.

Pathway analysis of DEGs (online supplemental file 10) demonstrated increased expression of genes related to RIG-I and TLR signalling in CD19+ B and CD4+ T cells, respectively, with a significant increase in antiviral pathways and IFN-I signalling. In T cells, pathway analysis also demonstrated significantly increased gene expression linked to RA.

Analysis of genes whose expression correlated with DMSs at relevant loci demonstrated multiple IRGs, such as *IFI44L*, *RSAD2* and *Mx1*, online supplemental file 11. Of interest to RA pathophysiology, there was increased expression of *PARP9* and *EPSTI1* in B cells and *STAT1* in CD4+ T cells in IGS high patients, which negatively correlated with methylation DMSs

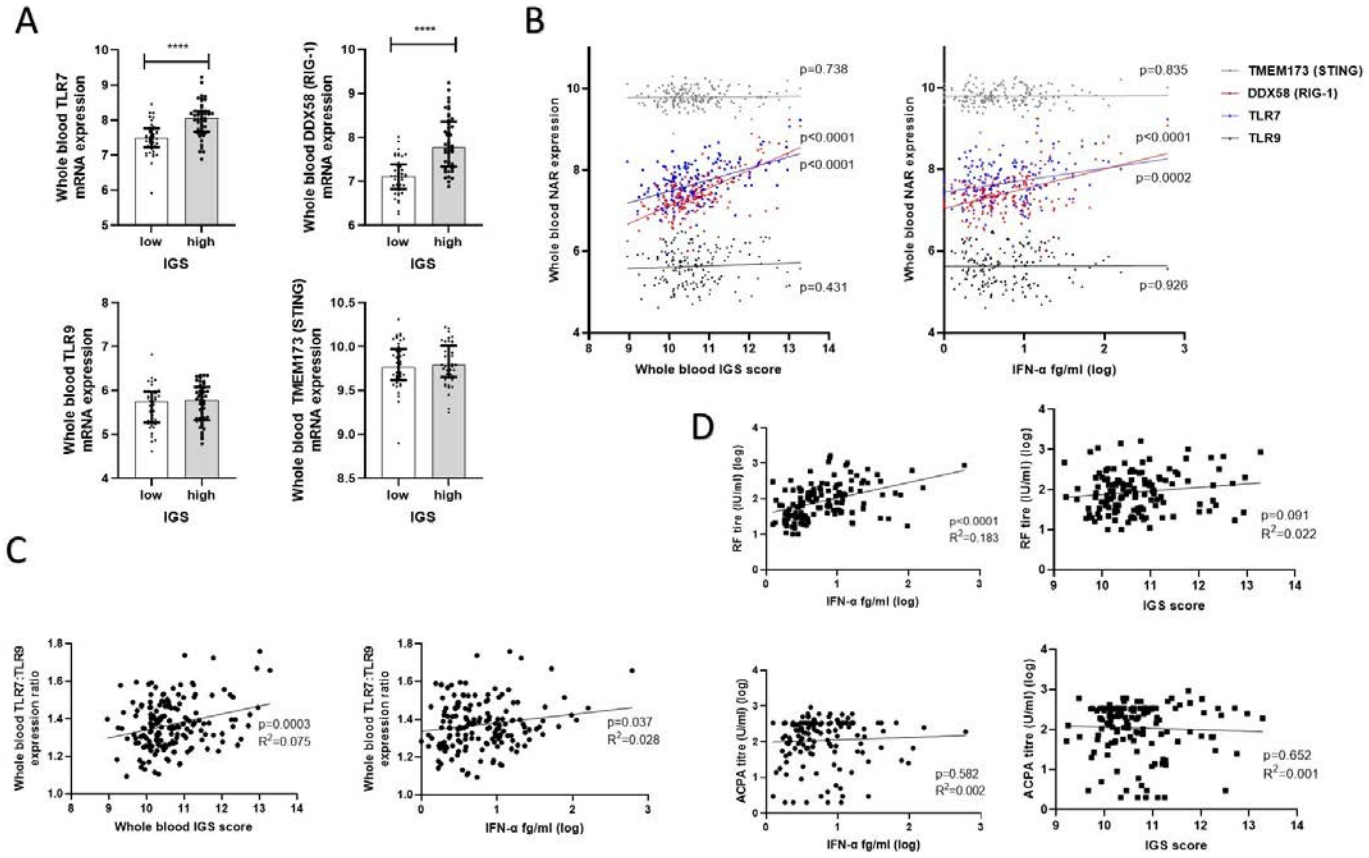

Figure 3 IFNs, signalling pathways and autoantibody titres. Whole blood expression of nucleic acid receptors (NARs) was examined in early RA TACERA cohort with respect to IFN-α/the IGS. (A) Expression of surface and cytosolic nucleic acid receptors, TLR7, TLR9 DDX58 (RIG-1) and TMEM173 (STING) were examined between IGS high and low patients, n=43 in each cohort. Median values with interquartile ranges are shown. Mann-Whitney U tests were performed. (B) Linear regression between the whole blood IGS or circulating IFN-α and whole blood mRNA expression of TLR7, TLR9 DDX58 (RIG-1) and TMEM173 (STING), n=164. P values are depicted in the figure. (C) Linear regression between whole blood ratio of TLR7: TLR9 mRNA expression and the whole blood IGS score or circulating IFN-α (fg/ml) in 164 eRA patients. (D) Linear regression comparing circulating IFN-α and RF and ACPA titres in seropositive eRA patients (n=132). ****p<0.0001. ACPA, anticitrullinated protein/peptide antibody; eRA, early rheumatoid arthritis; IFN, interferon; IGS, gene signature.

(figure 4B). We validated increased expression of CD4+ *STAT1* transcript in IGS high patients at baseline in the independent TACERA cohort (p=0.003, Mann-Whitey U test) and showed that this was maintained at 6 months (p=0.02), figure 4C. TACERA PBMC *PARP9* and *EPSTI1* was examined in lieu of a CD19+ B cell-specific transcriptome, which again confirmed significant gene upregulation in the IGS high cohort at baseline (p=0.0002 and p<0.0001, respectively, Mann-Whitney U tests), again sustained at 6 months (p<0.0001 for both), figure 4C.

To examine the potential effect of these methylation changes on gene regulation and expression, the CD4+ T and CD19+ B cell DMSs were overlapped with chromatin state information for E043 T cell line and E032 B cell line, respectively. DMSs, particularly hypomethylated DMSs, were enriched in putative enhancer regions and regions flanking transcription start sites for both cell types in IGS high patients (figure 4D). In IGS high CD19+ B cells, hypermethylated DMSs were enriched in the binding sites of several transcription factors, including GATA3, ETS1 and NFATC2, whereas hypomethylated DMSs were enriched in binding sites of polycomb protein EZH2. In IGS high CD4+ T cell hypermethylated DMSs were enriched, among others, for p300 TFBSs and hypomethylated DMSs for HIF1αHI (figure 4E). Full list of TFBS enrichment is found in online supplemental file 12.

DISCUSSION

In a large, multicentre cohort of DMARD and glucocorticoid nave patients with eRA, we identify, for the first time, IFN-α as primarily responsible for IGS generation. We additionally validate the IGS as a clinically relevant prognostic biomarker in RA for refractory disease.[6 14 15] This was independent of conventional markers of disease activity and suggests that IFN-related pathways drive disease persistence. We implicate lymphocyte epigenetic reprogramming as an underpinning mechanism.

Directly linking the IGS and IFN-α has been historically challenging due to difficulties directly measuring circulating IFN-α.[3 12] Therefore, our demonstration of the IGS in eRA positively associating with circulating IFN-α protein, and not other classes of IFN or other circulating cytokines, is an important step in dissecting the biological significance of the IGS and is in keeping with what has been reported in other rheumatic diseases.[16] Furthermore, both IGS and serum IFN-α levels fall in parallel with clinical response over 6 months, additionally supporting the role of IFN-α driving the IGS in eRA. Like others,[17] and in contrast to the IGS, we could not demonstrate an association of baseline IFN-α with longitudinal clinical outcomes. A potential explanation is that the IRG integrates activity over time of IFN-α, which itself has a short half-life.

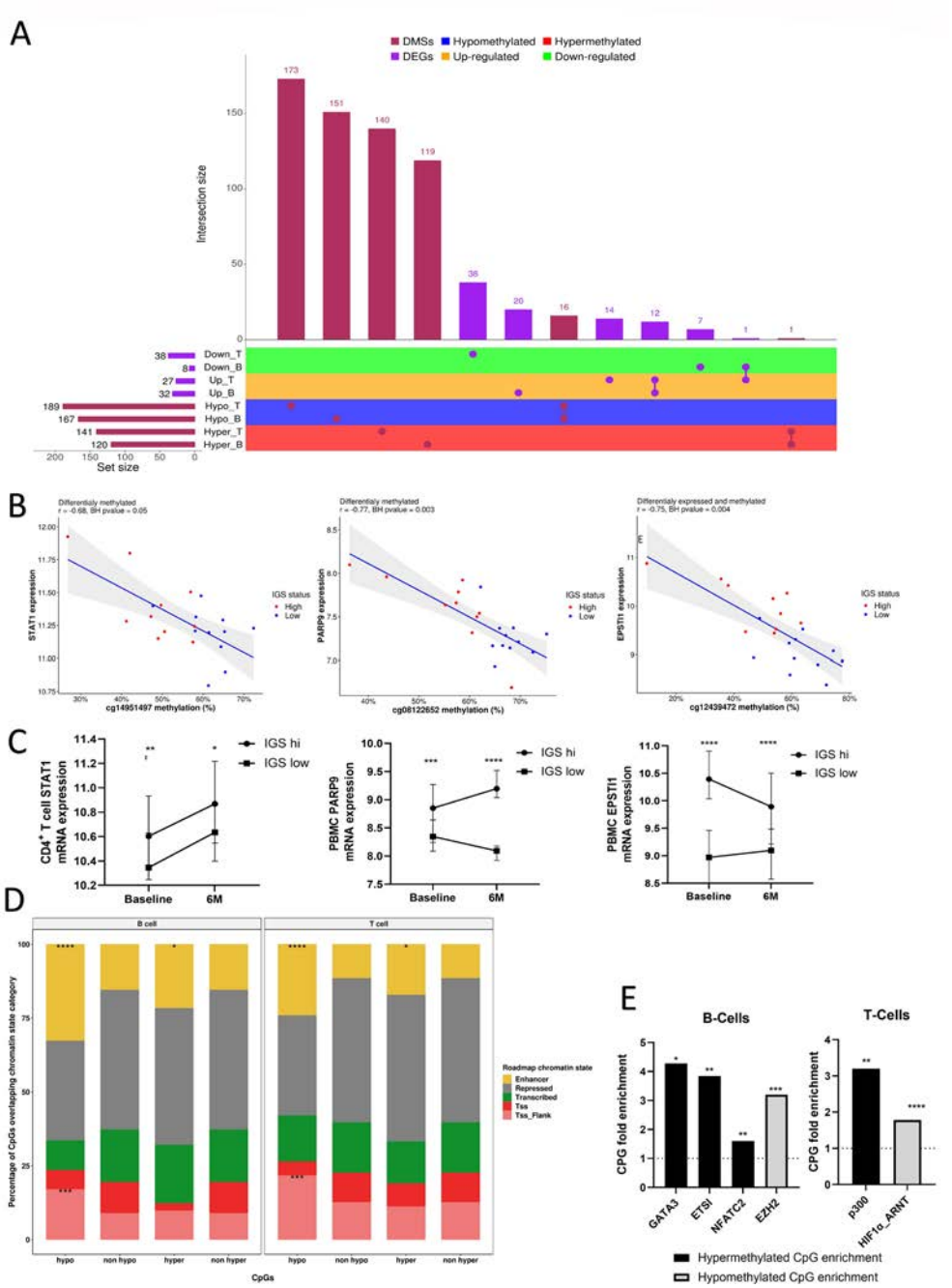

Figure 4 Differential methylation and expression of pathophysiologically relevant genes in eRA according to IGS. CD4+ T cells and CD19+ B cells were isolated from eRA patients (NEAC cohort) and their cell-specific transcriptome and methylome interrogated according to IGS status. (A) Upset plot[45] of differentially methylated sites (DMSs) and differentially expressed genes (DEGs) between IGS high and low early CD19+ B and CD4+ T cells and arranges the co-occurring variables into sets and with a bar chart of their frequency. The horizontal bar graph at the bottom left shows the total number of DEGs/DMSs that are altered in each cell subset between IGS high and IGS low cohorts. Joined red/purples circles to the right of these bar graphs indicate the same DEGs/DMSs were common to the IGS high/IGS low comparisons shown at the left. The vertical bar graph at the top quantitates the number of DEGs/DMSs with similar expression differences in the comparisons. 'Up' and 'Down' indicate increased expression or reduced expression in the IGS cohort respectively. (B) Scatterplots showing significant correlations (Benjamini-Hochberg (BH) adjusted p value (BHpval) <0.05) between gene expression and DNA methylation of exemplar genes in B and T cells of IGS high and low RA patients. R: Pearson correlation coefficient. (C) Baseline and 6 month (6M) expression of CD4+ T cell STAT1 and peripheral blood mononuclear cell (PBMC) PARP9 and EPSTI1 in IGS high and IGS low patients (n=41 for each) in a separate eRA cohort (RA-MAP TACERA). Median and error bars denoting 95% CI depicted. Mann-Whitney U tests performed between IGS high and IGS low cohorts at each time point. (D) Stacked bar plots indicating the relative distribution of the differentially methylated CPGs (DMS) between IGS high/low eRA patients as previously identified according to their chromatin state annotations. Chromatin states enrichments at DMSs that are hyper- or hypo-methylated in IGS high compared with IGS low RA patients are indicated for both cell types (Fisher's exact tests) along with standard expression for comparison. TSS: transcription start site; Tss_Flank: flanking a TSS. (E) Exemplar ENCODE and JASPAR transcription factor binding sites (TFBSs) that are significantly enriched (Fisher's exact test p<0.05) at CD4+ T and CD19+ B cell CPG sites detected as hyper-methylated or hypo-methylated in IGS high RA patients. CPG fold enrichment is displayed. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001. eRA, early rheumatoid arthritis; IGS, gene signature; NEAC, Newcastle Early Arthritis Clinic.

Our finding of comparable IFN-α levels in serum and synovial fluids implicates a systemic source potentially influencing synovial pathophysiology. Larger studies are required to confirm this, and examination of other inflammatory arthritides will shed further light on the role of IFN-α in synovial pathology. RA is a heterogeneous disease and, despite the association of baseline IGS with clinical outcome reported here, not all patients with a low IGS at inception fared well. This could well reflect the dominance of alternative disease pathways in some patients but does not reduce the prognostic value of a high IGS.

Contrasting with other IFNs, increased baseline levels of IFN-α suggest a pathophysiological role in eRA and, potentially, in disease initiation. The permissive effects of IFN-α on lymphocyte activation and development of autoimmune characteristics are well documented[1] and murine transfer of IFN-α secreting dendritic cells propagated a persistent inflammatory arthritis.[18] IFN-α associated with raised ESR in our cohort and in other autoimmune conditions[12] but is also relevant prior to onset of inflammation as an IGS predicts progression to RA in 'at risk' cohorts.[19–21] Furthermore, autoantibodies predate clinical presentation, and we demonstrate a clear association between IFN-α and RF titres in eRA consistent with previous observations in autoimmunity.[12 17] In contrast, there was no association between IFN-α and ACPA. This dichotomy likely reflects differences in autoantibody sources and generation. RF-producing B cells demonstrate activation of IFN-I pathways, whereas ACPA-producing B cells do not.[22] These data, alongside observations in other diseases, highlighting the role of type 1 interferons at disease onset,[23] cumulatively suggest IFN-α may promote or accelerate breach of tolerance in susceptible individuals. Indeed, RA twin studies hypothesise that environmental and stochastic factors may be more important than genetic factors in determining development of disease relevant autoantibodies.[24]

We attempted to identify the receptor(s), and thus potential environmental triggers, responsible for IFN-α release. Although the expression of all examined NARs can be increased following IFN-I exposure,[25] only RNA sensing pathways increased in association with a raised IGS/IFN-α, reflecting previous observations in autoimmunity.[12 26] Furthermore, the IGS was associated with TLR7:TLR9 imbalance, itself associated with heightened autoimmunity risk and breach of tolerance.[2 27] We feel our data are more consistent with RNA sensing pathways triggering IFN-α production and release, but we accept these are associations, and understanding the primary trigger(s) of IFN-α release in eRA remains a pressing priority for future study.

Altered DNA methylation regulates the innate antiviral immune response and hypomethylation of IRGs, such as we report, has also been demonstrated in autoimmunity.[7] Pretreatment with IFN-α in vitro enhances subsequent B cell activation[28] and pretreatment of macrophages with IFN-α prevented the silencing of NF-κB via effects on chromatin, thereby abolishing TNF-induced tolerance to TLR ligation and potentiating the proinflammatory function of TNF-α.[29] Similar chromatin changes were identified in systemic lupus erythematous patients, a condition where IFN-α levels are increased, highlighting the in vivo relevance of IFN-α-related epigenetic modifications.[29] In addition, IFN-α treatment of salivary gland tissues reduced DNA methyltransferases, which catalyse DNA methylation, and upregulated TET3 which is involved in demethylation.[30] Stratifying eRA T and B cells by IGS, we identified multiple DMSs. These preferentially mapped to enhancers and flank regions, thereby supporting their biological relevance. They also reflected TFBS patterns that favoured proliferative responses. Namely, B cell CpG enrichment inferred increased binding of EZH2, which is increased in cell proliferation and lymphoma[31] and reduced binding of (1) GATA3, suggesting increased cell proliferation[32], (2) ETS1 which is required to prevent autoimmune responses[33] and (3) NFATC2, involved in anergy, which, when reduced, causes a hyperproliferative phenotype.[34] In T cells, there was inferred reduction in p300 binding, which would impair Foxp3$^+$ T-regulatory cell function[35]; and enrichment of HIF1-α, which promotes Th17 differentiation and reduced Foxp3$^+$ expression.[36] Additionally, CPG methylation changes in IGS high patients associated with increased gene expression of *PARP9*, *EPSTI1* (B cells) and STAT1 (T-cells) and remained significantly differentially expressed at 6 months between IGS high and low patients in a distinct cohort despite a sustained fall in IGS/circulating IFN-α. PARP9 (BAL-1) can modify B cell proliferation and altered *PARP9* methylation, and expression has been implicated in RA pathogenesis[37]; ETSI promotes pathological B cell activation in Primary Sjogren's Syndrome (PSS)[38]; and STAT1 in T cells is a key mediator of inflammatory cytokine signalling and important in focal RA inflammatory infiltrates.[39] Our interpretation of these data is that IFN-α-induced perturbation of DNA methylation influences the immune system early in the natural history of an identifiable subpopulation of patients with RA, leading to adverse outcomes.

Further work is needed to support this, namely (1) longitudinal measurements to determine whether the observed methylation changes (and gene expression of correlated transcripts) are sustained over time where the IGS/IFN-α levels are not and (2) ex vivo confirmation of the propensity for IFN-α to induce relevant epigenetic changes in relevant cell populations. Indeed, although we have focused on T and B lymphocytes in view of their known relevance to RA pathophysiology, these effects are likely to extend to other cell subsets.[40 41]

Cumulatively, these data incriminate IFN-α as a key cytokine underpinning prognosis in RA and support the hypothesis of an IFN-α-driven epigenetic programming at disease onset that perpetuates pathological signalling pathways and refractoriness to therapy. Such phenomena could underpin the well-recognised window of opportunity in eRA by persistent dysregulation of proinflammatory pathways after a period of unopposed activity. JAK inhibitors modify IFN signalling as well as IFN-induced epigenetic programming in PSS.[30] Targeted administration of these or similar therapies in the early stages of RA, or in 'pre-RA' at-risk groups, may provide a precision medicine approach ultimately reducing clinical progression and patient morbidity. However, before adoption of stratification by IGS, factors to address include IRG selection and interlaboratory standardisation, as recently reviewed in Cooles and Isaacs.[42]

Sustained immune dysregulation secondary to IFN-I may have additional implications. For example, IFN-I-induced epigenetic modifications[29] are also present in patients with COVID-19[43] and might have relevance to 'long-covid' syndromes as well as autoantibody development and, potentially, autoimmunity.[44]

In conclusion, we identify, for the first time, IFN-α as primarily responsible for the IGS in eRA and validate a high IGS as a clinically relevant prognostic biomarker in eRA, portending refractory disease and implying a therapeutic window of opportunity for drugs that target IFN-α signalling. We additionally implicate lymphocyte epigenetic reprogramming as an underpinning mechanism with relevance for other IFN-α enriched states.

Author affiliations
[1]Translational and Clinical Research Institute, Newcastle University, Newcastle Upon Tyne, UK
[2]Laboratory of Dendritic Cell Immunobiology, Institut Pasteur, Paris, France

[3]Center for Translational Research, Institut Pasteur, Paris, France
[4]Centre for Translational Bioinformatics, William Harvey Research Institute, London, UK
[5]Bioinformatics Support Unit, Newcastle University Faculty of Medical Sciences, Newcastle Upon Tyne, UK
[6]Department of Statistical Science, University College London, London, UK
[7]Academic Department of Rheumatology, King's College London, London, UK
[8]Newcastle University Biosciences Institute, Newcastle University, Newcastle Upon Tyne, UK
[9]Musculoskeletal Research Group, The Freeman Hospital, Newcastle Upon Tyne, UK

Twitter Dennis W Lendrem @dennislendrem, Nicola J Maney @Nicolamaney and John D Isaacs @ProfJohnIsaacs

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Contributors FAHC devised the experiments, developed the concepts and wrote the first manuscript draft and acts as guarantor. DWL and JT assisted with RA-MAP TACERA data analysis and modelling. NN, AGP and LNR assisted with epigenetic analyses and support. DD, NJM, BM, CMAL and VB assisted with analysis of serum cytokines. AGP, NT, AEA and JD assisted with T and B cell data collection. GRS, MRB, DW, SN, RH assisted with RA-MAP TACERA data processing, QC and curation. APC supervised RA-MAP TACERA data collection. JDI provided direction and oversight to the whole project. All authors approved the final manuscript.

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ORCID iDs
Faye A H Cooles http://orcid.org/0000-0002-8529-3337
Jessica Tarn http://orcid.org/0000-0003-0083-8543
Dennis W Lendrem http://orcid.org/0000-0001-6268-5509
Nicola J Maney http://orcid.org/0000-0002-6079-2914
Arthur G Pratt http://orcid.org/0000-0002-9909-8209
John D Isaacs http://orcid.org/0000-0002-6103-7056

REFERENCES

1 Muskardin TLW, Niewold TB. Type I interferon in rheumatic diseases. *Nat Rev Rheumatol* 2018;14:214–28.
2 Lind NA, Rael VE, Pestal K, *et al*. Regulation of the nucleic acid-sensing Toll-like receptors. *Nat Rev Immunol* 2022;22:224–35.
3 Cooles FAH, Isaacs JD. The interferon gene signature as a clinically relevant biomarker in autoimmune rheumatic disease. *Lancet Rheumatol* 2022;4:e61–72.
4 de Jong TD, Sellam J, Agca R, *et al*. A multi-parameter response prediction model for rituximab in rheumatoid arthritis. *Joint Bone Spine* 2018;85:219–26.
5 de Jong TD, Snoek T, Mantel E, *et al*. Dynamics of the type I interferon response during immunosuppressive therapy in rheumatoid arthritis. *Front Immunol* 2019;10:902.
6 Cooles FAH, Anderson AE, Lendrem DW, *et al*. The interferon gene signature is increased in patients with early treatment-naive rheumatoid arthritis and predicts a poorer response to initial therapy. *J Allergy Clin Immunol* 2018;141:445–8.
7 Barrat FJ, Crow MK, Ivashkiv LB. Interferon target-gene expression and epigenomic signatures in health and disease. *Nat Immunol* 2019;20:1574–83.
8 Clark AD, Nair N, Anderson AE, *et al*. Lymphocyte DNA methylation mediates genetic risk at shared immune-mediated disease loci. *J Allergy Clin Immunol* 2020;145:1438–51.
9 RA-MAP Consortium. RA-MAP, molecular immunological landscapes in early rheumatoid arthritis and healthy vaccine recipients. *Sci Data* 2022;9:196.
10 Arnett FC, Edworthy SM, Bloch DA, *et al*. The American rheumatism association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;31:315–24.
11 Aletaha D, Neogi T, Silman AJ, *et al*. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League against rheumatism collaborative initiative. *Ann Rheum Dis* 2010;69:1580–8.
12 Reynolds JA, Briggs TA, Rice GI, *et al*. Type I interferon in patients with systemic autoimmune rheumatic disease is associated with haematological abnormalities and specific autoantibody profiles. *Arthritis Res Ther* 2019;21:147.
13 Meyer S, Woodward M, Hertel C, *et al*. AIRE-deficient patients harbor unique high-affinity disease-ameliorating autoantibodies. *Cell* 2016;166:582–95.
14 Rodríguez-Carrio J, Alperi-López M, López P, *et al*. Heterogeneity of the type I interferon signature in rheumatoid arthritis: a potential limitation for its use as a clinical biomarker. *Front Immunol* 2017;8:8.
15 Plant D, Maciejewski M, Smith S, *et al*. Profiling of gene expression biomarkers as a classifier of methotrexate nonresponse in patients with rheumatoid arthritis. *Arthritis Rheumatol* 2019;71:678–84.
16 Huijser E, Gopfert J, Brkic Z. Serum IFNalpha2 measured by single-molecule array associates with systemic disease manifestations in Sjogren's syndrome. *Rheumatology* 2021.
17 Stockfelt M, Lundell A-C, Hetland ML, *et al*. Plasma interferon-alpha is associated with double-positivity for autoantibodies but is not a predictor of remission in early rheumatoid arthritis-a spin-off study of the NORD-STAR randomized clinical trial. *Arthritis Res Ther* 2021;23:189.

18 Narendra SC, Chalise JP, Höök N, *et al*. Dendritic cells activated by double-stranded RNA induce arthritis via autocrine type I IFN signaling. *J Leukoc Biol* 2014;95:661–6.
19 Lübbers J, Brink M, van de Stadt LA, *et al*. The type I IFN signature as a biomarker of preclinical rheumatoid arthritis. *Ann Rheum Dis* 2013;72:776–80.
20 Macías-Segura N, Castañeda-Delgado JE, Bastian Y, *et al*. Transcriptional signature associated with early rheumatoid arthritis and healthy individuals at high risk to develop the disease. *PLoS One* 2018;13:e0194205.
21 Brink M, Lundquist A, Alexeyenko A, *et al*. Protein profiling and network enrichment analysis in individuals before and after the onset of rheumatoid arthritis. *Arthritis Res Ther* 2019;21:288.
22 Lu DR, McDavid AN, Kongpachith S, *et al*. T cell-dependent affinity maturation and innate immune pathways differentially drive autoreactive B cell responses in rheumatoid arthritis. *Arthritis Rheumatol* 2018;70:1732–44.
23 Lombardi A, Tsomos E, Hammerstad SS, *et al*. Interferon alpha: the key trigger of type 1 diabetes. *J Autoimmun* 2018;94:7–15.
24 Hensvold AH, Magnusson PKE, Joshua V, *et al*. Environmental and genetic factors in the development of anticitrullinated protein antibodies (ACPAs) and ACPA-positive rheumatoid arthritis: an epidemiological investigation in twins. *Ann Rheum Dis* 2015;74:375–80.
25 Rusinova I, Forster S, Yu S. Interferome v2.0: an updated database of annotated interferon-regulated genes. *Nucleic Acids Res* 2013;41(Database issue:D1040–6.
26 Maria NI, Steenwijk EC, IJpma AS, *et al*. Contrasting expression pattern of RNA-sensing receptors TLR7, RIG-I and MDA5 in interferon-positive and interferon-negative patients with primary Sjögren's syndrome. *Ann Rheum Dis* 2017;76:721–30.
27 Fillatreau S, Manfroi B, Dörner T. Toll-Like receptor signalling in B cells during systemic lupus erythematosus. *Nat Rev Rheumatol* 2021;17:98–108.
28 Akita K, Yasaka K, Shirai T, *et al*. Interferon α Enhances B Cell Activation Associated With FOXM1 Induction: Potential Novel Therapeutic Strategy for Targeting the Plasmablasts of Systemic Lupus Erythematosus. *Front Immunol* 2020;11:498703.
29 Park SH, Kang K, Giannopoulou E, *et al*. Type I interferons and the cytokine TNF cooperatively reprogram the macrophage epigenome to promote inflammatory activation. *Nat Immunol* 2017;18:1104–16.
30 Charras A, Arvaniti P, Le Dantec C, *et al*. JAK inhibitors suppress innate epigenetic reprogramming: a promise for patients with Sjögren's syndrome. *Clin Rev Allergy Immunol* 2020;58:182–93.
31 Li B, Chng W-J. EZH2 abnormalities in lymphoid malignancies: underlying mechanisms and therapeutic implications. *J Hematol Oncol* 2019;12:118.
32 Liu S, Chan HL, Bai F, *et al*. Gata3 restrains B cell proliferation and cooperates with p18INK4C to repress B cell lymphomagenesis. *Oncotarget* 2016;7:64007–20.
33 Sunshine A, Goich D, Stith A, *et al*. Ets1 controls the development of B cell autoimmune responses in a cell-intrinsic manner. *Immunohorizons* 2019;3:331–40.
34 Teixeira LK, Carrossini N, Sécca C, *et al*. NFAT1 transcription factor regulates cell cycle progression and cyclin E expression in B lymphocytes. *Cell Cycle* 2016;15:2346–59.
35 Liu Y, Wang L, Predina J, *et al*. Inhibition of p300 impairs Foxp3$^+$ T regulatory cell function and promotes antitumor immunity. *Nat Med* 2013;19:1173–7.
36 Guo X, Chen G. Hypoxia-Inducible factor is critical for pathogenesis and regulation of immune cell functions in rheumatoid arthritis. *Front Immunol* 2020;11:1668.
37 Zhu H, Wu L-F, Mo X-B, *et al*. Rheumatoid arthritis-associated DNA methylation sites in peripheral blood mononuclear cells. *Ann Rheum Dis* 2019;78:36–42.
38 Sun J-L, Zhang H-Z, Liu S-Y, *et al*. Elevated EPSTI1 promote B cell hyperactivation through NF-κB signalling in patients with primary Sjögren's syndrome. *Ann Rheum Dis* 2020;79:518–24.
39 Kasperkovitz PV, Verbeet NL, Smeets TJ, *et al*. Activation of the STAT1 pathway in rheumatoid arthritis. *Ann Rheum Dis* 2004;63:233–9.
40 Rodríguez-Ubreva J, de la Calle-Fabregat C, Li T, *et al*. Inflammatory cytokines shape a changing DNA methylome in monocytes mirroring disease activity in rheumatoid arthritis. *Ann Rheum Dis* 2019;78:1505–16.
41 de la Calle-Fabregat C, Rodríguez-Ubreva J, Ciudad L, *et al*. The synovial and blood monocyte DNA methylomes mirror prognosis, evolution, and treatment in early arthritis. *JCI Insight* 2022;7:158783. doi:10.1172/jci.insight.158783
42 Cooles FAH, Isaacs JD. The interferon gene signature as a clinically relevant biomarker in autoimmune rheumatic disease. *The Lancet Rheumatology* 2022;4:e61–72.
43 Lee JS, Park S, Jeong HW, *et al*. Immunophenotyping of COVID-19 and influenza highlights the role of type I interferons in development of severe COVID-19. *Sci Immunol* 2020;5:1554. doi:10.1126/sciimmunol.abd1554
44 Cañas CA. The triggering of post-COVID-19 autoimmunity phenomena could be associated with both transient immunosuppression and an inappropriate form of immune reconstitution in susceptible individuals. *Med Hypotheses* 2020;145:110345.
45 Lex A, Gehlenborg N, Strobelt H, *et al*. UpSet: visualization of intersecting sets. *IEEE Trans Vis Comput Graph* 2014;20:1983–92.

TRANSLATIONAL SCIENCE

Distinct stromal and immune cell interactions shape the pathogenesis of rheumatoid and psoriatic arthritis

Achilleas Floudas ,[1,2] Conor M Smith ,[3] Orla Tynan,[1,2] Nuno Neto,[4] Vinod Krishna,[5] Sarah M Wade ,[1,2] Megan Hanlon,[1,2] Clare Cunningham,[1,2] Viviana Marzaioli,[1,2] Mary Canavan,[1,2] Jean M Fletcher,[3] Ronan H Mullan,[6] Suzanne Cole,[5] Ling-Yang Hao,[5] Michael G Monaghan ,[4] Sunil Nagpal ,[5] Douglas J Veale ,[2] Ursula Fearon [1,2]

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[1]Molecular Rheumatology, Clinical Medicine, Trinity Biomedical Science Institute, Dublin, Ireland
[2]Eular Centre for Arthritis and Rheumatic Diseases, St Vincent's University Hospital, Univeristy College Dublin, Dublin, Ireland
[3]Translational Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland
[4]Department of Mechanical and Manufacturing Engineering, Trinity College Dublin, Dublin, Ireland
[5]Immunology, Janssen Research & Development, Spring House, PA, USA
[6]Department of Rheumatology, Tallaght University Hospital, Trinity College Dublin, Dublin, Ireland

Correspondence to
Professor Ursula Fearon, TCD, Dublin, Ireland; Fearonu@tcd.ie

DJV and UF are joint senior authors.

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ABSTRACT

Objectives Immune and stromal cell communication is central in the pathogenesis of rheumatoid arthritis (RA) and psoriatic arthritis (PsA), however, the nature of these interactions in the synovial pathology of the two pathotypes can differ. Identifying immune-stromal cell crosstalk at the site of inflammation in RA and PsA is challenging. This study creates the first global transcriptomic analysis of the RA and PsA inflamed joint and investigates immune-stromal cell interactions in the pathogenesis of synovial inflammation.

Methods Single cell transcriptomic profiling of 178 000 synovial tissue cells from five patients with PsA and four patients with RA, importantly, without prior sorting of immune and stromal cells. This approach enabled the transcriptomic analysis of the intact synovial tissue and identification of immune and stromal cell interactions. State of the art data integration and annotation techniques identified and characterised 18 stromal and 14 immune cell clusters.

Results Global transcriptomic analysis of synovial cell subsets identifies actively proliferating synovial T cells and indicates that due to differential λ and κ immunoglobulin light chain usage, synovial plasma cells are potentially not derived from the local memory B cell pool. Importantly, we report distinct fibroblast and endothelial cell transcriptomes indicating abundant subpopulations in RA and PsA characterised by differential transcription factor usage. Using receptor–ligand interactions and downstream target characterisation, we identify RA-specific synovial T cell-derived transforming growth factor (TGF)-β and macrophage interleukin (IL)-1β synergy in driving the transcriptional profile of FAPα$^+$THY1$^+$ invasive synovial fibroblasts, expanded in RA compared with PsA. In vitro characterisation of patient with RA synovial fibroblasts showed metabolic switch to glycolysis, increased adhesion intercellular adhesion molecules 1 expression and IL-6 secretion in response to combined TGF-β and IL-1β treatment. Disrupting specific immune and stromal cell interactions offers novel opportunities for targeted therapeutic intervention in RA and PsA.

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT?

⇒ Previous single cell RNA sequencing and flow cytometric analysis of sorted immune cells revealed the presence of peripheral helper T and follicular helper T cells and pathogenic B cells in the inflamed joint of patients with rheumatoid arthritis (RA).
⇒ THY1$^+$ sublining synovial fibroblasts are expanded in RA.
⇒ Notch signalling driven synovial fibroblast and EC crosstalk contributes to synovial inflammation.

WHAT DOES THIS STUDY ADD?

⇒ First study to perform transcriptomic analysis of unsorted synovial tissue single cell suspensions of the RA and psoriatic arthritis (PsA) inflamed joint.
⇒ First time characterisation of immune-stromal cell interactions via the utilisation of receptor–ligand interaction networks on a global scale in the inflamed joint for RA and PsA.
⇒ Identification of differential fibroblast subpopulation involvement in PsA compared with RA.
⇒ Evidence regarding the origin of the T cell and B cell populations in the joint, potentially impacting the current paradigms.

HOW MIGHT THIS IMPACT ON CLINICAL PRACTICE OR FUTURE DEVELOPMENTS?

⇒ The data presented in this study will impact our understanding of RA and PsA synovial inflammation pathogenesis and will reveal new opportunities for targeted therapeutic intervention based on inhibition of specific cell–cell interactions.

INTRODUCTION

Rheumatoid arthritis (RA) and psoriatic arthritis (PsA) are common autoimmune and autoinflammatory diseases of unknown aetiology characterised by complex synovial pathology with a detrimental effect on the patient's quality of life.[1 2] RA and PsA are characterised by a spectrum of clinical manifestations that can be similar in both conditions, however, there are significant differences at a number of levels including clinical, anatomical, genetic, cellular and molecular.[1–3] The most defined differences focus on the presence/absence

of autoantibodies, synovial vascular morphology, the pattern of periarticular inflammation, bone erosion and new bone formation at the entheseal complex of peripheral and spinal joints.[1–8] These differences may explain certain distinct clinical manifestations of the two diseases, and more importantly, may account for different responses to specific therapies impacting on disease outcome and prognosis.[1–8] The complexity of synovial inflammation, associated with different pathotypes, is further increased by immune and stromal cell involvement.[3–8] Recent implementation of single cell transcriptomic analysis of sorted synovial cells has revealed the diverse cellular landscape of the RA synovial stromal and immune cell compartments.[9] While these studies have identified unique synovial cell clusters, increasing our understanding of potential pathogenetic mechanisms involved in RA, no studies to date have examined the synovial landscape of PsA, in addition to characterising the differential and complex immune-stromal cell crosstalk that may define the distinct synovial pathotypes observed in RA and PsA.

T cells have been implicated in RA and PsA synovial pathogenesis. Synovial polyfunctional CD4 and CD8 T cells expressing multiple pro-inflammatory cytokines simultaneously, associate with disease progression in PsA, with polyfunctional CD4 T cell responses recently reported in the synovial tissue of patients with RA.[8 10] Synovial T cell functional plasticity is also highlighted by PD-1^{high}CXCR5^{-} peripheral helper T (Tph) cells sharing features with follicular helper T (Tfh) cells in promoting B cell antibody responses in RA.[7] Clonally expanded CXCR3-expressing memory CD8 T cells with diverse phenotypes have been identified in the synovial fluid of patients with PsA and CD8 T cell clonal convergence between patients provides evidence for common MHC-I–antigen complex involvement and potential for T cell–stromal cell crosstalk.[11] Along with T cells, macrophages are the predominant immune cells in synovial tissue. Macrophages form distinct subsets in the joint of patients with RA and exhibit immune regulatory and pro-inflammatory features, with MerTK^{+} macrophages associated with disease remission.[12] Tissue-resident synovial macrophages characterised by expression of CX3CR1 form a physical barrier at the lining layer of the joint.[13] These self-renewing macrophages have characteristics akin to epithelial cells and contribute to the homeostasis of the joint.[13]

Similarly to the diverse profile and roles of immune cells involved in synovial inflammation, emerging evidence suggests specific fibroblast cell subsets contribute to RA disease pathogenesis.[6] FAPα and THY1 define two functionally distinct synovial fibroblasts with FAPα^{+} THY1^{+} fibroblasts mediating bone erosion whereas FAPα^{+} THY1^{-} fibroblasts contribute to inflammation via the production of chemokines that promote immune cell trafficking to the inflamed joint.[14]

Despite recent advances in the resolution of the RA synovial tissue composition, several key questions remain unanswered, while additionally, the cellular landscape of PsA has not been explored at this level. To achieve precision medicine in RA and PsA, minimise lost time with exploratory treatments and reduce potential adverse effects, a better understanding of specific cell–cell interactions in RA and PsA is required. In this study transcriptomic analysis of intact RA and PsA synovial tissue cell suspensions was performed allowing for characterisation of immune-stromal cell interactions and the identification of overlapping and differential pathways of inflammation.

The cellular landscape of RA and PsA reveals points of convergence and distinct underlying mechanisms of synovial inflammation with utilisation of receptor–ligand interaction networks providing evidence of T cell and macrophage synergy in shaping the transcriptome of proinflammatory fibroblasts in RA.

RESULTS

Single cell RNA sequencing reveals distinct synovial tissue immune and stromal cell clusters in patients with RA and PsA

Following the implementation of novel, strict, quality control measures (as described in the Methods section), we analysed a total of 178.196 cells derived from four patient with RA and five patient with PsA synovial tissue samples. Clinical characteristics of the patients at time of arthroscopic surgery are summarised in online supplemental table 1. Following data integration with Harmony to minimise sample to sample variation, cells were divided into nine megaclusters akin to distinct cell types. These megaclusters include: fibroblasts (88 953 cells), endothelial cells (24 207 cells), pericytes (4182 cells), macrophages (25 315 cells), dentritic cells (DC) (4103 cells), B cells (5902 cells), plasma cells (3098 cells), T cells (18 420 cells) and natural killer T cells (NKT) (1517 cells) (figure 1A). The annotation of the distinct cell type clusters was based on manual (prior knowledge-defined) and automated (scCATCH) identification of cell type-specific markers. Cell type-specific markers were identified following comparison of expression values of a specific cluster to all other synovial cells, leading to the generation of a list of cluster-specific markers (figure 1B).[15] The identified cell type-specific megaclusters were divided further resulting in a total of 18 stromal cell clusters (11 fibroblast clusters, 6 endothelial cell clusters and 1 pericyte cluster) and 13 immune cell clusters (3 macrophage clusters, 5 T cell clusters and 2 B cell and 2 plasma cell clusters) (figure 1C). Distribution of expression on a single cell level using non-linear, stochastic Uniform Manifold Approximation and Projection for Dimensionality Reduction (UMAP) and expression level per cell type-specific megacluster of key markers is shown in figure 1D,E.

Differential fibroblast cluster distribution between PsA and RA synovial tissue samples

Recent studies have highlighted that patient with RA synovial fibroblasts are highly heterogeneous with a newly described synovial subset, characterised by expression of FAP and THY1, exhibiting effector function via the secretion of pro-inflammatory cytokines.[14] Analysis of the fibroblast megacluster of 88.953 cells, resulted in the identification of 11 distinct fibroblast clusters in RA and PsA synovial biopsies (figure 2 and online supplemental figure S2). Importantly, there is differential abundance of the synovial fibroblast clusters (expressed as frequency of each cluster as part of all synovial fibroblasts per sample), separating PsA from patient with RA samples with a significantly higher abundance of F1 fibroblasts in PsA (***$p<0.001$) and F8, F9 and F11 fibroblasts in RA (**$p=0.006$, ***$p<0.001$, ***$p<0.001$, respectively) (figure 2A,D). We then examined the expression of FAP and THY1 by synovial fibroblast clusters, with the enriched RA F11 synovial fibroblast cluster harbouring the highest number of FAPα-expressing and/or THY1-expressing cells and the enriched in PsA F1 synovial fibroblast cluster showing almost no FAP or THY1 expressing cells (figure 2B). Expression of THY1 and FAP by cells of a specific fibroblast cluster is an under-representation of co-expressing cells due to dropouts in the sampling of RNA and sequencing. Therefore, to examine the degree of FAP and THY1 co-expression, we performed data imputation.[16] Data imputation algorithms use the transcriptional profile of neighbouring cells to infer the expression of genes that may be affected by increased sparsity.[17] Fibroblasts co-expressing

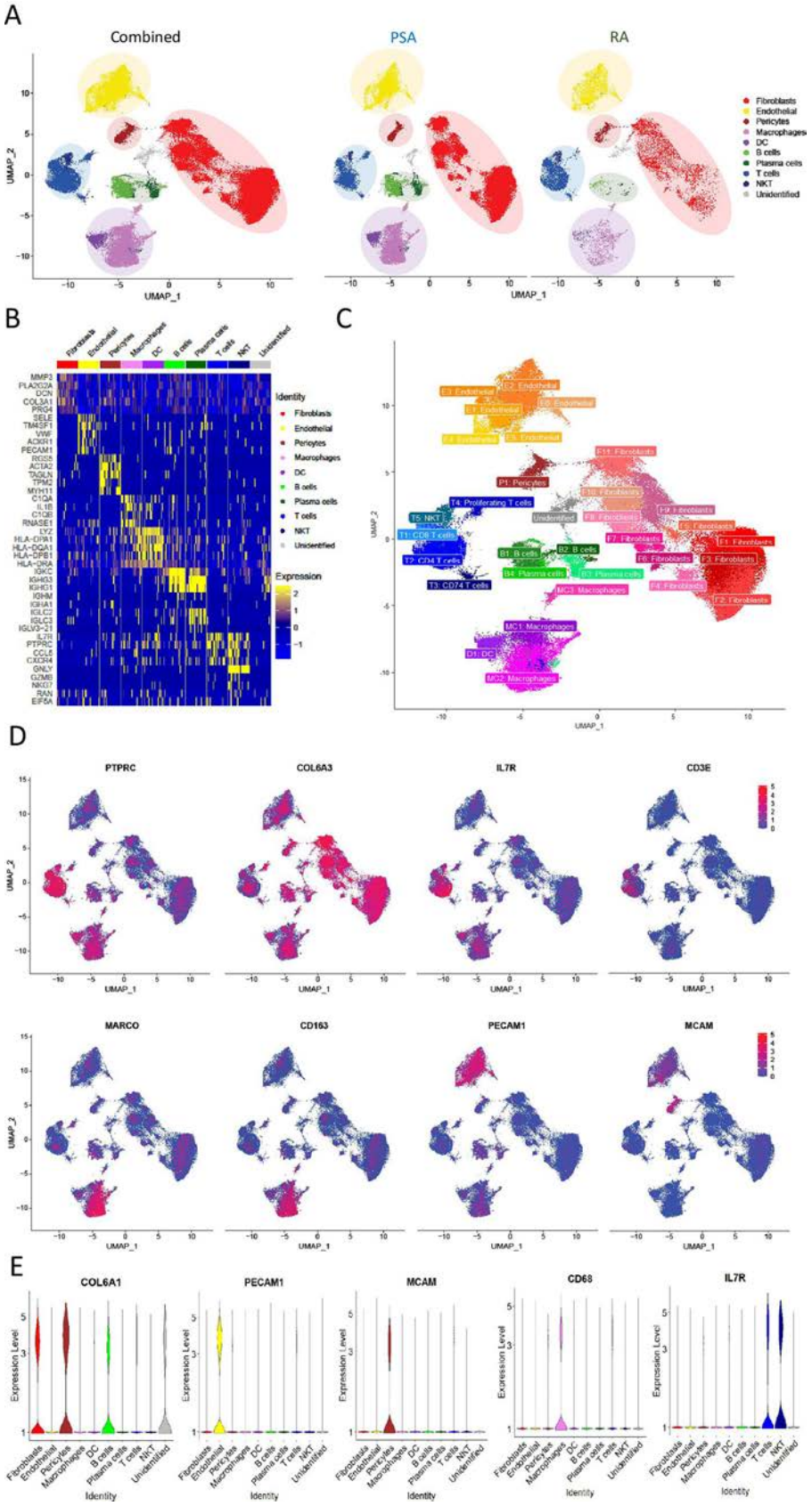

Figure 1 High dimensionality single cell RNA sequencing analysis identifies specific cell clusters in patient with RA and PsA synovial tissue biopsies. (A) UMAP representation of 9 'mega'-clusters based on 178 196 cells across all cell types and synovial tissue biopsies (n=4 and 5 for patient with RA and PsA biopsies, respectively). (B) Differential gene expression analysis identifies upregulated or downregulated marker genes of the observed mega clusters. (C) Division of the nine identified mega clusters into a total of 33 subclusters. (D) Feature plots for the expression and distribution of the indicated genes in all cells. (E) Violin plots depicting log normalised expression per cluster of key markers used in cluster annotation. DC, dentritic cells NKT, natural killer T cells, IL, interleukin; PsA, psoriatic arthritis; RA, rheumatoid arthritis; UMAP, Uniform Manifold Approximation and Projection.

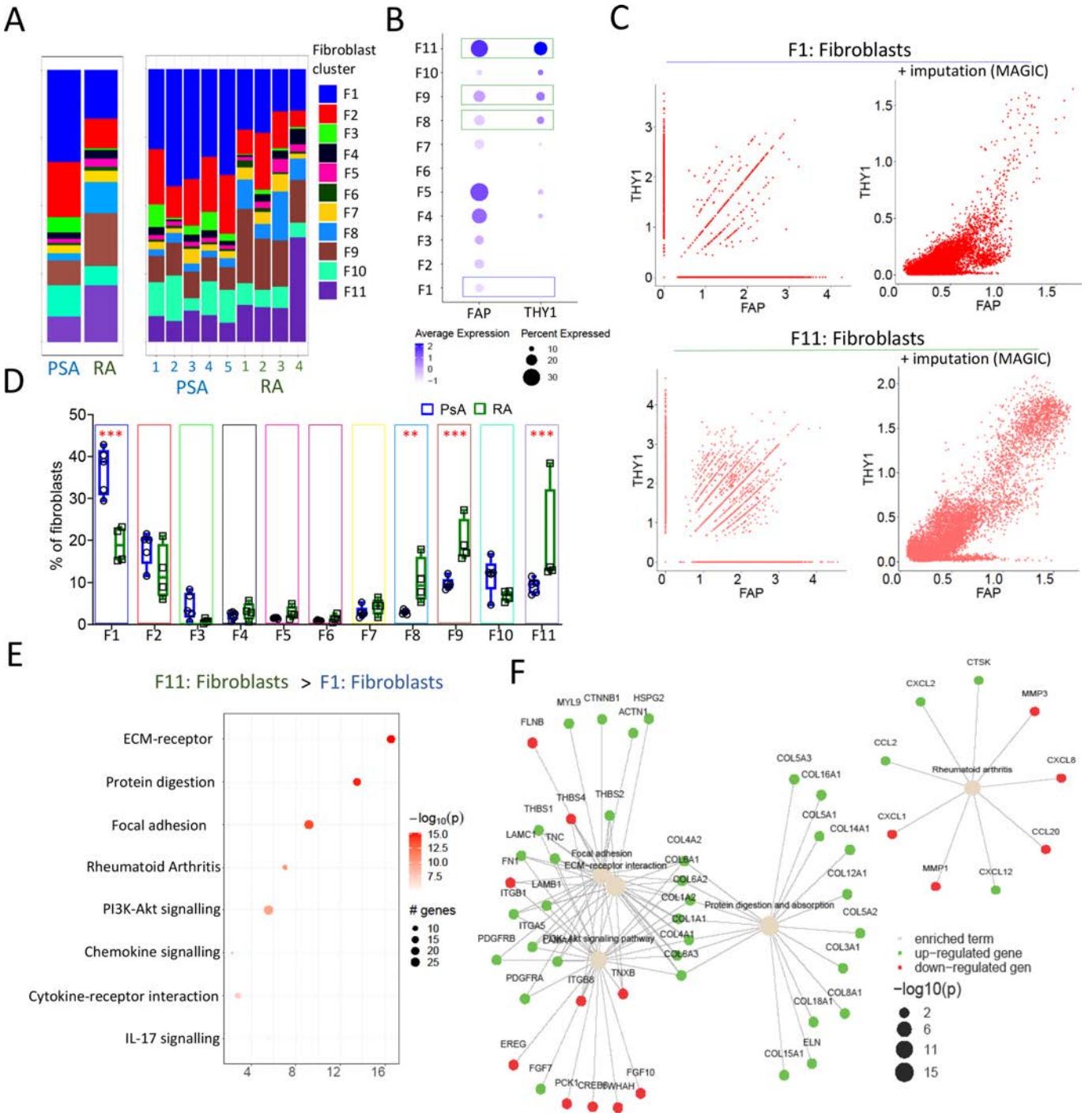

Figure 2 Distinct fibroblast cluster distribution in RA compared with PsA synovial biopsies. (A) Abundance of fibroblast clusters in patient with RA and PsA synovial biopsies. (B) Expression and percentage of positive cells per fibroblast cluster for FAP and THY1. (C) Scatterplots showing the relation between THY1 and FAP expressing cells before and after data imputation for RA and PsA fibroblast cluster F1 and fibroblast cluster F11. Fibroblast clusters with significantly different abundances between RA and PsA are indicated by green (higher in RA) and blue (higher in PsA) boxes. (D) Frequency of fibroblast clusters (calculated as a percentage of all fibroblasts/sample) in patient with PsA and RA synovial biopsies (n=4–5), data are presented as box and whiskers plots (min to max), symbols represent individual samples, statistical significance was determined by two-way analysis of variance with Sidak's multiple comparisons test (***p<0.001, **p=0.0062). (E) Analysis of pathways enriched in fibroblast cluster 11 compared with fibroblast cluster 1, colour intensity represents significance and dot size the number of genes within each pathway that are differentially expressed. (F) Term plot of the indicated pathways with significant enrichment in fibroblast cluster 11 compared with fibroblast cluster 1. Colour indicates up or downregulation of specific genes within the pathway and dot size represents statistical significance of change. ECM, extracellular matrix; IL, interleukin; PsA, psoriatic arthritis; RA, rheumatoid arthritis.

FAP and THY1 are found in the enriched RA F11 cluster but not in the enriched PsA F1 cluster (figure 2C). Due to the differential abundance of clusters F11 and F1 between RA and PsA synovial tissue samples, we performed pathway enrichment analysis using differentially expressed genes between fibroblast cluster F11 and cluster F1. Pathway enrichment identified several pathways,

previously implicated in RA pathogenesis, which are enriched in F11 compared with F1 fibroblasts. These pathways include the extracellular matrix (ECM) receptor, focal adhesion and RA pathways (figure 2E). Interestingly principal component analysis (PCA) of enriched pathways in synovial fibroblasts, revealed a separation of the fibroblast clusters, with F1 and F11 synovial fibroblasts on opposite ends of the spectrum (online supplemental figure S2C). Common genes and the upregulation or downregulation of specific members of these pathways in the comparison between F11 and F1 fibroblasts are shown in figure 2F.

Differential abundance and distinct transcriptional profile of specific endothelial cell clusters between PsA and RA

A pivotal first step of synovial inflammation in RA and PsA is increased angiogenesis which facilitates immune cell infiltration into the synovial tissue. Of the six endothelial and one pericyte cell cluster, endothelial cell cluster E1 is significantly (*p=0.02) elevated in RA compared with PsA (figure 3A,B, online supplemental figure S3). Interestingly, E1 endothelial cell cluster shows the highest expression of the VEGF receptors VEGFR1 (FLT1) and VEGFR2 (KDR) and high expression of NOTCH family members, specifically NOTCH4, NOTCH1 and their ligand, DLL4 (figure 3C). VEGF and NOTCH signalling result in fate decisions of endothelial cell specialisation towards stalk, tip or intermediate cell phenotypes that impact angiogenesis.[18] In order to identify regulators of the E1 transcriptional profile in PsA and RA, we performed transcription factor (TF) usage estimation by analysing the expression of known, TF-regulated genes that are differentially expressed between PsA and RA. Interestingly, PsA and RA E1 endothelial cells show stark differences in TF usage, with TEA domain 1 (TEAD1) and myocyte enhancer factor 2A being the highest scored TF in RA E1 cells (figure 3D).[19] Contrary to RA, PsA E1 cells show potential involvement of FOXP1 (figure 3D). The differences in endothelial cell TF usage, are potentially a reflection of differential transcriptional regulation, indicative of the distinct synovial angiogenesis in RA and PsA. Angiogenesis is the result of a highly regulated, orchestrated process, characterised by cell–cell interactions that define the fate and specialisation of endothelial cells.[20] In order to examine potential cell interactions of endothelial cells belonging to cluster E1, differentially expressed receptors of cluster E1 were identified. Based on prior knowledge of receptor–ligand interaction potential, the heatmap of figure 3E depicts the top ligands for receptors expressed by endothelial cell cluster E1 (figure 3E). We then assessed the expression on all synovial cells of the top ligands for receptors of cluster E1 (figure 3F). Interestingly, endothelial cell cluster E1 shows potential for interaction with other endothelial cell clusters due to the high expression of several ligands by endothelial cells. While, limited, specific interactions between E1 cells and synovial fibroblasts and immune cells can be inferred from the extent of potential receptor ligand interactions (figure 3F).

The VEGF receptors, FLT1 and KDR and the VEGF-binding neuropilin-1, which modulates KDR expression, are upregulated in RA compared with PsA E1 endothelial cells[21](figure 3G). Angiogenic NOTCH4 is upregulated by endothelial cells in response to VEGF, and previous histological analysis has revealed high NOTCH4 expression in the synovial tissue of patients with RA and PsA.[22] Consistent with the upregulated VEGF receptor expression by RA E1 endothelial cells, NOTCH4 shows higher expression in RA compared with PsA (figure 3G). Platelet And Endothelial Cell Adhesion Molecule 1 (PECAM1), involved in endothelial cell adhesion and motility during angiogenesis, and podocalyxin, a key modulator of apical-basal endothelial cell polarisation and lumen formation, are also upregulated in RA compared with PsA E1 endothelial cells[23 24] (figure 3G). The potential capacity of endothelial cells to interact with stromal and immune cells of the joint, and the identified transcriptomic differences between PsA and RA, indicate that the altered synovial blood vessel morphology between the two disease pathotypes is potentially the result of complex alterations in endothelial cell–cell crosstalk.

Identification of IL-1B expressing synovial macrophage cell cluster in RA and PsA synovial tissue

Macrophages are the most abundant immune cells of the synovial tissue with known protective, as well as pro-inflammatory, roles in RA disease pathogenesis.[12 13] The abundance, calculated as the frequency of each cell cluster as part of all macrophages/DC per sample, of the three identified synovial tissue macrophage and one synovial DC clusters are comparable in PsA and RA (figure 4A,C). Interestingly, the macrophage cell cluster MC1, shows high level of *IL-1B* expression (figure 4B). Pathway enrichment analysis of the MC1 cluster of macrophages, shows enrichment of pathogenic signalling pathways including the tumor necrosis factor (TNF), IL-17, chemokine and cytokine–cytokine receptor pathways (figure 4D). PsA MC1 cluster macrophages use reduced myelocytomatosis proto-oncogene (MYC) compared with their RA counterparts (figure 4E). MYC can dictate the activation threshold for macrophages and early metabolic reprogramming by suppressing their response to lipopolysaccharide (LPS)-dependent stimulation, instead MYC has been shown to induce genes associated with an M2 transcriptional profile.[25 26] To investigate which synovial cells have the highest potential to respond to the MC1 cluster macrophage-derived IL-1β, the expression of the IL-1β receptor, *IL-1R1* was assessed. Synovial fibroblast clusters showed increased expression of *IL-1R1* compared with other synovial cells, however, not all fibroblasts exhibited the same level of *IL-1R1* expression (figure 4F). Interestingly, the F11 cluster, enriched in $FAP^{+}THY1^{+}$ synovial fibroblasts has higher expression of *IL-1R1* compared with fibroblast cluster F1 (figure 4F).

Limited *in situ* synovial T cell proliferation and high expression of TGFB1 in RA

We identified five clusters of synovial T cells, T1: CD8 T cells, T2: CD4 T cells, T3: CD74 T cells, T4: proliferating T cells and T5: NKT cells, in the synovial tissue of patients with PsA and RA. T cell cluster abundances, calculated as the frequency of each cell cluster as part of all T cells per sample, did not differ between PsA and RA except for a significant (*p=0.016) enrichment of T cell cluster T1: CD4 T cells in RA compared with PsA (figure 5A). T cells are key mediators of synovial inflammation, however, whether the primary mechanism of T cell accumulation in the synovial tissue is migration, or *in situ* synovial tissue expansion, remains poorly understood.[27] MKI67, a marker of cell proliferation, was primarily detected in synovial T cell cluster T4 (figure 5B). Computational analysis of transcriptional profiles associated with cell proliferation based on the relative expression of 54 G2/M phase associated genes and 43 s phase associated genes, revealed that the T4 cluster was the only actively proliferating T cell cluster (figure 5C). T cell cluster T4, represents only 1.4%±0.6% and 8.8%±5.17% of synovial T cells in RA and PsA, respectively. Pathway enrichment analysis of synovial tissue T cells revealed differential enrichment of

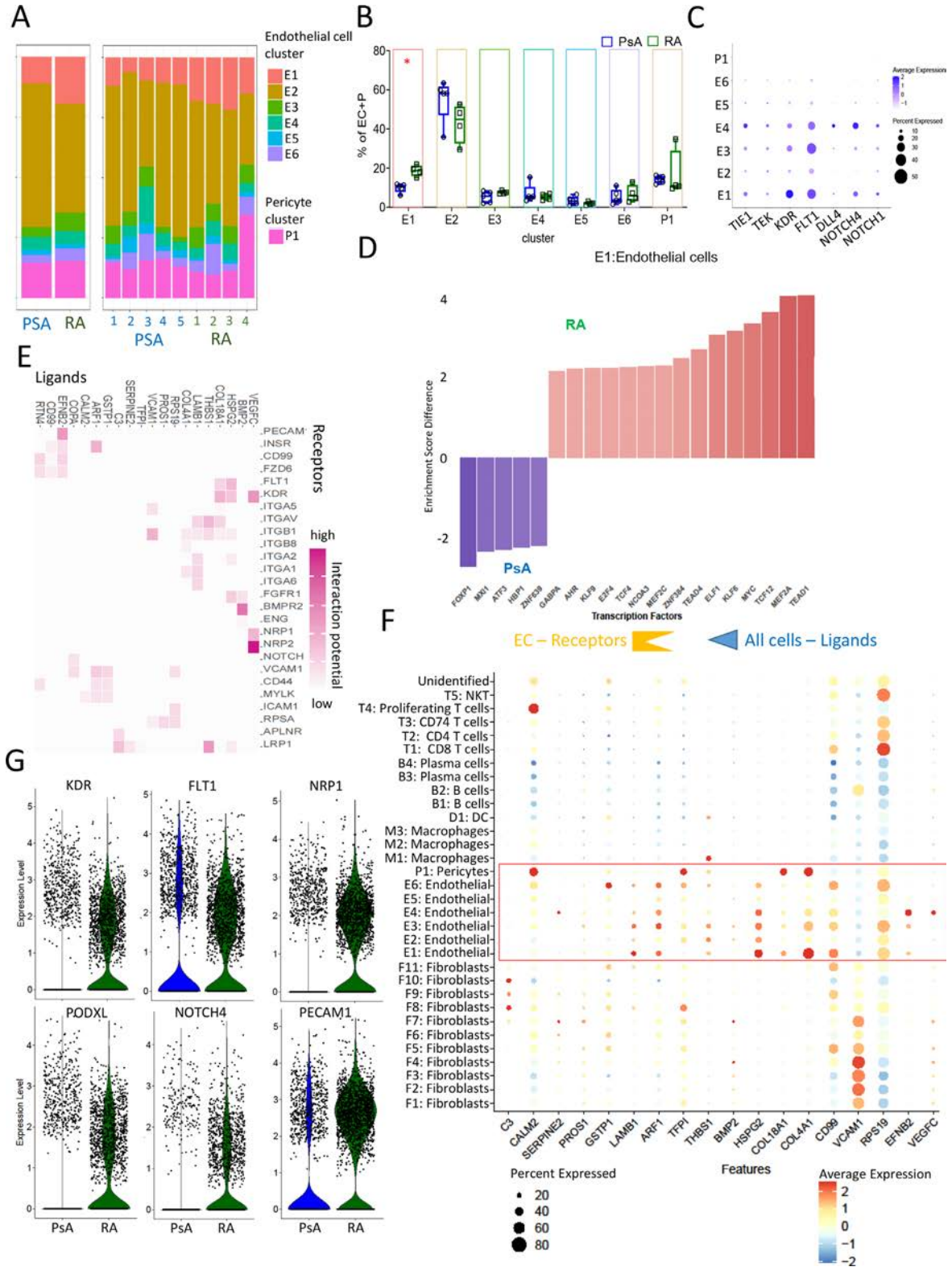

Figure 3 Distinct endothelial cell profiles between patient with PsA and RA synovial biopsies. (A) Abundance of endothelial and pericyte cell clusters in patient with RA and PsA synovial biopsies. (B) Frequency of endothelial cell clusters in patient with PsA and RA synovial biopsies (n=4–5), data are presented as box and whiskers plots (min to max), symbols represent individual samples, statistical significance was determined by two-way analysis of variance with Sidak's multiple comparisons test, *p=0.019. (C) Dotplot for the average scaled expression levels of angiopoietin receptor (TIE1 and TEK), VGF receptor (KDR and FLT1) and notch signalling elements (DLL4, NOTCH4 and NOTCH1). Dot size represents the percentage of cells per cluster expressing the indicated genes. (D) DoRothEA analysis of transcription factor usage by endothelial cell cluster E1 cells, based on expression of known downstream ligands. VIPER score difference between RA and PsA is shown. (E) Top 20 ligands with known and predicted interactions with receptors expressed by E1: endothelial cell cluster. (F) Dotplot depicting the potential sources of top ligands for cells of the E1 endothelial cell cluster. (G) Violin plots for the expression of VEGF receptor (KDR and FLT1), neuropilin-1 (NRP1), podocalyxin (PODXL), NOTCH4 and CD31 (PECAM1) by RA and PsA E1: endothelial cell cluster. DC, dentritic cells; NKT, natural killer cells; PsA, psoriatic arthritis; RA, rheumatoid arthritis.

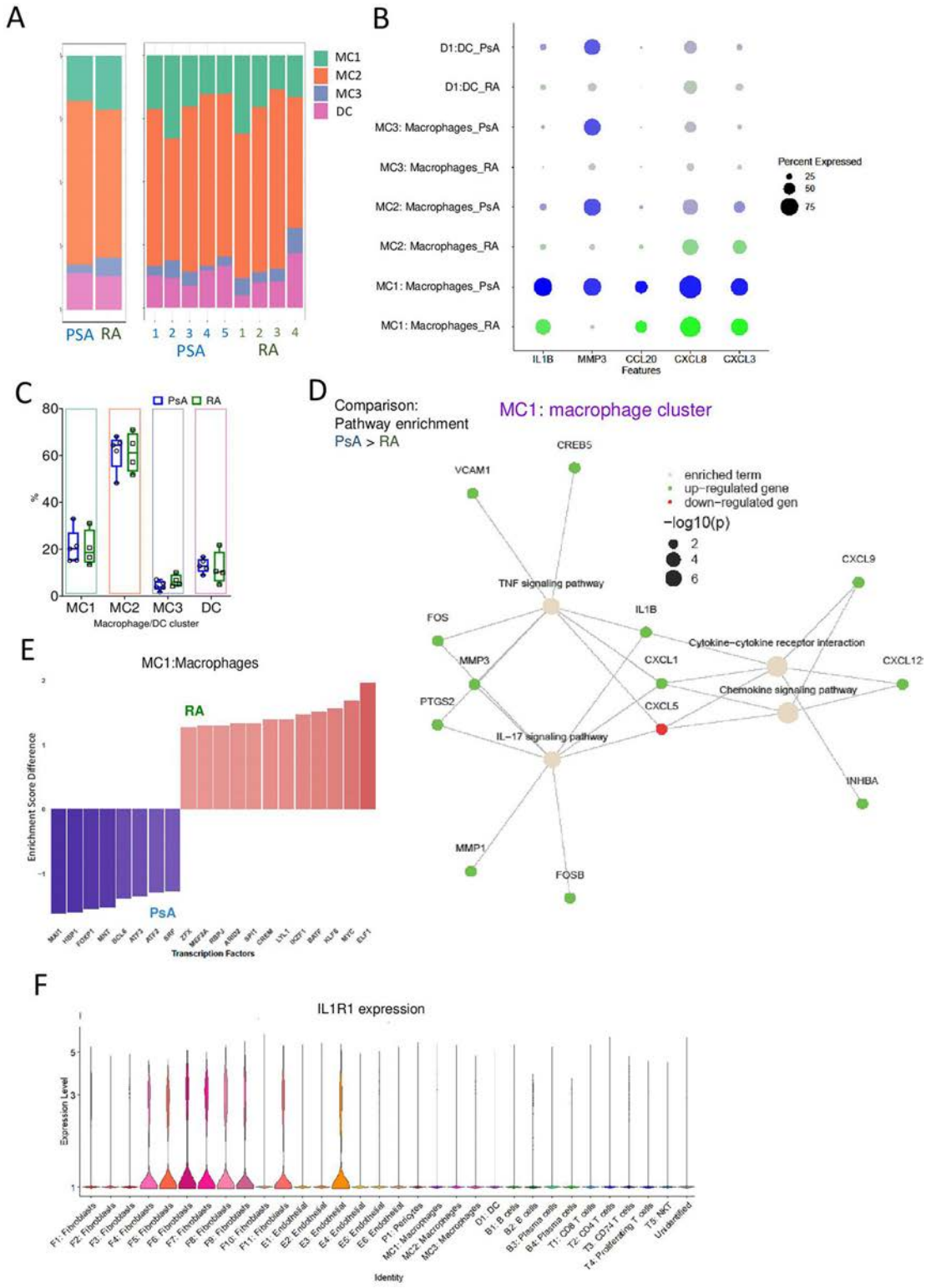

Figure 4 Distinct macrophage cell transcriptomic profiles between patient with PsA and RA synovial biopsies. (A) Abundance of macrophage and DC cell clusters in patient with RA and PsA synovial biopsies. (B) Dot plot for the indicated markers in PsA (blue) and RA (green) macrophage and DC clusters. (C) Frequency of macrophage and DC cell clusters (calculated as a percentage of all macrophage and DC cells per sample) in patient with PsA and RA synovial biopsies (n=4–5), data are presented as box and whiskers plots (min to max), symbols represent individual samples, statistical significance was determined by two-way analysis of variance with Sidak's multiple comparisons test, *p<0.05 were considered significant. (D) Term plot of the indicated pathways with significant enrichment in PsA compared with RA macrophage cluster MC1 following pathway enrichment analysis. Colour indicates up or downregulation of specific genes within the pathway and dot size represents significance. (E) Estimation of transcription factor activity by macrophages of cluster MC1 in RA compared with PsA. Transcription factor usage is estimated based on the differentially expressed genes with prior knowledge of genes regulated by specific transcription utilising bioinformatics package DoRothEA. Transcription factor enrichment score difference between RA and PsA is shown. (F) Violin plots for the normalised expression of IL-1R1 (IL-1B receptor) by all identified synovial cell clusters. IL, interleukin; PsA, psoriatic arthritis; RA, rheumatoid arthritis.

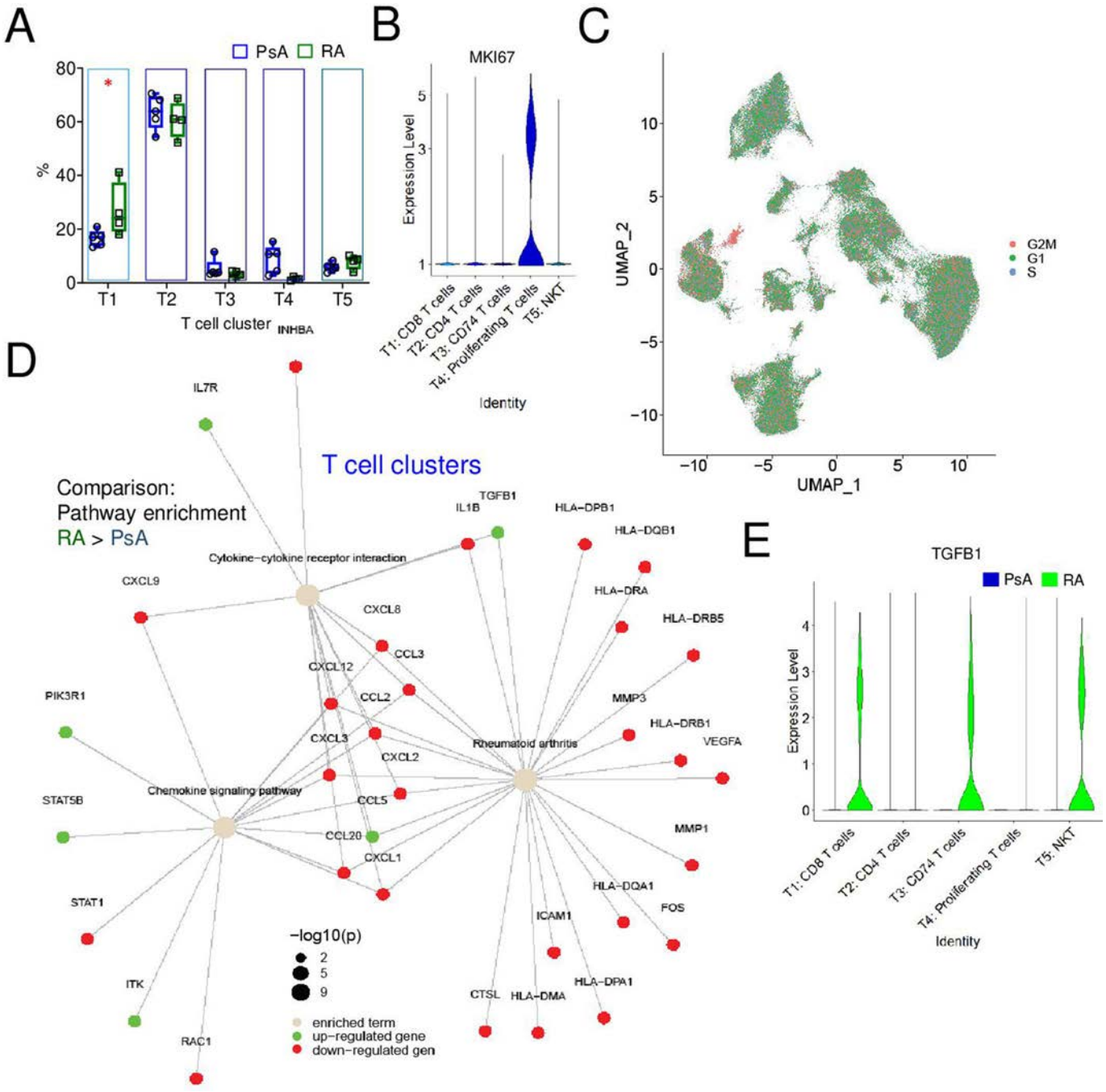

Figure 5 Patient with RA synovial T cells express TGFB1. (A) Frequency of T cell clusters (calculated as a percentage of all T cells per sample) in patient with PsA and RA synovial biopsies (n=4–5), data are presented as box and whiskers plots (min to max), symbols represent individual samples, statistical significance was determined by two-way analysis of variance with Sidak's multiple comparisons test, *p=0.016. (B) Violin plot for the log normalised expression of MKI67 by synovial T cell clusters. (C) Computational cell cycle analysis of synovial cells, based on the relative expression of 54 G2/M phase associated genes and 43 s phase associated genes, depicting cells in different stages of the cell cycle. (D) Term plot of the indicated pathways with significant enrichment in RA compared with PsA T cell clusters following pathway enrichment analysis. Colour indicates up or down regulation of specific genes within the corresponding pathway and dot size represents significance. (E) Violin plot for the log normalised expression of TGFB1 by RA and PsA synovial T cell clusters. HLA, human leukocyte antigen; IL, interleukin; PsA, psoriatic arthritis; RA, rheumatoid arthritis; TGF, transforming growth factor; UMAP, Uniform Manifold Approximation and Projection.

genes of the cytokine–cytokine receptor interaction and chemokine signalling pathways. Additionally, Tph/Tfh associated gene expression and indicative of tissue residency and early activation expression of CD69 are higher in RA compared with PsA synovial T cells (online supplemental figure S5)[9]. Demarcation of synovial T cell subsets and chemokine expression of PsA and RA T cells may be indicative of differential T cell involvement, however extensive further analysis and subclustering of T cells is required. Interestingly, transforming growth factor (TGF) B1 expression was increased in RA compared with PsA synovial T cells (figure 5D). Inhibition of TGF-β can limit synovial fibroblast hyperplasia in murine models of RA.[28 29] With T cells being a critical source of TGF-β, TGFB1 expression was examined further. Patient with RA synovial tissue T cells of clusters

T1, T3 and T5 exhibited high expression of TGFB1 compared with their PsA counterparts (figure 5E). TGF-β is a pleiotropic cytokine and determining its role in RA disease pathogenesis has been challenging. However, previous studies have identified that signalling pathways associated with TGF-β are enriched in RA but not osteoarthritis (OA) synovial fibroblasts and TGF-β1 messenger RNA expression correlates with patient with RA C-reactive protein (CRP) levels.[30]

The majority of synovial tissue plasma B cells are potentially not derived from synovial tissue memory B cells

We identified four clusters of B cells, clusters B1 and B2 consisting primarily of memory B cells and clusters B3 and B4 consisting of plasma cells. Relative abundances expressed as the frequency of each cluster as a percentage of the total B cells for each sample did not differ between patient with PsA and RA synovial tissue (figure 6A). PCA plot of all enriched pathways per cluster following pathway enrichment analysis shows separation of B cells and plasma cells with plasma cell clusters grouping together while B cell clusters appear more dissimilar in the pathways that are being used (figure 6B). Despite the absence of any noticeable difference in the abundance of synovial B cell clusters between PsA and RA, specific pathways including the B cell receptor (BCR) signalling pathway were enriched in RA, compared with PsA, B cells (figure 6C). Ectopic lymphoid structure formation is a characteristic of aberrant RA synovial inflammation. It has been hypothesised that synovial plasma B cells emerge in the aforementioned structures as a result of *in situ* memory B cell differentiation.[31] To evaluate this hypothesis, we examined κ and λ light chain usage by synovial tissue B cells. Due to allelic exclusion, a process that ensures B cells express one monospecific BCR following rearrangement of the light chains in early stages of B cell development, B cells express either a κ or a λ light chain.[32] Synovial tissue B cells of cluster B1 and B2 and plasma cells of cluster B4 showed high expression of the κ light chain constant region (IGKC). Contrary to the majority of synovial plasma cells, cluster B3 demonstrates a clear preference for the λ light chain constant region (IGLC2) (figure 6D). Due to reports of a small population of B cells with dual BCR expression, the relationship between IGKC and IGLC2 expression was examined. Indeed, dual κ-expressing and λ-expressing synovial B cells were identified without data imputation. These B cells were primarily confined within the B2 B cell cluster (figure 6E). To assess the potential progression of synovial memory B cells to plasma cells, trajectory analysis was performed. Trajectory analysis uses gene expression to reconstruct the progression of cells along a lineage.[33] Pseudotime, a measure of the distance of the cells from the starting point of the trajectory is used to infer the progression of the cells from the basal condition.[34] The starting point of the trajectory was decided based on maximum pseudotime from B cells to plasma cells (figure 6F). Analysis of groups of co-regulated genes (modules) on the trajectory shows separation of plasma and B cell clusters (figure 6F). Interestingly, different gene modules achieve high scores between B cell cluster 1 and 2 (figure 6G). Plotting the dynamics of IGKC and IGLC2 expression as a function of pseudotime accentuates the separation between κ light chain-expressing B cells and λ light chain-expressing plasma cells (figure 6H). The distribution of IGKC and IGLC2 expression in relation to pseudotime invites the question of whether synovial B cells revise their BCR from κ to λ light chain, a phenomenon previously only observed in very early stages of B cell development.[35] Therefore, the pseudotime was divided into segments and expression of the differentially expressed genes of pseudotime segment B (pseudotime distance 1 to 2) was evaluated as a function of pseudotime. Interestingly, differentially expressed genes of segment B showed high expression only in segment B (figure 6I).

Synovial T cell-derived TGF-β and macrophage IL-1β drive the transcriptome of proinflammatory synovial fibroblasts

As this study included unsorted synovial tissue single cell suspensions, it had the advantage of being able to examine potential networks of immune-stromal cell interaction involved in RA and PsA, thus reflecting the joint microenvironment. Synovial fibroblast clusters F1 and $FAP^{+}THY1^{+}$ F11, enriched in PsA and RA, respectively, were assigned the role of receiver cells to generate receptor–ligand interaction networks (figure 7A,B). Importantly, examination of the top receptor–ligand interactions (receptors expressed by fibroblasts; ligands expressed by all other synovial cells), indicate that the transcriptional profile of the proinflammatory fibroblast cluster F11 is potentially driven by synovial T cell derived TGFB and macrophage derived IL1B (figure 7B). Top 20 ligands with high receptor–ligand interaction potential with F11 fibroblast-expressed receptors include IL1B, TGFB, migration inhibitory factor (MIF), vascular cell adhesion molecule 1 (VCAM1) and NOTCH ligand Jagged1 (JAG1) (figure 7C). To assess the influence of IL1B and TGFB on the transcriptome of the fibroblasts of cluster F11 in RA and PsA, we used machine learning with random forest generation to evaluate to what extent IL1B and TGFB can predict the top per cent of differentially expressed genes of cluster F11 positioned downstream of the IL1B and TGFB receptors (figure 7D). IL1B but not TGFB could significantly (*p=0.028) predict the expression of downstream genes of fibroblast cluster F11 in PsA (figure 7D). Conversely, neither TGFB nor IL1B, could predict the expression of downstream genes of fibroblast cluster F11 in RA, however, the combination of both TGFB and IL1B shows high significance (***p<0.001) in predicting the downstream expression of differentially expressed genes of F11 fibroblasts in RA (figure 7D).

Transcription factor usage analysis based on expression of known transcription factor-regulated genes, support a potentially increased usage of MYC and HIF1A by in RA F11 fibroblasts compared with PsA (figure 7E).

IL-1β and TGF-β synergistically drive metabolic adaptation of patient with RA synovial fibroblast and pro-inflammatory markers

The transcriptomically identified synergy of IL-1β and TGF-β in RA was assessed by in vitro characterisation of patient with RA synovial fibroblasts treated with combination of IL-1β and TGF-β. Previous studies have shown intercellular adhesion molecules 1 (ICAM-1) is upregulated in lining layer fibroblasts and facilitates tissue invasion and immune cell adhesion.[36] Flow cytometric analysis of patient with RA synovial fibroblast showed no increase in ICAM-1 expression in response to TGF-β, however a significant increase in ICAM-1 (***p=0.0004) following treatment with IL-1β was observed (figure 8A). Importantly, the combined treatment with TGF-β and IL-1β resulted in a significant (**p=0.0047) increase in ICAM-1 expression compared with IL-1β only treated synovial fibroblasts (figure 8A). Fibroblasts are the main source of IL-6 in RA with pro-inflammatory synovial fibroblast secreting high levels of IL-6 in response to TNF-α.[6 37] Similarly to the expression of ICAM-1, treatment of synovial fibroblasts with TGF-β did not lead to increased IL-6 secretion compared with untreated synovial fibroblasts, however

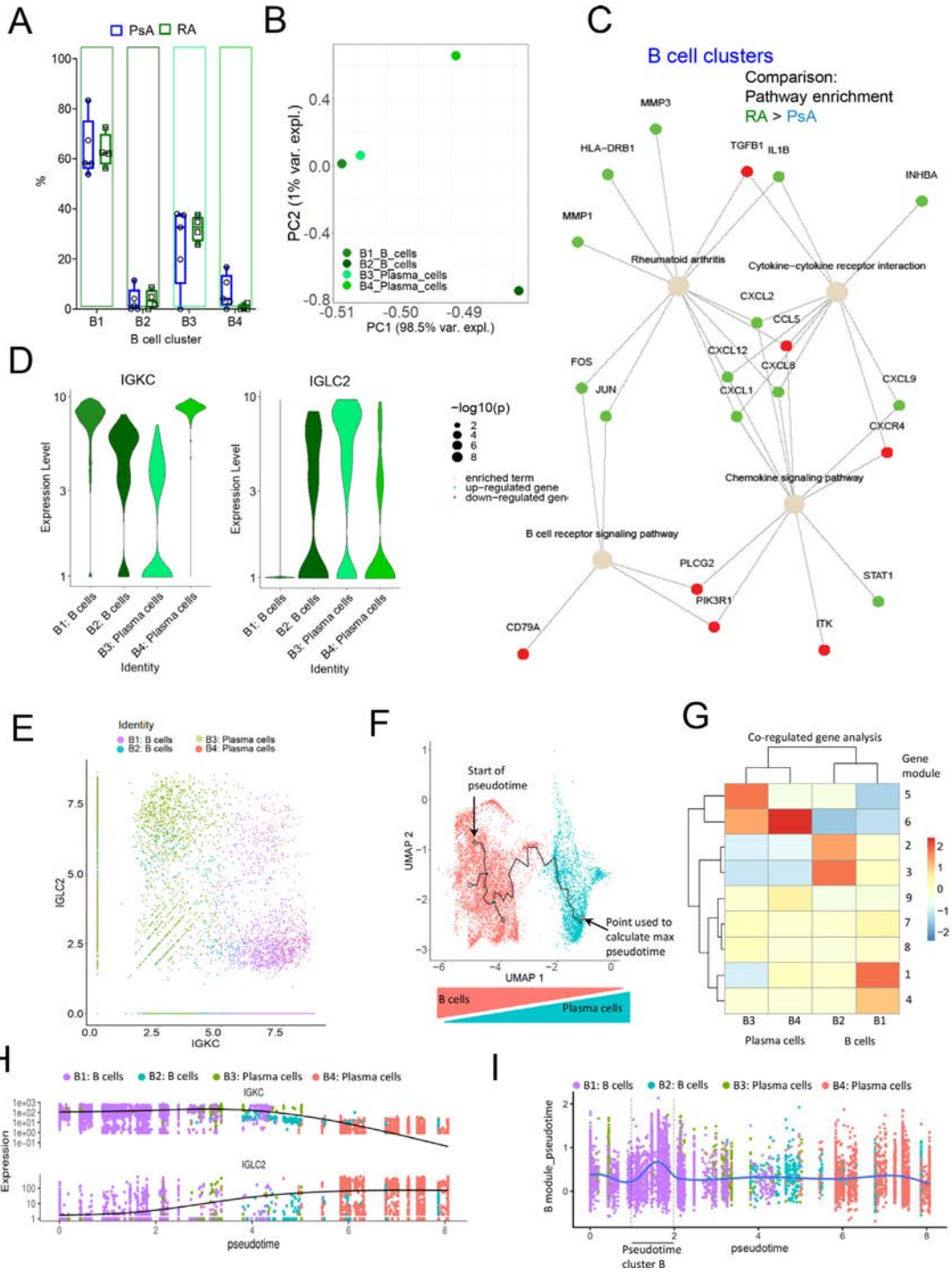

Figure 6 Synovial plasma cells show biased usage of antibody λ over κ light chains. (A) Abundance of B cell and plasma cell clusters in patient with RA and PsA synovial biopsies, the frequency of each cluster was calculated as a percentage of all B cells and plasma cells per sample in PsA and RA synovial biopsies (n=4–5). Data are presented as box and whiskers plots (min to max), symbols represent individual samples, statistical significance was determined by two-way analysis of variance with Sidak's multiple comparisons test, *p<0.05 were considered significant. (B) Principal component analysis plot of enriched pathways following pathway enrichment analysis of the identified B cell and plasma cell clusters, shows separation of B cells and plasma cells. (C) Term plot of the indicated pathways with significant enrichment in RA compared with PsA B cell clusters following pathway enrichment analysis. (D) Violin plot for the log normalised expression of IGKC (κ chain) and IGLC2 (λ chain) by the identified clusters. (E) Scatter plot of the relation between IGKC and IGLC2 expression of al B cell clusters. (F) Trajectory analysis of B cell and plasma cell clusters, arrows indicate starting point of pseudotime analysis. Due to the branching of the trajectory, in order to identify starting point of pseudotime analysis, analysis was initially performed with the indicated starting point. The highest pseudotime difference was then identified and used as the new starting point so trajectory analysis progresses from B cells to plasma cells. (G) Heatmap of co-regulated genes expressed per cluster as a function of pseudotime. Co-regulated genes were found using the *find_gene_modules* function in Monocle3 which runs UMAP on the genes rather than cells to group genes into modules using Louvain community analysis. (H) Expression of IGKC and IGLC2 as a function of pseudotime. (I) Differentially expressed genes of pseudotime fragment 1–2 expressed over the length of pseudotime. PsA, psoriatic arthritis; RA, rheumatoid arthritis; UMAP, Uniform Manifold Approximation and Projection.

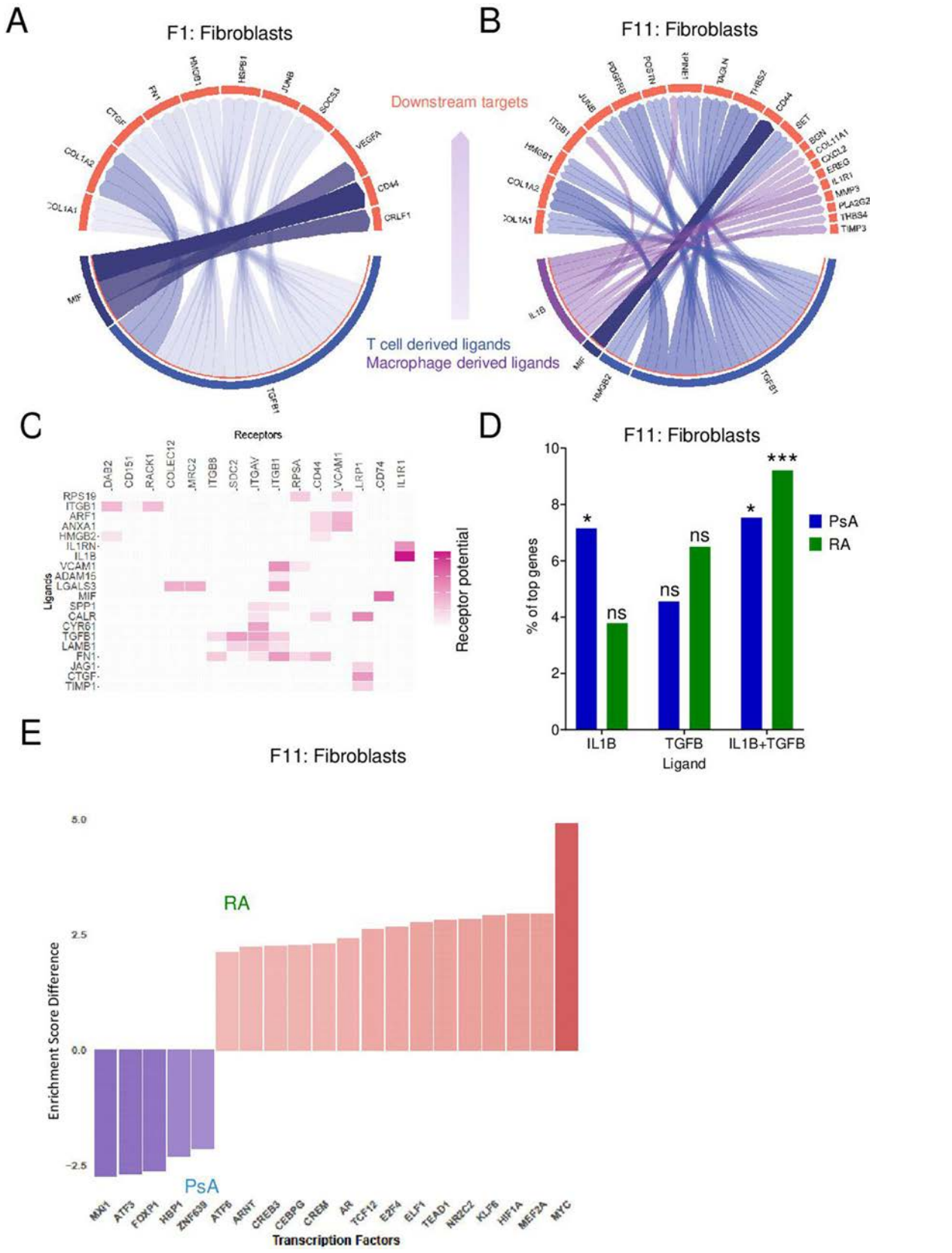

Figure 7 Identification of ligand receptor interactions that promote proinflammatory fibroblasts. (A) Circo plot depicting the top ligand and downstream target interaction for enriched in PsA synovial fibroblast cluster F1. (B) Circo plot depicting the top ligand and downstream target interaction for enriched in RA, proinflammatory synovial fibroblast cluster F11. (C) Heatmap of ligand receptor interactions for synovial fibroblast cluster F11. (D) Percentage of gene targets downstream of IL1B, TGFB1 or IL1B+TGFB as part of the top targets regulated by F11 fibroblast cluster receptors. Asterisks indicate significance of ligand-target interactions. (E) DoRothEA analysis of transcription factor usage by RA compared with PsA fibroblast cell cluster F11, based on expression of known downstream ligands, VIPER score difference between RA and PsA is shown. IL, interleukin; PsA, psoriatic arthritis; RA, rheumatoid arthritis; TGF, transforming growth factor.

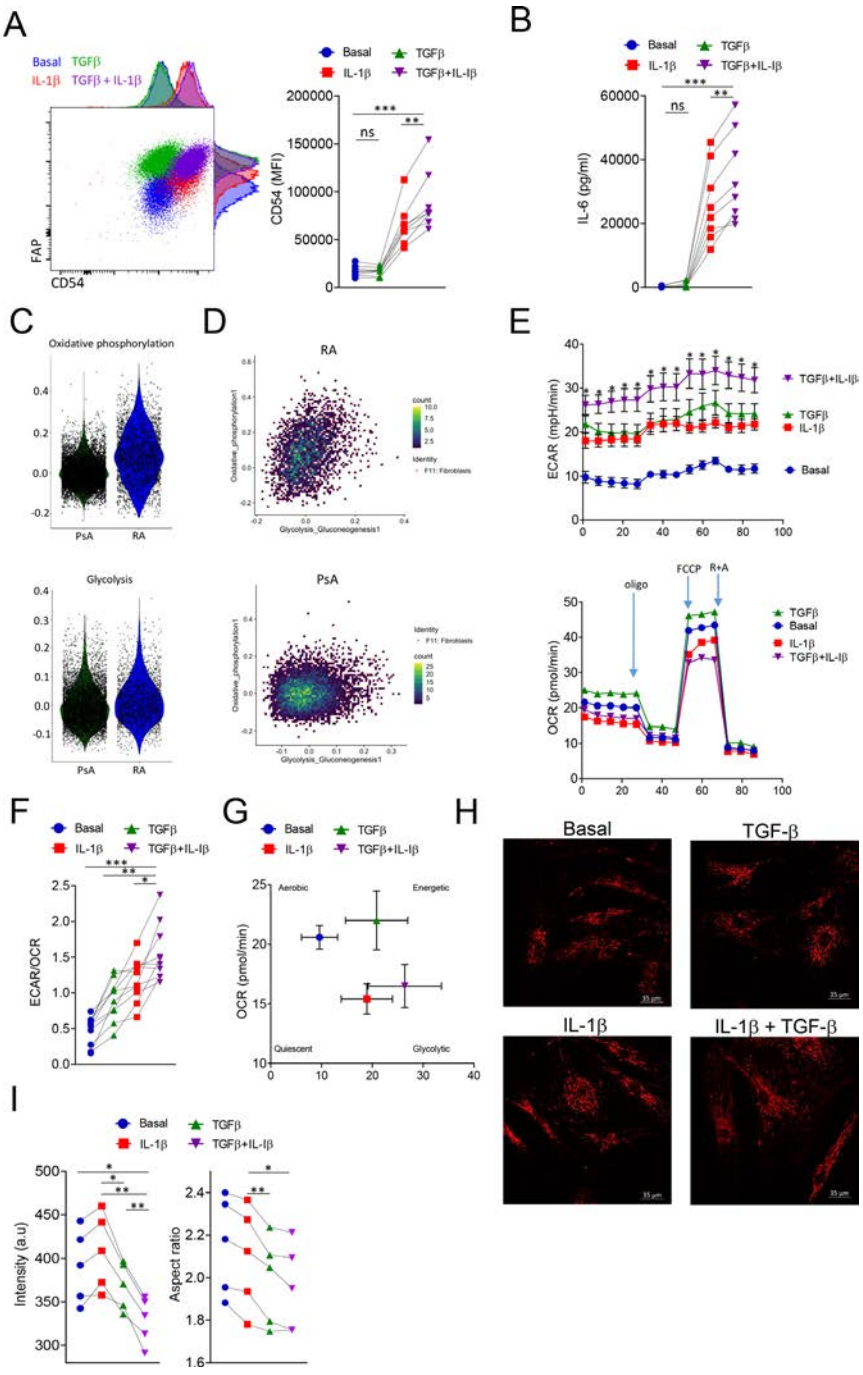

Figure 8 Effect of IL-1β and TGF-β on patient with RA synovial fibroblasts. (A) Flow cytometric analysis of CD54 (ICAM-1) expression by patient with RA synovial fibroblasts following treatment with IL-1β, TGF-β or a combination of both. Statistical significance was determined by one-way analysis of variance (ANOVA), symbols indicate individual samples (n=8), **p=0.0047, ***p=0.0007. (B) IL-6 secretion by patient with RA synovial fibroblasts treated under the conditions indicated. Statistical significance was determined by one-way ANOVA, symbols indicate individual samples (n=8), **p=0.0018, ***p=0.0008. (C) Violin plots of gene module expression scores in F11 fibroblasts, generated using the *AddModuleScore* function in Seurat, for oxidative phosphorylation-related and glycolysis-related genes derived from the Kegg gene sets 'hsa00010' and 'hsa00190'. (D) Scatter plot of the relationship between oxidative phosphorylation and glycolysis gene module expression scores in the F11 fibroblast cluster. Scatter plots are coloured by density and Pearson correlation scores estimate the relationship between oxidative phosphorylation and glycolysis in RA-derived and PsA-derived F11 fibroblasts. (E) Seahorse bioenergetic analysis of patient with RA synovial fibroblast, ECAR and OCR measurements are shown under basal conditions or following treatment with TGF-β, IL-1 β or a combination of both. Statistical significance was determined with two-way ANOVA, p<0.05 was considered significant, n=10, points and lines represent mean values. (F) ECAR to OCR ratio of patient with RA fibroblasts for the indicated conditions, statistical significance was determined by one-way ANOVA, n=10, **p=0.0014, *p=0.019, ***p<0.0001. (G) Bioenergetic profile graph of patient with RA synovial fibroblasts under the indicated treatments, n=10. (H) Representative multiphoton microscope images of TMRM stained synovial fibroblast mitochondria under the indicated conditions. (I) Fluorescent intensity of TMRM and mitochondrial aspect ratio. Statistical significance was determined with one-way ANOVA, p<0.05 were considered significant. ECAR, extracellular acidification rate; IL, interleukin; OCR, oxygen consumption rate; PsA, psoriatic arthritis; RA, rheumatoid arthritis; TGF, transforming growth factor; TMRM,tetramethylrhodamine methyl ester perchlorate.

the combined treatment with TGF-β and IL-1β resulted in a significant (**p=0.0018) increase in IL-6 compared with IL-1β only treated synovial fibroblasts (figure 8B). Dysregulation of synovial fibroblast metabolism with increased reliance on glycolysis has previously been associated with fibroblast pathogenic behaviour, interestingly, T cell derived soluble mediators have also been shown to enact metabolic switch of fibroblast towards glycolysis.[38–40] Bioinformatic characterisation of gene modules based on oxidative phosphorylation or glycolysis reference pathways, deposited on Kyoto Encyclopedia of Genes and Genomes (KEGG) (pathways hsa00190 and hsa00010, respectively) showed on a transcriptional level, increased glycolysis and oxidative phosphorylation involvement in RA synovial fibroblasts of cluster F11 compared with PsA (figure 8C,D). To evaluate on a functional level that the potential synergistic effect of TGF-β and IL-1β regulate a pro-inflammatory synovial fibroblast we next used real-time Seahorse metabolic profiling to examine the extracellular acidification rate (ECAR) which measures glycolysis and the comparable oxygen consumption rate (OCR) which measured oxidative phosphorylation (figure 8E). While both TGF-β and IL-1β alone increased ECAR, the combined TGF-β and IL-1β treatment leads to a significant (*p<0.05) increase in all ECAR measurements (baseline glycolysis, glycolytic capacity, glycolytic reserve) compared with IL-1β only treated synovial fibroblasts (figure 8E). While there was no significant difference in the OCR profile in response to TGF-β and IL-1β stimulation alone, the combination of TGF-β and IL-1β, resulted in a decrease in maximal spare respiratory capacity compared with either alone. This led to an increase in the ECAR/OCR ratio, indicative of the cell's reliance on glycolysis over oxidative phosphorylation, which is significantly higher in TGF-β and IL-1β treated synovial fibroblast compared with IL-1β or TGF-β alone (*p=0.029 and **p=0.0014, respectively) (figure 8F). The metabolic energy map demonstrated a shift in the overall metabolic profile of synovial fibroblasts where the combination of TGF-β and IL-1β resulted in a highly glycolytic synovial fibroblast phenotype (figure 8G). This shift in metabolic profile was paralleled by changes in synovial fibroblast mitochondrial function and morphology in response to TGF-β and IL-1β. Combined treatment with TGF-β and IL-1β resulted in significantly reduced tetramethylrhodamine methyl ester (TMRM) staining intensity compared with TGF-β or IL-1β (**p=0.0056, **p=0.0024, respectively), indicative of reduced mitochondrial membrane potential (figure 8H1). Decreased mitochondrial aspect ratio is indicative of reduced mitochondrial fusion, TGF-β and IL-1β treated synovial fibroblasts had significantly (*p=0.028) reduced aspect ratio compared with IL-1β only treated fibroblasts (figure 8H1) and aligns with the increased reliance of TGF-β and IL-1β treated fibroblasts to glycolysis over oxidative phosphorylation, as mitochondrial fusion supports oxidative phosphorylation.[41]

DISCUSSION

Synovial inflammation in RA and PsA has a complex aetiology and is defined as the outcome of several underlying immunological mechanisms. Despite recent advances and increased availability of therapeutic options due to the introduction of biologics, patients often undergo exploratory treatments until they show an adequate response.[42] For patients to experience sustained remission, achieving remission early is fundamental, therefore, lost time at initial stages of disease can have serious, lasting effects for the patients' quality of life.[43] Even when successful therapeutic intervention is achieved, long-term toxicity can have an impact on the patient.[44] To advance towards precision medicine, it is crucial that we achieve a better understanding of the complex immune environment of the inflamed joint. The complexity of RA and PsA pathogenesis is confounded by multifaceted synovial and stromal cell interactions. Identifying and therapeutically targeting specific immune-stromal cell interactions has the potential to greatly reduce toxicity and improve therapeutic outcomes for both patients with RA and PsA. While significant advances have been made with the introduction of single-cell RNA sequencing (scRNAseq) and other omic approaches in RA, to our knowledge, this is the first time that a transcriptomic analysis of intact synovial single cell suspensions of the inflamed joint in RA and PsA has been performed,[9] allowing for in-depth comparative cellular analysis of these two pathotypes.

Using high numbers of cells from intact synovial biopsy single cell suspensions for scRNAseq analysis offers distinct advantages. Previous studies have used sorted immune or stromal cells on the basis of CD45 expression or sorted specific populations, while prior knowledge of the cells included can expedite cluster analysis and annotation, it makes the generation of cell–cell interaction networks challenging.[11] By not sorting synovial cells prior to RNAseq analysis, we remove an important potential source of variation between experimental data and the *in situ* environment of the joint. Additionally, cell frequencies of the populations analysed more faithfully mirror their relative abundances in the inflamed joint and allow for the generation of cell–cell interaction networks between immune and stromal cells.

The resulting transcriptomic analysis of the inflamed joint in RA and PsA revealed several previously unappreciated aspects of synovial inflammation. Limited T cell proliferation indicates that infiltrating T cells may be have a more important role in maintaining synovial T cells than previously anticipated. Additionally, differential light chain expression by synovial memory and plasma B cells leads to the hypothesis that in part, synovial plasma cells are recruited to the inflamed joint. Importantly, we identified differential abundance of synovial fibroblasts and their transcriptome in RA and PsA, alluding to disease specific mechanisms of synovial inflammation.

Recent studies have identified the existence of synovial fibroblasts with distinct functional characteristics in RA.[14] Mizoguchi *et al*, have used RNAseq analysis of sorted synovial fibroblasts from two patient with RA and two patient with OA samples to identify three populations of synovial fibroblasts based on the expression of CD34 and THY1.[6] Synovial fibroblasts negative for CD34 but expressing THY1 are expanded in RA and potentially contribute to synovial inflammation via the production of pro-inflammatory cytokines.[6] Dividing synovial fibroblasts into functionally distinct subsets is an emerging field of study, an additional categorisation of synovial fibroblasts into distinct populations has been proposed where synovial fibroblasts are divided into two populations based on expression of FAP and THY1.[14] FAP^+THY1^+ RA synovial fibroblasts express elevated levels of pro-inflammatory cytokines and chemokines including IL-6, chemokine (C-C motif) ligand 5 (CCL5) and CCL2, and are necessary to maintain synovial inflammation in a murine model of RA.[14] Available information on distinct functions of synovial fibroblasts in PsA is scarce, however, recent studies show that PsA synovial fibroblasts can promote angiogenesis through regulation of endothelial cells.[45] Previous studies have identified FAP^+THY1^+ synovial fibroblasts as pro-inflammatory with increased expression of C-C chemokine receptor type 2 (CCR2) and reduced expression of matrix metalloproteinase-3 (MMP3) compared with $THY1^-$ synovial fibroblasts, supporting our analysis of F11 compared with F1 synovial fibroblasts. FAP

expression has been detected on synovial fibroblasts at early stages of inflammation in RA, indicating possible contribution of FAP^+THY1^+ fibroblasts early in disease pathogenesis, however, little is known regarding functionally distinct synovial fibroblast clusters in PsA.[46] Herein, we report increased abundance of $THY1^-$ synovial fibroblast cluster F1 in PsA compared with RA, indicative of differences in fibroblast involvement in synovial inflammation between the two pathotypes.

Extensive angiogenesis is a characteristic of both RA and PsA, required to support the egress of immune cells and O_2 from the periphery to the otherwise hypoxic environment of the inflamed joint.[47] Despite the central role of angiogenesis in RA and PsA, morphological differences are evident with PsA synovial blood vessels presenting a tortuous, elongated and dilated phenotype, similar to that observed in tumour vasculature.[45] Endothelial cell contribution to synovial inflammation is more complex and extends beyond pathogenic angiogenesis. Recent studies show a stromal crosstalk between synovial fibroblasts and endothelial cells in RA, with the latter providing NOTCH3-activating ligands to promote THY1-expressing synovial fibroblasts.[20] In our analysis, five transcriptionally distinct synovial endothelial cell clusters were identified, however, only one cluster showed evidence of differential abundance between RA and PsA. Interestingly, VEGF receptor expression and NOTCH expression is higher in RA compared with PsA E1 endothelial cells. VEGF and NOTCH signalling cascades decide the fate of endothelial cell specialisation towards stalk, tip or intermediate cell phenotypes that impact angiogenesis. Differences in the angiogenic process in RA and PsA are additionally reflected by the differential TF usage with FOXP1, a TF that is required for neoangiogenesis and endothelial cell sprouting, upregulated in PsA compared with RA, while master regulator of endothelial cell metabolic reprograming during sprouting, TEAD1, shows enhanced usage in RA compared with PsA.[19 48 49] Importantly, endothelial cells harbour high potential for interaction not only with distinct synovial fibroblast clusters but also with immune cells. Endothelial cell clusters show a plethora of potential interactions with other endothelial cell clusters; interactions that could be pivotal in the organisation of new blood vessels contributing to the pathogenesis of RA and PsA.

There is a great body of evidence regarding autoantibody involvement in RA disease pathogenesis.[50] In addition to autoantibodies, novel functions of synovial B cells and synovial B cell populations have recently been described.[4 51 52] The presence of ectopic lymphoid structures in RA has led to the popular hypothesis that synovial plasma cells are generated in the synovial tissue from clonally expanded, peripheral blood B cell infiltrates.[53] However, no direct connection leading from synovial B cells to plasma cells has previously been described. B cells are monospecific and express a BCR consisting of two identical heavy and two identical light chains, the monospecificity of the B cell is ensured by the process of allelic exclusion.[54] Following successful functional rearrangement of the heavy chain-encoding allele immunoglobulin heavy chain (IGH), the light chain-encoding loci are rearranged. Rearrangement of the light chain starts at the κ chain locus and, if no functional κ light chain emerges, recombination proceeds with the λ chain locus.[55] Due to this process, the ratio of κ/λ chain usage by antibodies is biased towards κ light chains (κ/λ, 2:1).[55] Interestingly, in patients with RA, anti–citrullinated protein antibody (ACPA)-expressing B cells show increased bias towards λ light chains.[56] In agreement with this study, synovial tissue plasma cells show a clear preference for the expression of IGLC2 compared with IGKC. Surprisingly, most synovial B cells express IGKC, which indicates that synovial plasma cells are not derived from synovial B cells. It has to be noted that several rounds of BCR editing can result in a transition from κ light chain usage to λ light chain, this process however, has been reported at the very early stages of B cell development and there is no direct evidence to suggest that it can occur after the onset of somatic hypermutation.[35 57] Despite the effectiveness of allelic exclusion, dual BCR-expressing B cells can emerge.[58 59] Expression of two BCRs with different specificities could help autoreactive B cells evade central tolerance mechanisms. The data presented in this study suggest that synovial plasma cells are not derived from synovial B cells, therefore the role of ectopic lymphoid organs may be secondary to the differentiation of plasma cells. However, the presence of a small population of λ light chain expressing B cells or BCR editing in the synovial tissue could be contributing in the emergence of λ light chain expressing synovial plasma cells. Further studies are required to evaluate the origin of synovial plasma cells and assess their connection to plasma cells recruited from the periphery, and dual $\kappa^+\lambda^+$ light chain-expressing B cells.

The most abundant immune cells of the inflamed joint are synovial macrophages with distinct protective as well as pro-inflammatory roles in RA disease pathogenesis.[13 60 61] In this analysis we have identified three macrophage and one DC cluster. Importantly we describe a macrophage population with high pathogenic capacity, characterised by high expression of IL1B. While present in similar abundances in both RA and PsA, IL1B-expressing macrophages are differentially regulated between RA and PsA, with increased nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation potential in PsA compared with RA. IL-1β, could be responsible for inducing pro-inflammatory programming by synovial fibroblasts, therefore, we assessed the expression of the IL-1β receptor by all synovial cells. Interestingly, highest degree of IL-1β receptor expression is observed by synovial fibroblasts and endothelial cells, however, not all fibroblast and endothelial cell clusters express the IL-1β receptor to a similar degree, thus, certain fibroblast and endothelial cell populations, such as fibroblast cluster F11, are more susceptible to programming by IL-1β.

Polyfunctional T cell responses with a bias towards Th17-like and Th1 have been reported in PsA and RA, respectively.[8 62] Even within RA, synovial T cell cytokine contributions are not uniform and are indicative of discrete endotypes of disease.[27] Further characterisation of T cell subsets and their potential for crosstalk with stromal cells in RA and PsA could significantly increase our understanding of T cell involvement in synovial inflammation. Specific RA T cell populations show high expression of the immunomodulatory TGF-B1 inhibition of TGF-β can limit synovial fibroblast hyperplasia in murine models of RA and, due to synergistic effects with other cytokines, it could be an attractive target for future therapeutic intervention.[28 29]

Despite current efforts, there is no consensus on the proportion and function of synovial fibroblasts in RA or PsA. An additional level of complexity is added when attempting to decipher the interactions that dictate the pro-inflammatory attributes of synovial fibroblasts. Recent studies have suggested a stromal crosstalk between endothelial cells and synovial fibroblasts as the driving force of the transcriptome of potentially pathogenic synovial fibroblasts.[20] Indeed, decoding cell–cell interactions at the site of inflammation in RA and PsA can lead to the identification of novel avenues of targeted therapeutic intervention; therefore, instead of targeting systemic immunological pathways or entire immune populations, specific context-dependent cell–cell interactions can be disrupted leading to resolution of inflammation with minimal side effects for the patient. Herein

we describe one such potential immune-stromal cell interaction: synovial T cell TGF-β and macrophage-derived IL-1β synergistically drive the transcriptome, cell adhesion molecule expression, pro-inflammatory cytokine secretion and metabolic profile of potentially pathogenic fibroblasts that are enriched in RA but not in PsA.

Sample heterogeneity may impact some of the findings, and while both patients with RA and PsA had comparable active moderate to high disease activity based on disease activity score-28 (DAS28) and disease activity index for psoriatic arthritis (DAPSA), respectively, and all biopsies were obtained from the same joint type, analysis of additional samples would allow for the assessment of transcriptomic profiles, disease status and response to treatment in RA and PsA. Additionally, while we have performed functional characterisation of synovial tissue fibroblasts following treatment with IL-1β and TGF-β, to validate the bioinformatically identified cellular crosstalk, further targeted in vitro studies will be required for the assessment of endothelial cell transcriptomic differences in RA and PsA and their potential impact on the characteristic vascular morphology of the two disease pathotypes. The balance between synovial T cell proliferation potential and homing from the periphery will require further flow cytometric analysis and proliferation assays. The herein presented scRNAseq and complementary functional assays importantly, highlight the need for the implementation of novel antibody multiplexing techniques using DNA barcoded antibodies allowing for target co-detection by indexing and spatial transcriptomic analysis for further characterisation of the proposed receptor–ligand interactions.

The first analysis of patient with intact RA and PsA synovial tissue single-cell suspensions is a significant step towards precision medicine and reveals previously unappreciated aspects of synovial inflammation. The potential reliance of the synovial T cell and plasma cell pools on renewal from the periphery and the identification of immune-stromal cell interactions can become a paradigm shift in the development of novel therapeutic options for inflammatory arthritis.

METHODS

Patient sample collection and study approval

Patients with RA and PsA (defined by the American College of Rheumatology (ACR) and CASPAR Criteria, respectively) were recruited from the Rheumatology Department, St. Vincent's University Hospital, UCD and Tallaght University Hospital, TCD. Patient with RA and PsA synovial tissue samples from knee joints with active inflammation were obtained under local anaesthetic using Wolf 2.7 mm needle arthroscopy or ultrasound guided biopsy as previously described, please see online supplemental table S1 for patient clinical characteristics.[63] Patients with RA and PsA had comparable moderate to high disease activity (DAS28 4.6±1.1 and DAPSA 24.2±4.9, respectively) and biopsies had lymphocyte infiltrates and lining layer hyperplasia as scored by a clinical pathologist. The research was performed in accordance with the Declaration of Helsinki.

Synovial tissue sample preparation

Synovial tissue single cell suspensions were generated following enzymatic and mechanical digestion of the synovial biopsies as described previously.[27] Briefly, approximately 15 synovial biopsies per patient were digested using the GentleMACS Tumor Dissociation Kit, human (Miltenyi Biotech) as per manufacturers' instructions. Immediately after isolation, biopsies are washed with RPMI (Merck) before being placed in 4.7 mL RPMI supplemented with 200 µl of enzyme H, 100 µl enzyme R and 25 µl enzyme A in a gentleMACS C Tube followed by initial mechanical disruption of the tissue using programme h_tumor_01 on a gentleMACS Dissociator. Samples are enzymatically digested for a total of 1 hour at 37°C under continuous rotation using the MACSmix Tube Rotator with further applications of the gentleMACS Dissociator at the halfway point and at the end of the 1 hour incubation. The cell suspension is then passed through a 70 µM cell strainer. Viability of the cells is assessed with trypan blue exclusion staining and immediately cryopreserved in sterile filtered 10% dimethyl sulfoxide (DMSO)/fetal bovine serum (FBS) at a concentration of 1×10^6 cells per mL scRNAseq.

Frozen synovial biopsy cell suspensions were thawed quickly in a 37°C waterbath and transferred to sterile tubes with warm RPMI media (10% FBS). After washing and counting, a dead cell removal kit (Miltenyi cat#130-090-101) was implemented to increase viability. Using the Chromium Next GEM Single Cell 3' Reagent Kits V.3.1 (10X Genomics), cells were loaded onto the GEM Chips. The 10X Genomics Chromium Next GEM Single Cell 3' user manual was followed for all steps to generate complementary DNA (cDNA) libraries for each sample. cDNA quantifications and quality control were determined using the Agilent TapeStation. Final libraries were normalised, quantified (Illumina/ROX low, Kappa Biosystems), pooled based on 40 k reads/sample. Pooled libraries were sequenced on the Illumina NovaSeq using and S2 NovaSeq 6000 Reagents V.1 kit and a 100-cycle sequencing run.

ScRNA-seq data analysis

Initial processing

The gene expression raw sequencing data for the synovial tissue single cell suspensions were processed using Cell Ranger V.3.1 (10X Genomics, California, USA), with the 10X human transcriptome GRCh38.3.0.0 serving as a reference. Single-cell reads for each sample were converted to Seurat objects using the R package Seurat (V.4.0.3) in R (V.4.1). For each object representing an individual patient synovial tissue sample, data were filtered with genes detected in less than 3 cells, excluded from downstream analysis. Empty droplets were removed with function EmptyDrops (DropletUtils, V.1.12.1, code available here: https://github.com/MarioniLab/DropletUtils), followed by removal of cell doublets. Cell doublets were removed by using a newly described computational approach—DoubletFinder. DoubletFinder intersects transcriptional data and a data set specific artificial population of doublets, generated by averaging gene expression of randomly selected pairs of cells, in order to identify cell doublets (code available here: https://github.com/chris-mcginnis-ucsf/DoubletFinder).[64] Apoptotic cells were removed by eliminating cells with a mitochondria associated gene expression of over 25%.[9] One patient sample with a high frequency of cells over the mitochondrial gene expression threshold was completely excluded from further analysis due to potentially compromised/stressed live cells. (Data scaling) and normalisation were performed with the newly described sctransform (V.0.3.2) package. Sctransform uses non-heuristic approaches in order to scale the data based on Pearson residuals of a negative binomial regression, as such, it is superior to the widely used unique molecular identifier method since it is less susceptible to technical variations associated with widely different sequencing depths between deeply and shallowly sequenced cells of the same data set (code available here: https://github.com/satijalab/sctransform).[65] This approach resulted in

178 804 cells from four patient with RA and five patient with PsA synovial tissue samples available for downstream cell clustering and analysis.

Clustering of major cell populations

PCA using the sctransform scaled data identified variable genes as input. Prior to clustering, integration of the Seurat objects representing synovial tissue samples from nine patients was performed with Harmony (V.0.1.0) (code available here: https://github.com/harmony-one/harmony).[66] Harmony reduces variation associated with technical differences between samples that may otherwise, 'mask' biological differences, this is achieved by cell specific correction of the cell's PCA coordinates. Clusters were identified with FindCluster function of Seurat and visualised on a UMAP plot (code available here: https://github.com/satijalab). Clustering efficiency was independently assessed by calculating the observed to expected edge weight ratios for all pairs of clusters (online supplemental figure S6). These ratios were calculated with function *pairwiseModularity* of package *bluste*r of all off-diagonal cluster pairings had less observed to expected edge weight ratios compared with cells belonging to the same cluster. Further analysis of cluster stability was performed with bootstrapping the data in order to calculate the probability of a cell being randomly co-assigned to two clusters (function *bootstrapStability*, package *bluster*), clustering was efficient and stable. Cell identity was calculated with automated and prior knowledge approaches. SignleR and scCATCH were unsuccessful in annotating the majority of the cell clusters primarily due to a lack of synovial tissue specific data in reference data sets.[15] Therefore, several clusters were annotated based on differential gene expression (DEG) profiles. DEGs were derived using the FindMarkers function of Seurat with Wilcoxon test and p values adjusted by Bonferroni correction (code available here: https://github.com/satijalab/seurat). DEGs were filtered on the basis of a minimum 0.25 of the cell cluster expression, a minimum log2 fold change of 0.5 and a p value below 0.05. Cell numbers per cluster per patient are included in online supplemental file 3.

Trajectory analysis

Trajectory analysis of synovial tissue B cells was performed with Monocle3 (V.1.0). Co-regulated genes over pseudotime were identified by the find gene modules function. Additional analysis was performed by clustering cells in pseudotime fragments and then identifying DEG per pseudotime clustered cells (code available here: https://github.com/cole-trapnell-lab/monocle-release). DEG of specific pseudotime fragments were used as modules and their expression assessed over the complete pseudotime.

Cell cycle analysis

Cell cycle analysis was performed by scoring the relative expression of 54 G2/M phase associated genes and 43 s phase associated genes as per function CellCycleScoring of package Seurat. The base code used can be found as part of the cell cycle analysis vignette here: https://github.com/satijalab/seurat/blob/master/vignettes.

Cell–cell interaction analysis

Cell–cell interactions were identified with the nichenetr (V.1.0) package following the nichenetr vignette and code available here (https://github.com/saeyslab/nichenetr/tree/master/vignettes).[67] Potential cell–cell interactions were identified based on gene expression and predetermined, based on prior-knowledge, receptor–ligand interaction pathways. One cell cluster was assigned the role of the 'receiver' population with its expression data intersected with known receptors and all other cells were assigned the role of 'sender' cells with their expression data intersected with known ligands. Receptors, downstream target genes of interest and ligands were based on DEG between a defined condition of interest and a reference condition. To evaluate to what extent TGF-β and IL-1β may regulate the differences between RA and PsA F11 fibroblasts a multi-ligand random forest model was used. This model uses the regulatory potential scores of TGF-β and IL-1β to predict the transcriptional programme of RA F11 fibroblasts and PsA F11 fibroblasts. The per cent of RA-specific or PsA-specific genes which belong to the 5% most strongly predicted targets were visualised and a one-sided Fisher's exact test was used to test the significance of the association between the RA-specific and PsA-specific genes and whether they are part of the 5% most strongly predicted targets.

Pathway analysis

Pathway enrichment analysis was performed with pathfindR (V.1.6.2) active subnetwork analysis.[68] DEG identified by FindMarkers were filtered based on log2 fold change and adjusted p value followed by run_pathfinR based on the KEGG database. Gene modules of oxidative phosphorylation-related and glycolysis-related genes were generated using the gauge package (https://bioconductor.org/packages/release/bioc/html/gage.html) to access the Kegg gene sets 'hsa00010 Glycolysis/Gluconeogenesis' and 'hsa00190 Oxidative Phosphorylation'. The *AddModuleScore* function in Seurat calculated the average expression of each gene module in each disease group on a single cell level and subtracted the aggregate expression of 100 control genes. Gene modules were visualised by violin plot and scatterplot and Pearson correlation scores were calculated.

TF usage analysis

TF usage was estimated with package dorothea (V.1.4.1) with human regulons A, B, C.[69] For visualisation, viper score differences between RA and PsA cell clusters were calculated.

Data visualisation

Plots are generated via ggplot2 (V.3.3.5), pheatmap (V.1.0.12), Seurat (V.4.0.3) and pathfindR (V.1.6.2) functions in R (V.4.0.1). Cell cluster abundance box and whisker plots (min to max) were generated in Prism based on relative frequency data; each symbol represents an individual sample.

Data availability and public access

All raw and processed files as well clinical information for each sample are deposited on national center for biotechnology information (NCBI), ascension number GSE200815 and are publically available without any restrictions of their subsequent use. Additionally, while detailed vignettes and base code is available on the bioinformatics platform GitHub (as indicated in methods) for all packages used in the analysis, if specific parts of the code are needed, they will become available on reasonable request.

Cellular bioenergetic function analysis

To examine the metabolic profile of IL-1B/TGF-B stimulated RA-fibroblast like synoviocytes (RAFLS), OCR and ECAR, reflecting oxidative phosphorylation and glycolysis, respectively, were measured using the Seahorse-XFe96 analyser (Seahorse Biosciences). RAFLS were seeded at 15 000 cells per well in a 94-well cell culture XFe microplate (Seahorse Biosciences) and

allowed to adhere overnight. Following this, cells were then treated with either IL-1B (1 ng/mL), TGF-B (10 ng/mL), or a combination of IL-1B (1 ng/mL) and TGF-B (10 ng/mL) for 24 hours. Cells were then washed with assay medium (unbuffered DMEM supplemented with 10 mM glucose, pH-7.4) before incubation with assay medium for 1 hour at 37°C in a non-CO2 incubator. Basal oxidative phosphorylation/glycolysis was calculated by the average of three baseline OCR/ECAR measurements, respectively, obtained before injection of specific metabolic inhibitors; oligomycin (ATP-synthase-inhibitor), (2 µg/mL; Seahorse Biosciences, UK) trifluorocarbonylcyanide phenylhydrazone (FCCP) (mitochondrial uncoupler) (5 µM; Seahorse Biosciences) and antimycin A (complex-III inhibitor) (2 µM; Seahorse Biosciences). Oligomycin was injected to evaluate both the maximal glycolytic rate and ATP synthesis, determined by subtracting the amount of respiration left after oligomycin injection from baseline OCR. FCCP was injected to evaluate the maximal respiratory capacity (average of three measurements following injection) and respiratory reserve. Maximal respiratory capacity was determined by subtracting baseline OCR from FCCP-induced OCR and the respiratory reserve (baseline OCR subtracted from maximal respiratory capacity).

Cytokine measurements

To assess the effects of treatment with IL-1B and TGF-B singly and in combination on the production of pro-inflammatory mediators by the RAFLS, RAFLS were seeded in 24-well plates at a density of 5×10^5 per well and allowed to attach overnight. Cells were then incubated in 1% RPMI-1640 for 24 hours and subsequently stimulated with IL-1B (1 ng/mL), TGF-B (10 ng/mL), or a combination of IL-1B (1 ng/mL) and TGF-B (10 ng/mL). Supernatants were then harvested and levels of IL-6 (IL-6, R&D systems, UK,) were determined according to manufacturer's conditions.

Flow cytometric analysis

Surface marker expression of RAFLS following stimulation with IL-1B (1 ng/mL), TGF-B (10 ng/mL), and IL-1B (1 ng/mL)+TGF B (10 ng/mL) was analysed by multiparameter flow cytometry. For extracellular staining, cells were seeded at 5×10^5 cells/well in a 24-well plate and stimulated with 1% complete Roswell Park Memorial institute medium (cRPMI) supplemented with the specific cytokines IL-1B and TGF-B prior to staining. For the gating strategy, the cells were initially gated based on forward and side scatter and doublets were removed. LIVE/DEAD fixable NIR (Thermo Fisher) viability dye was used to eliminate dead cells. To eliminate non-specific binding of monoclonal antibodies to the Fc-γ receptor (FcγR), samples were blocked with a human FcγR-binding inhibitor (TruStain FcX Receptor blocking solution (BioLegend)) prior to antibody staining. The following antibodies were used in combination to investigate surface markers expressed by the stimulate RAFLS: Podoplanin FITC (Clone NC-08) (BioLegend), Human FAP Alexa Fluor 700 (Clone 427819) (RnD), CD90 Brilliant Violet 421 (Clone 5E10) (BioLegend), CD34 Brilliant Violet 510 (Clone 581) (BioLegend), CD54 Brilliant Violet 605 (Clone HA58) (BD), CD45 Brilliant Violet 650 (Clone HI30) (BioLegend), CD146 Brilliant Violet 711 (Clone P1H12) (BioLegend), HLA-DR Brilliant Violet 785 (Clone L243) (BioLegend), FAS-L PE (Clone NOK-1) (BioLegend) and CD309 PE/Cy7 (Clone 7D4-6) (BioLegend). Samples were acquired using the LSR Fortessa Flow Cytometer (BD) and analysed using FlowJo (V.10) software. Fluorescent minus one gating controls used were appropriate.

Tetramethylrhodamine methyl ester staining and analysis

Mitochondria Imaging was performed using a custom upright (Olympus BX61WI) laser multiphoton microscopy system equipped with a pulsed (80 MHz) titanium: sapphire laser (Chameleon Ultra, Coherent, USA), water-immersion 25× objective (Olympus, 1.05NA) and temperature controlled stage at 37°C. Fibroblasts were seeded in 35 mm petri-dishes and stained at 37°C for 30 min with 250 nM of tetramethylrhodamine methyl ester and then washed with phosphate-buffered saline. Two-photon excitation was performed at 850 nm and fluorescence emission was collected at 580–638 nm. Fluorescence images were acquired and quantified for their intensity and mitochondria morphology using CellProfiler with a custom built project pipeline, script available on request.[70 71]

Patient and public involvement

In this study we analysed specific immune and stromal cells obtained from patients with RA and PsA. While no patients were involved in setting the research question, the outcome measures or recruitment plans for the study, the group have hosted a number of patient information evenings where we have described the research, current project and the importance of patient engagement. No patients were asked to advice on interpretation or writing up of results, however, in collaboration with patient partners we developed a series of patient partnership workshops where lay dissemination of the study research to relevant patient groups was performed, with patient feedback now incorporated in future studies.

Twitter Sarah M Wade @Sarah_M_Wade, Mary Canavan @mary_canavan and Michael G Monaghan @drmgmonaghan

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Contributors Guarantor, AF,UF, Conceptualisation, AF, UF, DJV; methodology, AF, CMS, OT; software, AF, CMS, VK; validation, AF, CMS, OT; formal analysis, AF, UF, DJV, SN, CMS, OT, NN; investigation, AF, UF, DJV; resources, UF, DJV; data curation, AF, UF, DJV, VK, MC, SN, OT, NN, MGM, VM, JMF, SC, MH, CC, SMW, L-YH; writing—original draft preparation, AF, CMS, UF, DJV; writing—review and editing, AF, CMS, OT, UF, DJV; visualisation, AF, UF, DJV, CMS, NN; supervision, UF, DJV; project administration, UF, DJV; funding acquisition, UF, DJV.

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Competing interests None declared.

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Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available in a public, open access repository. Data are available upon reasonable request. All raw and processed files as well clinical information for each sample are deposited on national center for biotechnology information (NCBI), ascension number GSE200815 and are publically available without any restrictions of their subsequent use. Additionally, while detailed vignettes and base code is available on the bioinformatics platform GitHubgithub (as indicated in methods) for all packages used in the analysis, if specific parts of the code are needed, they will become available upon reasonable request.

ORCID iDs
Achilleas Floudas http://orcid.org/0000-0003-1690-5595
Conor M Smith http://orcid.org/0000-0003-1701-1665
Sarah M Wade http://orcid.org/0000-0003-2179-3903
Michael G Monaghan http://orcid.org/0000-0002-5530-4998
Sunil Nagpal http://orcid.org/0000-0001-6090-8780
Douglas J Veale http://orcid.org/0000-0003-2802-4971
Ursula Fearon http://orcid.org/0000-0001-8084-0429

REFERENCES

1 Veale DJ, Fearon U. The pathogenesis of psoriatic arthritis. *Lancet* 2018;391:2273–84.
2 Orr C, Vieira-Sousa E, Boyle DL, *et al*. Synovial tissue research: a state-of-the-art review. *Nat Rev Rheumatol* 2017;13:630.
3 Lewis MJ, Barnes MR, Blighe K, *et al*. Molecular portraits of early rheumatoid arthritis identify clinical and treatment response phenotypes. *Cell Rep* 2019;28:2455–70.
4 Floudas A, Neto N, Marzaioli V, *et al*. Pathogenic, glycolytic PD-1+ B cells accumulate in the hypoxic RA joint. *JCI Insight*. In Press 2020;5. doi:10.1172/jci.insight.139032. [Epub ahead of print: 05 Nov 2020].
5 Gao W, McGarry T, Orr C, *et al*. Tofacitinib regulates synovial inflammation in psoriatic arthritis, inhibiting STAT activation and induction of negative feedback inhibitors. *Ann Rheum Dis* 2016;75:311–5.
6 Mizoguchi F, Slowikowski K, Wei K, *et al*. Functionally distinct disease-associated fibroblast subsets in rheumatoid arthritis. *Nat Commun* 2018;9:789.
7 Rao DA, Gurish MF, Marshall JL, *et al*. Pathologically expanded peripheral T helper cell subset drives B cells in rheumatoid arthritis. *Nature* 2017;542:110–4.
8 Wade SM, Canavan M, McGarry T, *et al*. Association of synovial tissue polyfunctional T-cells with DAPSA in psoriatic arthritis. *Ann Rheum Dis* 2019;78:350–4.
9 Zhang F, Wei K, Slowikowski K, *et al*. Defining inflammatory cell states in rheumatoid arthritis joint synovial tissues by integrating single-cell transcriptomics and mass cytometry. *Nat Immunol* 2019;20:928–42.
10 Foley C, Floudas A, Canavan M. Increased T cell plasticity with dysregulation of T follicular helper, T peripheral helper and T regulatory cell responses in children with JIA and Down syndrome-associated arthritis. *Arthritis Rheumatol* 2019.
11 Penkava F, Velasco-Herrera MDC, Young MD, *et al*. Single-cell sequencing reveals clonal expansions of pro-inflammatory synovial CD8 T cells expressing tissue-homing receptors in psoriatic arthritis. *Nat Commun* 2020;11:4767.
12 Alivernini S, MacDonald L, Elmesmari A, *et al*. Distinct synovial tissue macrophage subsets regulate inflammation and remission in rheumatoid arthritis. *Nat Med* 2020;26:1295–306.
13 Culemann S, Grüneboom A, Nicolás-Ávila José Ángel, *et al*. Locally renewing resident synovial macrophages provide a protective barrier for the joint. *Nature* 2019;572:670–5.
14 Croft AP, Campos J, Jansen K, *et al*. Distinct fibroblast subsets drive inflammation and damage in arthritis. *Nature* 2019;570:246–51.
15 Shao X, Liao J, Lu X, *et al*. scCATCH: automatic annotation on cell types of clusters from single-cell RNA sequencing data. *iScience* 2020;23:100882.
16 Karlsson E, Magić I, Bostner J, *et al*. Revealing different roles of the mTOR-Targets S6K1 and S6K2 in breast cancer by expression profiling and structural analysis. *PLoS One* 2015;10:e0145013.
17 Hou W, Ji Z, Ji H, *et al*. A systematic evaluation of single-cell RNA-sequencing imputation methods. *Genome Biol* 2020;21:218.
18 Koon YL, Zhang S, Rahmat MB, *et al*. Enhanced Delta-Notch lateral inhibition model incorporating intracellular Notch heterogeneity and tension-dependent rate of Delta-Notch binding that reproduces sprouting angiogenesis patterns. *Sci Rep* 2018;8:9519.
19 Arderiu G, Espinosa S, Peña E, *et al*. PAR2-SMAD3 in microvascular endothelial cells is indispensable for vascular stability via tissue factor signaling. *J Mol Cell Biol* 2016;8:255–70.
20 Wei K, Korsunsky I, Marshall JL, *et al*. Notch signalling drives synovial fibroblast identity and arthritis pathology. *Nature* 2020;582:259–64.
21 Soker S, Takashima S, Miao HQ, *et al*. Neuropilin-1 is expressed by endothelial and tumor cells as an isoform-specific receptor for vascular endothelial growth factor. *Cell* 1998;92:735–45.
22 Gao W, Sweeney C, Walsh C, *et al*. Notch signalling pathways mediate synovial angiogenesis in response to vascular endothelial growth factor and angiopoietin 2. *Ann Rheum Dis* 2013;72:1080–8.
23 Cao G, O'Brien CD, Zhou Z, *et al*. Involvement of human PECAM-1 in angiogenesis and in vitro endothelial cell migration. *Am J Physiol Cell Physiol* 2002;282:C1181–90.
24 Pelton JC, Wright CE, Leitges M, *et al*. Multiple endothelial cells constitute the tip of developing blood vessels and polarize to promote lumen formation. *Development* 2014;141:4121–6.
25 Bae S, Park PSU, Lee Y, *et al*. MYC-mediated early glycolysis negatively regulates proinflammatory responses by controlling IRF4 in inflammatory macrophages. *Cell Rep* 2021;35:109264.
26 Pello OM, De Pizzol M, Mirolo M, *et al*. Role of c-myc in alternative activation of human macrophages and tumor-associated macrophage biology. *Blood* 2012;119:411–21.
27 Floudas A, Canavan M, McGarry T, *et al*. Acpa status correlates with differential immune profile in patients with rheumatoid arthritis. *Cells* 2021;10. doi:10.3390/cells10030647. [Epub ahead of print: 14 Mar 2021].
28 Sakuma M, Hatsushika K, Koyama K, *et al*. TGF-beta type I receptor kinase inhibitor down-regulates rheumatoid synoviocytes and prevents the arthritis induced by type II collagen antibody. *Int Immunol* 2007;19:117–26.
29 Bira Y, Tani K, Nishioka Y, *et al*. Transforming growth factor beta stimulates rheumatoid synovial fibroblasts via the type II receptor. *Mod Rheumatol* 2005;15:108–13.
30 Pohlers D, Beyer A, Koczan D, *et al*. Constitutive upregulation of the transforming growth factor-beta pathway in rheumatoid arthritis synovial fibroblasts. *Arthritis Res Ther* 2007;9:R59.
31 Corsiero E, Nerviani A, Bombardieri M, *et al*. Ectopic lymphoid structures: powerhouse of autoimmunity. *Front Immunol* 2016;7:430.
32 Vettermann C, Schlissel MS. Allelic exclusion of immunoglobulin genes: models and mechanisms. *Immunol Rev* 2010;237:22–42.
33 Van den Berge K, Roux de Bézieux H, Street K, *et al*. Trajectory-based differential expression analysis for single-cell sequencing data. *Nat Commun* 2020;11:1201.
34 Trapnell C, Cacchiarelli D, Grimsby J, *et al*. The dynamics and regulators of cell fate decisions are revealed by pseudotemporal ordering of single cells. *Nat Biotechnol* 2014;32:381–6.
35 Perfetti V, Vignarelli MC, Palladini G, *et al*. Insights into the regulation of immunoglobulin light chain gene rearrangements via analysis of the kappa light chain locus in lambda myeloma. *Immunology* 2004;112:420–7.
36 Szekanecz Z, Haines GK, Lin TR, *et al*. Differential distribution of intercellular adhesion molecules (ICAM-1, ICAM-2, and ICAM-3) and the MS-1 antigen in normal and diseased human synovia. their possible pathogenetic and clinical significance in rheumatoid arthritis. *Arthritis Rheum* 1994;37:221–31.
37 Valin A, Del Rey MJ, Municio C, *et al*. IL6/sIL6R regulates TNFα-inflammatory response in synovial fibroblasts through modulation of transcriptional and post-transcriptional mechanisms. *BMC Mol Cell Biol* 2020;21:74.
38 de Oliveira PG, Farinon M, Sanchez-Lopez E, *et al*. Fibroblast-Like synoviocytes glucose metabolism as a therapeutic target in rheumatoid arthritis. *Front Immunol* 2019;10:10.
39 Kvacskay P, Yao N, Schnotz J-H, *et al*. Increase of aerobic glycolysis mediated by activated T helper cells drives synovial fibroblasts towards an inflammatory phenotype: new targets for therapy? *Arthritis Res Ther* 2021;23:56.
40 Biniecka M, Canavan M, McGarry T, *et al*. Dysregulated bioenergetics: a key regulator of joint inflammation. *Ann Rheum Dis* 2016;75:2192–200.
41 Yao C-H, Wang R, Wang Y, *et al*. Mitochondrial fusion supports increased oxidative phosphorylation during cell proliferation. *Elife* 2019;8. doi:10.7554/eLife.41351. [Epub ahead of print: 29 Jan 2019].
42 Schork NJ. Personalized medicine: time for one-person trials. *Nature* 2015;520:609–11.
43 Ajeganova S, Huizinga T. Sustained remission in rheumatoid arthritis: latest evidence and clinical considerations. *Ther Adv Musculoskelet Dis* 2017;9:249–62.
44 Kohler BM, Gunther J, Kaudewitz D, *et al*. Current therapeutic options in the treatment of rheumatoid arthritis. *J Clin Med* 2019;8.
45 Fromm S, Cunningham CC, Dunne MR, *et al*. Enhanced angiogenic function in response to fibroblasts from psoriatic arthritis synovium compared to rheumatoid arthritis. *Arthritis Res Ther* 2019;21:297.
46 Choi IY, Karpus ON, Turner JD, *et al*. Stromal cell markers are differentially expressed in the synovial tissue of patients with early arthritis. *PLoS One* 2017;12:e0182751.
47 Elshabrawy HA, Chen Z, Volin MV, *et al*. The pathogenic role of angiogenesis in rheumatoid arthritis. *Angiogenesis* 2015;18:433–48.
48 Mammoto A, Muyleart M, Kadlec A, *et al*. YAP1-TEAD1 signaling controls angiogenesis and mitochondrial biogenesis through PGC1α. *Microvasc Res* 2018;119:73–83.
49 Liu M, Zhou J, Liu X, *et al*. Targeting monocyte-intrinsic enhancer reprogramming improves immunotherapy efficacy in hepatocellular carcinoma. *Gut* 2020;69:365–79.
50 Derksen VFAM, Huizinga TWJ, van der Woude D. The role of autoantibodies in the pathophysiology of rheumatoid arthritis. *Semin Immunopathol* 2017;39:437–46.
51 Amara K, Clay E, Yeo L, *et al*. B cells expressing the IgA receptor FcRL4 participate in the autoimmune response in patients with rheumatoid arthritis. *J Autoimmun* 2017;81:34–43.
52 Amara K, Clay E, Yeo L, *et al*. Immunoglobulin characteristics and RNAseq data of FcRL4+ B cells sorted from synovial fluid and tissue of patients with rheumatoid arthritis. *Data Brief* 2017;13:356–70.
53 Kim HJ, Krenn V, Steinhauser G, *et al*. Plasma cell development in synovial germinal centers in patients with rheumatoid and reactive arthritis. *J Immunol* 1999;162:3053–62.
54 Martin VG, Wu Y-CB, Townsend CL, *et al*. Transitional B Cells in Early Human B Cell Development - Time to Revisit the Paradigm? *Front Immunol* 2016;7:546.
55 González D, van der Burg M, García-Sanz R, *et al*. Immunoglobulin gene rearrangements and the pathogenesis of multiple myeloma. *Blood* 2007;110:3112–21.

56 Slot LM, Vergroesen RD, Kerkman PF, *et al*. Light chain skewing in autoantibodies and B-cell receptors of the citrullinated antigen-binding B-cell response in rheumatoid arthritis. *PLoS One* 2021;16:e0247847.
57 Halverson R, Torres RM, Pelanda R. Receptor editing is the main mechanism of B cell tolerance toward membrane antigens. *Nat Immunol* 2004;5:645–50.
58 Giachino C, Padovan E, Lanzavecchia A. kappa+lambda+ dual receptor B cells are present in the human peripheral repertoire. *J Exp Med* 1995;181:1245–50.
59 Pelanda R. Dual immunoglobulin light chain B cells: Trojan horses of autoimmunity? *Curr Opin Immunol* 2014;27:53–9.
60 Kim S-J, Chang HJ, Volin MV, *et al*. Macrophages are the primary effector cells in IL-7-induced arthritis. *Cell Mol Immunol* 2020;17:728–40.
61 Udalova IA, Mantovani A, Feldmann M. Macrophage heterogeneity in the context of rheumatoid arthritis. *Nat Rev Rheumatol* 2016;12:472–85.
62 Basdeo SA, Cluxton D, Sulaimani J, *et al*. Ex-Th17 (nonclassical Th1) cells are functionally distinct from classical Th1 and Th17 cells and are not constrained by regulatory T cells. *J Immunol* 2017;198:2249–59.
63 Ng CT, Biniecka M, Kennedy A, *et al*. Synovial tissue hypoxia and inflammation in vivo. *Ann Rheum Dis* 2010;69:1389–95.
64 McGinnis CS, Murrow LM, Gartner ZJ. DoubletFinder: doublet detection in single-cell RNA sequencing data using artificial nearest neighbors. *Cell Syst* 2019;8:329–37.
65 Hafemeister C, Satija R. Normalization and variance stabilization of single-cell RNA-seq data using regularized negative binomial regression. *Genome Biol* 2019;20:296.
66 Korsunsky I, Millard N, Fan J, *et al*. Fast, sensitive and accurate integration of single-cell data with harmony. *Nat Methods* 2019;16:1289–96.
67 Browaeys R, Saelens W, Saeys Y. NicheNet: modeling intercellular communication by linking ligands to target genes. *Nat Methods* 2020;17:159–62.
68 Ulgen E, Ozisik O, Sezerman OU. pathfindR: an R package for comprehensive identification of enriched pathways in omics data through active Subnetworks. *Front Genet* 2019;10:858.
69 Lee AYS, Reimer D, Zehrer A. Expression of membrane-bound CC chemokine ligand 20 on follicular T helper cells in T-B-Cell conjugates. *Front Immunol* 2017;8:8.
70 McQuin C, Goodman A, Chernyshev V, *et al*. CellProfiler 3.0: next-generation image processing for biology. *PLoS Biol* 2018;16:e2005970.
71 Rees DJ, Roberts L, Carla Carisi M, *et al*. Automated quantification of mitochondrial fragmentation in an in vitro Parkinson's disease model. *Curr Protoc Neurosci* 2020;94:e105.

CLINICAL SCIENCE

MRI lesions of the spine in patients with axial spondyloarthritis: an update of lesion definitions and validation by the ASAS MRI working group

Xenofon Baraliakos ,[1] Mikkel Østergaard,[2] Robert GW Lambert,[3] Iris Eshed ,[4] Pedro M Machado ,[5,6,7] Susanne Juhl Pedersen,[2] Ulrich Weber ,[8] Manouk de Hooge ,[9] Joachim Sieper ,[10] Denis Poddubnyy ,[10] Martin Rudwaleit ,[11] Désirée van der Heijde ,[12] Robert BM Landewé ,[13] Walter P Maksymowych[14]

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For numbered affiliations see end of article.

Correspondence to
Professor Walter P Maksymowych, Medicine, Alberta Health, Edmonton, T6R 2G8, Canada;
walter.maksymowych@carearthritis.com

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ABSTRACT

Objectives Spinal MRI is used to visualise lesions associated with axial spondyloarthritis (axSpA). The ASAS MRI working group (WG) updated and validated the definitions for inflammatory and structural spinal lesions in the context of axSpA.

Methods After review of the existing literature on all possible types of spinal MRI pathologies in axSpA, the group (12 rheumatologists and two radiologists) consented on the required revisions of lesion definitions compared with the existing nomenclature of 2012. In a second step, using 62 MRI scans from the ASAS classification cohort, the proposed definitions were validated in a multireader campaign by global (absent/present) and detailed (inflammation and structural) lesion assessment at the vertebral corner (VC), vertebral endplate, facet joints, transverse processes, lateral and posterior elements. Intraclass correlation coefficient (ICC) was used for analysis.

Results Revisions were made for both inflammatory (bone marrow oedema, BMO) and structural (fat, erosion, bone spur and ankylosis) lesions, including localisation (central vs lateral), extension (VC vs vertebral endplate) and extent (minimum number of slices needed), while new definitions were suggested for the type of lesion based on lesion maturity (VC monomorphic vs dimorphic). The most reliably assessed lesions were VC fat lesion and VC monomorphic BMO (ICC (mean of all 36 reader pairs/overall 9 readers): 0.91/0.92; 0.70/0.67, respectively.

Conclusions The lesion definitions for spinal MRI lesions compatible with SpA were updated by consensus and validated by a group of experienced readers. The lesions with the highest frequency and best reliability were fat and monomorphic inflammatory lesions at the VC.

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT?

⇒ The usage of spinal MRI has increased and the understanding of how to interpret both inflammatory and structural spinal lesions in the context of clinical symptoms in axSpA and differential diagnoses has advanced significantly.

WHAT DOES THIS STUDY ADD?

⇒ The ASAS MRI working group revised the existing definitions of spinal MRI lesions where needed, to increase the understanding of their interpretation in the context of axSpA.

HOW MIGHT THIS IMPACT ON CLINICAL PRACTICE OR FUTURE DEVELOPMENTS?

⇒ These results may be used in ongoing efforts for re-evaluation of the definition of a 'positive' MRI of the spine in the context of axSpA versus non-SpA.

INTRODUCTION

Imaging of the sacroiliac joints (SIJ) and the spine are important tools for the correct recognition of axSpA.[1 2] Radiographs have been used for decades for imaging of structural changes of both the SIJ and spine, which occur due to long-standing disease.[3] With the introduction of MRI, the inflammatory nature of the disease was visualised and recognised as an objective manifestation of active disease.[4] Imaging of the SIJ has been included in the classification criteria of axSpA as a key objective criterion, next to human leucocyte antigen B27.[5] On the other hand, imaging of the spine, although more frequently performed in daily practice for identification of any cause of back pain, has not yet been included in these criteria. One main reason for this is that spinal changes on both MRI and radiography are considered to occur later in the course of the disease.[6] Furthermore, although descriptions of spinal MRI lesions were published over 20 years ago and the first definition of a 'positive MRI' highly suggestive of axSpA was published by the ASAS/OMERACT MRI group almost a decade ago,[2] the sensitivity and specificity of the definitions have been a matter of debate.[7]

Since that publication from 2012,[2] the usage of MRI of the spine has increased and the understanding of how to define and interpret both inflammatory and structural spinal lesions in the context of clinical symptoms in axSpA and differential diagnoses has advanced significantly.[7–11]

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Based on the progress with MRI interpretation in general and in the field of axSpA, in particular, the ASAS MRI working group (WG) decided to revise the existing axSpA-related definitions of these lesions where needed, in order to increase the understanding of spinal MRI interpretation in the disease. This publication presents the most recent update on this topic, not only dealing mainly with the updated definitions but also providing information on lesions where an update was not necessary, for completeness.

METHODS

Preparatory steps

The ASAS MRI WG for the spine consisted of 12 rheumatologists and two radiologists. In a first step, a literature review on published descriptions on inflammatory and structural changes in the MRI of the spine was performed based on a PubMed search using the terms: spondyloarthritis, MRI, spine, inflammation, bone marrow oedema (BMO), erosion, sclerosis, fat metaplasia, ankylosis as a narrative review. The results were presented to the group in face-to-face meetings and discussed based on all findings reported in the assessed literature.

After presentation of the results to all ASAS members and incorporation of feedback by the ASAS assembly, the WG finalised the wording on the description of the lesions and agreed on a set of reference images for each lesion, in a virtual meeting. Furthermore, the group agreed on the study design and the study-specific interactive electronic case report form (eCRF).[12] As a second step, the validation of the lesion descriptions was conducted in a multireader exercise with experts in the field of spinal MRI.

Image resources

Spinal MRIs were available from 62 patients, who were all participants in the ASAS classification cohort.

Usage of the eCRF for evaluation of spinal MRI lesions in the spine

Details on the eCRF platform have been published recently.[13] More specifically, for the spinal application, information from the global impression was entered first as to whether the spine scan findings were consistent with axSpA, and whether there were degenerative lesions in the segments of the cervical and lumbar spine. After the global assessment, not only more detailed information on the presence of inflammatory but also postinflammatory structural changes for all possible lesion types were collected for each segment, taking into account the anatomical localisation of the lesions according to individual discovertebral units.

Statistical analysis

Descriptive statistics were performed for the frequencies of the different spinal lesions not only for each individual reader but also for the majority reader (≥5/9) and ≥2-reader data.

Reliability for the total number of the different types of lesions at the level of the patient was assessed by intraclass correlation coefficient (ICC 2.1 (two-way random effects, absolute agreement, single rater/measurement MedCalc V.12.6)).

RESULTS

Overarching considerations and general consensus on MRI lesions in the spine

After review and discussion of the literature, the group decided on specific overarching principles for reviewing spinal MRIs in the context of axSpA (box 1). A major difference from previous reports is the subdivision of lesion definitions according to central and lateral slice anatomical locations in the thoracic and lumbar spinal segments on a sagittal MRI. The *central sagittal* slices include those that visualise the spinal canal. The pedicle may be partially seen but is not continuous between the vertebral body and posterior elements. The *lateral sagittal* slices are located lateral to the spinal canal. These slices do not include the visualisation of the spinal canal, and either the pedicle must be continuous between vertebral body and posterior elements, or the slice is lateral to the pedicle.

Lesion definitions

Active lesions

These are divided into lesions involving or not involving the vertebral bodies (figures 1 and 2). Definitions of lesions are provided in box 1. For vertebral body lesions, active inflammatory lesions are considered as present if BMO is located at the VCs or endplates. The terms BMO and osteitis are considered to be equivalent. Similar to the previous publication,[2] it was agreed (based on expert opinion on the morphology of the lesion and taking into account possible image artefacts) that each of the inflammatory lesions described has to be visible in at least two or more consecutive sagittal slices. This rule does not apply to: (1) lateral slices and facet or posterior element lesions, which can be considered present on a single slice and (2) dimorphic lesions (explanation see below), which may be considered present on a single slice, provided their structural component is visible in at least two slices, while in all other circumstances, their appearance must be present on two or more slices (box 1).

Inflammatory lesions are specified for the different anatomical localisations, such as the VCs (anterior/posterior corner inflammatory lesions, also known as anterior or posterior spondylitis, figures 1 and 2). Inflammatory activity at the VCs is subdivided into two types (box 1, figure 1): in a monomorphic corner lesion, the increased inflammatory signal extends to the cortex of the corner. In a dimorphic corner lesion, the increased inflammatory signal does not extend to the cortex of the corner but does extend to both the endplate and the anterior/posterior border of the vertebra. At the corner itself, there may be an erosion, sclerosis or a fat lesion.

Additional lesions affecting the vertebral bodies are the vertebral endplate inflammatory lesion (figure 2), and the thoracic lateral inflammatory lesion (a lesion located posteriorly in a lateral slice is also known as arthritis of the costovertebral joints) (figure 1), which is only recorded for the thoracic spine (box 1).

Inflammatory lesions that are not involving the vertebral body include the facet joint inflammatory lesion (also known as facet joint arthritis) and the posterior element inflammatory lesion (*including enthesitis of spinal ligaments*) (figure 1), but excluding the pedicle, facet processes and pars interarticularis.

Structural lesions

Structural lesions refer to the clear presence of typical findings such as fat lesions, erosions, sclerosis, syndesmophytes or ankylosis located at the vertebrae (box 1). All types of structural lesions may present solely or accompanied/surrounded by BMO (box 1, figure 2). Most of the observations can only be seen clearly on sequences sensitive for fat signal, specifically T1-weighted (T1W) spin echo without fat suppression. An update on structural lesions was felt necessary for erosions, syndesmophytes and ankylosis, while the definition of fat lesions remained unchanged.[2]

Box 1 Assessments in spondyloarthritis International Society MRI Working Group consensus definitions for MRI lesions in the spine of patients with axial spondyloarthritis

A. Overarching principles

1. All definitions of inflammatory lesions relate to their appearance on the water-sensitive sagittal T2-weighted fat-suppressed (T2FS) or sagittal short tau inversion recovery (STIR) images in the sagittal orientation. In both, an increased water content is seen as an increased signal intensity.
2. All definitions of structural lesions relate to their appearance on the fat-sensitive sagittal T1-weighted (T1W) MR images in the sagittal orientation.
3. The appearance of all lesions must be highly suggestive of spondyloarthritis.
4. The term 'increased signal in bone marrow' refers to a signal intensity higher than the 'normal bone marrow signal'. The bone marrow signal in the centre of the vertebra, if normal, constitutes the reference for designation of normal signal or, alternatively, in the centre of the closest available normal vertebra.
5. Based on anatomical location, the images of the thoracic and lumbar spine on a sagittal MRI scan may be divided into 'central' and 'lateral' slices, which are defined as follows:
   a. Central sagittal slices: the sagittal slices that include the spinal canal. The pedicle may be partially seen but is not continuous between the vertebral body and posterior elements.
   b. Lateral sagittal slices: the sagittal slices that are located lateral to the spinal canal. These slices do not include the spinal canal, and either the pedicle must be continuous between vertebral body and posterior elements or the slice is lateral to the pedicle.
6. The maximum sagittal slice thickness is 4 mm.

B. MRI spine lesions indicating activity

These observations are made on MRI sequences that are sensitive for the detection of disease activity such as T2-weighted sequences with fat suppression that are sensitive for free water such as STIR or T2FS or T1W sequences with fat suppression that are sensitive for contrast enhancement such as T1FS post-Gd.

Inflammatory lesions

These can be divided into:

a. Vertebral body inflammatory lesion:
   A. Vertebral corner inflammatory lesion (*also known as anterior/posterior spondylitis*): increased signal in bone marrow in a water-sensitive sequence at the vertebral corner, in at least two continuous sagittal slices. These can be subdivided into anterior and posterior vertebral corner lesions. There are two types:
      1. Regular corner lesion or type A: increased signal extends to the corners.
      2. 'Irregular' corner lesion or type B: increased signal does not cover the whole corner but extends to both the endplate and the anterior/posterior border of the vertebra. Notes: in the corner itself often an erosion, sclerosis or a fat lesion is present. If inflammation (bone marrow oedema) is only visible on one slice, a type B lesion may be scored on that single slice, provided the structural component of the lesion is visible in at least two slices. In all other circumstances, the appearance of the type B lesion must be present on two or more slices.
   B. Vertebral endplate inflammatory lesion including the intervertebral disc (*also known as aseptic spondylodiscitis*): increased signal in bone marrow in a water-sensitive sequence adjacent to the vertebral endplate that involves the vertebral endplate but not the vertebral corner.
   C. Thoracic lateral inflammatory lesion (a lateral inflammatory lesion located in the posterior part of the slice is *also known as arthritis of the costovertebral joints*) (applies to thoracic spine only). Increased signal in bone marrow on STIR/T2FS sequence adjacent to the endplate in at least one lateral sagittal slice.
b. Vertebral inflammatory lesions not involving the vertebral body
   A. Facet joint inflammatory lesion (*also known as facet joint arthritis*): increased signal in bone marrow in at least one sagittal slice in a water-sensitive sequence in at least one facet of a facet joint.
   B. Posterior element inflammatory lesion (*including enthesitis of spinal ligaments and costotransverse joint inflammation*): increased signal in bone marrow in at least one sagittal slice in a water-sensitive sequence in one of the other posterior elements at which there are ligamentous or muscular attachments, or at the costotransverse joint (the pedicle, facet processes and pars interarticularis are excluded).

C. MRI spine lesions indicating structural change

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

1. Bone erosion: full-thickness loss of the dark appearance of cortical bone *and* loss of normal bright appearance of adjacent bone marrow on T1w images in at least one sagittal slice. Only erosions involving the vertebral corners are assessed. Erosions can be subdivided into anterior and posterior corner erosions.
2. Focal fat lesion: focal increased signal in bone marrow on T1w images in at least two sagittal slices. Only fat lesions involving the vertebral corners are assessed. Fat lesions can be subdivided into anterior and posterior corner fat lesions.
3. Bone spur in the direction of the anterior or posterior longitudinal ligament *(also known as syndesmophytes):* Bright signal on T1w images extending vertically from the vertebral corner towards the adjacent vertebral corner, seen in at least one sagittal slice. Bone spurs do not reach the adjacent vertebra and can be subdivided into anterior and posterior corner bone spurs (located in anterior and posterior corners, respectively). Notes: bone spurs should not be scored as related to SpA (ie, syndesmophytes) in the presence of disc degeneration.
4. Ankylosis: bright signal on T1w images extending from a vertebra and being continuous with the adjacent vertebra. This can be divided into:
   a. Vertebral corner ankylosis: ankylosis involving the vertebral corner, in at least one sagittal slice. This can be

Continued

Continued

Box 1 Continued

subdivided into anterior and posterior corner ankylosis (located in anterior and posterior corners, respectively).
b. Vertebral endplate ankylosis: ankylosis involving the endplate, but neither the anterior nor the posterior vertebral corner.
c. Facet joint ankylosis: ankylosis of a facet joint.

Erosions

Erosion is defined as full-thickness loss of the dark appearance of cortical bone *and* loss of the normal bright appearance of adjacent bone marrow on T1w images in at least one sagittal slice. Only erosions involving the VCs are assessed and these can be subdivided into anterior and posterior corner erosions. Although erosions may affect vertebral endplates, these are not considered sufficiently specific for axSpA to warrant inclusion in the list of axSpA-associated lesions (box 1, figure 3).

Bone spurs/syndesmophytes

This description includes the bony outgrowths at the anterior, posterior or lateral corners of vertebral bodies that do not reach the adjacent vertebra. Their origin of growth is at the attachment site of the annulus fibrosus (box 1, figure 4). These are defined as bright signal on T1w images extending vertically from the VC towards the adjacent VC, seen in at least one sagittal slice. Bone spurs should not be scored as related to SpA (ie, syndesmophytes) in the presence of disc degeneration.

Ankylosis

This finding represents the bony fusion at the attachment sites of the annulus fibrosus (bridging syndesmophytes) and/or bony fusion across the intervertebral disc (box 1, figure 4) or across apophyseal joints (figure 5) or costovertebral joints (figure 5). Vertebral is defined as bright signal on T1w images extending from a vertebra and being continuous with the adjacent vertebra on at least a single sagittal slice.

Fat lesions

These lesions are defined as focal increased signal in bone marrow on T1w images in at least two consecutive sagittal slices. Similar to bone marrow oedema, it was agreed (based on expert opinion on the morphology of the lesion and taking into account possible image artefacts) that each of the lesions described has to be visible in at least two consecutive sagittal

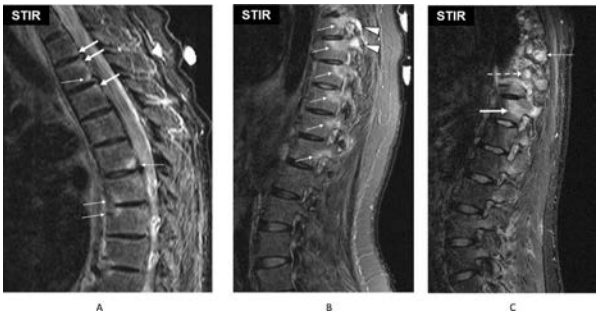

Figure 1 Signs of active changes in the lumbar spine of two patients with axial spondyloarthritis: (A) Anterior and posterior spondylitis with monomorphic (thick arrows) and dimorphic (thin arrows) lesions. (B) Thoracic lateral inflammatory lesions (arrows). Facet joint lesions (arrowheads). (C) Lateral inflammatory lesion (thick arrow), inflammation in the rib (dashed arrow), inflammation in the transverse process (thin arrow). Provided courtesy of the Canada-Denmark MRI Working Group (*Lambert et al*[9]).

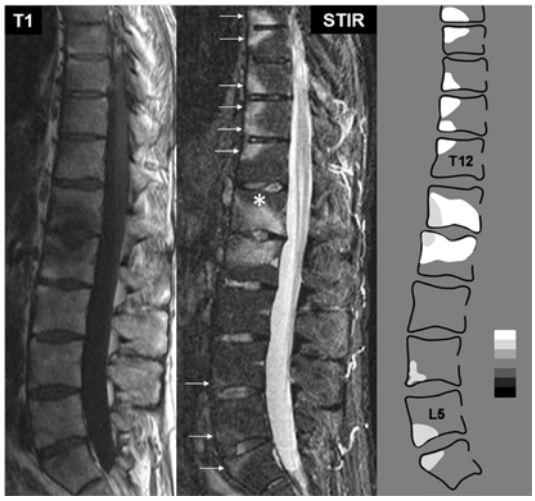

Figure 2 Signs of active changes in the lumbar spine of a patient with axial spondyloarthritis: Examples of anterior vertebral corner inflammatory lesions are prominent in the thoracic spine and more subtle in the lumbar spine (arrows), with an area of spondylodiscitis at L1–L2 (asterisk). Provided courtesy of the Canada-Denmark MRI Working Group (Lambert *et al*[9]).

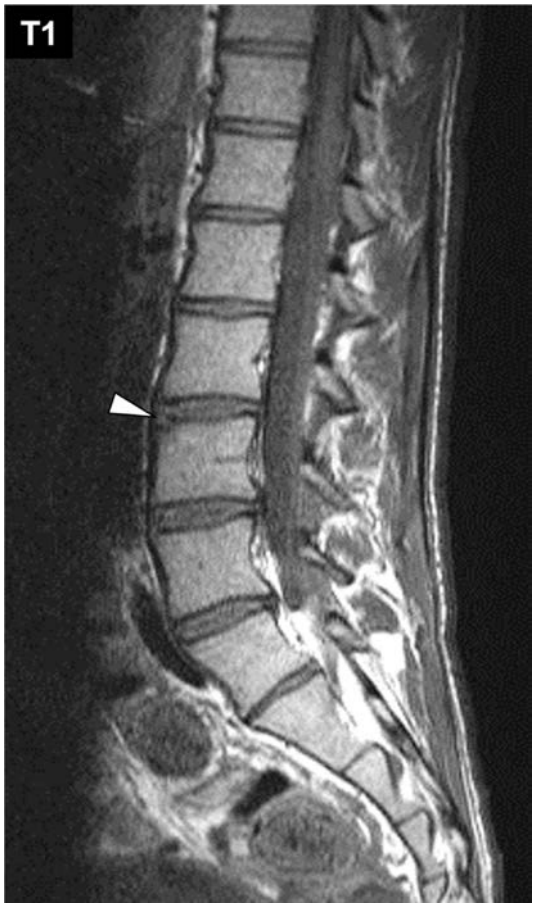

Figure 3 Signs of structural change in the lumbar spine of patients with axial spondyloarthritis: vertebral corner erosion (arrowhead) in the lumbar spine. Provided courtesy of the Canada-Denmark MRI Working Group (Østergaard *et al*[8]).

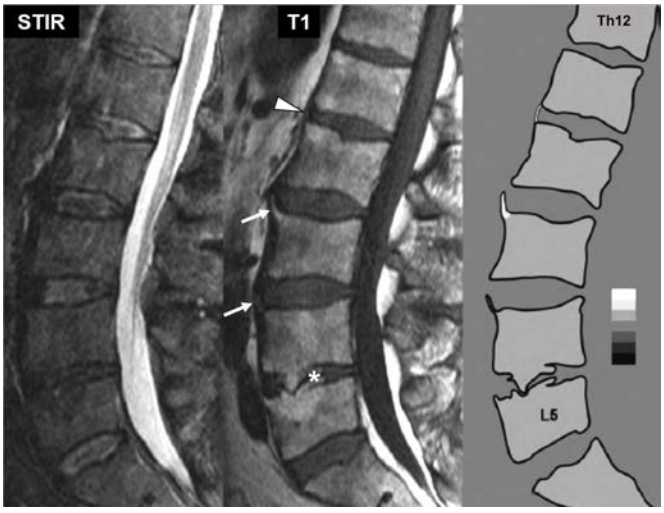

Figure 4 Signs of structural changes in the lumbar spine of patients with axial spondyloarthritis: bone growth with origin at the attachment site of the annulus fibrosus of different size (arrows) including a segment with bridging syndesmophytes/ankylosis (arrowhead) or within the intervertebral disc (asterisk). Provided courtesy of the Canada-Denmark MRI Working Group. (*Østergaard et al*[8]). STIR, sagittal short tau inversion recovery.

slices. Only fat lesions involving the VCs are assessed and these can be subdivided into anterior and posterior corner fat lesions (box 1, figure 6). Although fat lesions may occur adjacent to vertebral endplates, these are not considered sufficiently specific for axSpA to warrant inclusion in the list of axSpA-associated lesions.

Sclerosis

Similar to fat lesions, sclerosis also represents a stage of local tissue transformation due to chronic occurrence of inflammation and is seen at the area of the VCs. It is defined as low signal at a VC on all MRI sequences, but the lesion is infrequent, the MRI appearance is hard to interpret, and it is not considered sufficiently specific for axSpA to warrant inclusion in the list of axSpA-associated lesions.

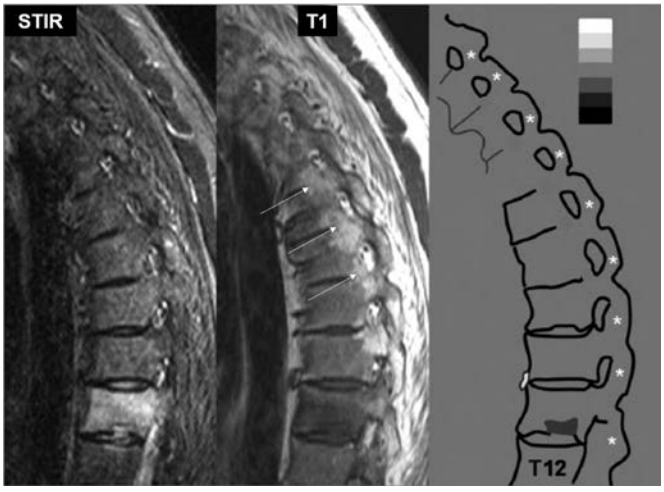

Figure 5 Signs of structural changes in the thoracic spine of patients with axial spondyloarthritis: Facet joint (asterisks) and posterior intervertebral ankylosis (arrows). Image provided courtesy of Canada-Denmark MRI Working Group (Østergaard *et al*[8]). STIR, sagittal short tau inversion recovery.

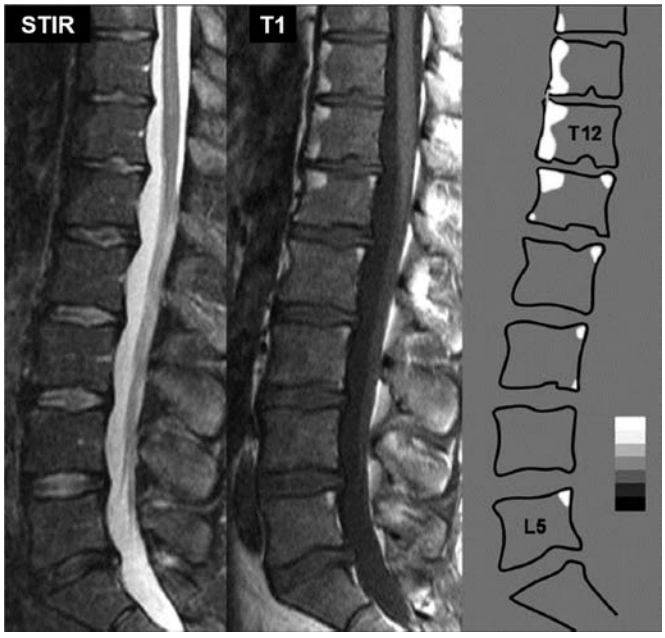

Figure 6 Signs of structural changes in the lumbar spine of a patient with axial spondyloarthritis: fat lesions in the anterior and posterior vertebral corners. Provided courtesy of the Canada-Denmark MRI Working Group (Østergaard *et al*[8]). STIR, sagittal short tau inversion recovery.

Degenerative lesion of the intervertebral disc

In parallel to the typical lesions suggestive of axSpA, degenerative disc lesions may occur. The agreed definition for such lesions is the presence of irregularity of both vertebral endplates and reduction of intervertebral disc height by ≥50%. The presence of any of the inflammatory and structural lesions described above at the site of a degenerating disc lesion should not be regarded as indicative of axSpA.

Frequency of pathologic lesions and reliability of spinal lesion identification

Based on the agreement of either ≥2/9 or ≥5/9 readers, the most frequently observed lesions were the monomorphic BMO corner lesion and the corner fat lesion (table 1).

For the reliability of lesion assessment, agreement of all 36 reader pairs and for all 9 readers for fat lesions was (0.88) (0.08) and 0.87 (0.82–0.91), respectively, and for BMO 0.69 (0.12) and 0.68 (0.58–0.77), respectively (table 2).

On the level of the patient, agreement of reader pairs (mean ICC (SD)) and for all nine readers (ICC (95% CI) for fat lesions at VCs was 0.91 (0.06) and 0.92 (0.89–0.94), respectively. In comparison, agreement for monomorphic BMO lesions was 0.70 (0.10) and 0.67 (0.60–0.76), respectively, while agreement for dimorphic BMO lesions was much lower (table 2).

DISCUSSION

The main aim of this publication was the update of the definitions of spinal lesions related to axSpA, by the ASAS MRI WG.

The characteristic MRI findings in the spine of patients with axSpA were described over 20 years ago and definitions of spinal MRI lesions were first published in 2009.[8 9] The first definition of a positive spinal MRI for inflammation and structural changes was made by ASAS in 2012,[2] arising from the evidence that inflammation in the spine may also occur in parallel and also before or even without inflammation in the SIJ. In addition

Table 1 Frequency of the different lesion types and number of lesions based on the agreement of ≥2/9 and the majority (≥5/9) readers

Lesion type and anatomic lesion localisation		Number (%) of lesions with agreement	Number of cases where≥2/9 readers agree	Number of cases where≥5/9 readers agree
Bone marrow oedema	Corner lesion	≥1	29 (46.8%)	16 (25.8%)
		≥2	19 (30.6%)	14 (22.6%)
		≥3	15 (24.2%)	11 (17.7%)
		≥4	12 (19.4%)	7 (11.3%)
		≥5	7 (11.3%)	6 (9.7%)
	Type A corner lesion	≥1	29 (46.8%)	16 (25.8%)
		≥2	19 (30.6%)	12 (19.4%)
		≥3	14 (22.6%)	9 (14.5%)
		≥4	11 (17.7%)	5 (8.1%)
		≥5	7 (11.3%)	5 (8.1%)
	Type B corner lesion	≥1	7 (11.3%)	3 (4.8%)
		≥2	4 (6.5%)	3 (4.8%)
		≥3	2 (3.2%)	2 (3.2%)
		≥4	0 (0%)	0 (0%)
		≥5	0 (0%)	0 (0%)
	Vertebral body: vertebral endplate	≥1	6 (9.7%)	2 (3.2%)
		≥2	3 (4.8%)	0 (0%)
		≥3	1 (1.6%)	0 (0%)
		≥4	0 (0%)	0 (0%)
		≥5	0 (0%)	0 (0%)
	Vertebral body: lateral region	≥1	5 (8.1%)	3 (4.8%)
		≥2	3 (4.8%)	2 (3.2%)
		≥3	2 (3.2%)	0 (0%)
		≥4	2 (3.2%)	0 (0%)
		≥5	0 (0%)	0 (0%)
	Facet joint	≥1	6 (9.7%)	0 (0%)
		≥2	2 (3.2%)	0 (0%)
		≥3	1 (1.6%)	0 (0%)
		≥4	0 (0%)	0 (0%)
		≥5	0 (0%)	0 (0%)
	Posterior elements	≥1	8 (12.9%)	3 (4.8%)
		≥2	7 (11.3%)	2 (3.2%)
		≥3	4 (6.5%)	0 (0%)
		≥4	4 (6.5%)	0 (0%)
		≥5	2 (3.2%)	0 (0%)
Fat lesions	Corner lesion	≥1	21 (33.9%)	12 (19.4%)
		≥2	14 (22.6%)	10 (16.1%)
		≥3	10 (16.1%)	7 (11.3%)
		≥4	8 (12.9%)	7 (11.3%)
		≥5	7 (11.3%)	5 (8.1%)
Erosion	Corner	≥1	5 (8.1%)	0 (0%)
		≥2	5 (8.1%)	0 (0%)
		≥3	1 (1.6%)	0 (0%)
		≥4	1 (1.6%)	0 (0%)
		≥5	1 (1.6%)	0 (0%)
Bone spur	Corner	≥1	10 (16.1%)	2 (3.2%)
		≥2	7 (11.3%)	2 (3.2%)
		≥3	5 (8.1%)	2 (3.2%)
		≥4	3 (4.8%)	2 (3.2%)
		≥5	2 (3.2%)	1 (1.6%)

Continued

Table 1 Continued

Lesion type and anatomic lesion localisation		Number (%) of lesions with agreement	Number of cases where≥2/9 readers agree	Number of cases where≥5/9 readers agree
Ankylosis	Corner	≥1	1 (1.6%)	1 (1.6%)
		≥2	2 (3.2%)	0 (0%)
		≥3	2 (3.2%)	0 (0%)
		≥4	2 (3.2%)	0 (0%)
		≥5	1 (1.6%)	0 (0%)
	Intervertebral	≥1	1 (1.6%)	0 (0%)
		≥2	0 (0%)	0 (0%)
		≥3	0 (0%)	0 (0%)
		≥4	0 (0%)	0 (0%)
		≥5	0 (0%)	0 (0%)
	Facet	≥1	2 (3.2%)	1 (1.6%)
		≥2	2 (3.2%)	1 (1.6%)
		≥3	2 (3.2%)	1 (1.6%)
		≥4	2 (3.2%)	1 (1.6%)
		≥5	1 (1.6%)	1 (1.6%)

VC, vertebral corner .

to that consensus statement, several other studies have been published in the meantime[7 10 11] partly confirming and partly questioning the former definitions, and identifying the potential for misleading interpretation if imaging is assessed without the clinical context.[14 15] One reason for this was the knowledge gained on the relationship between lesions found on MRI and subsequent structural progression on conventional radiographs.[16–18] Another reason was the practical aspect arising from the technical improvement of MR image quality over time,[19] which provides improved insight into the lesions found in patients with axSpA in comparison to patients with chronic back pain without a diagnosis of axSpA. One step towards a more precise terminology and validation of the SpA-related lesions was the recently published update of definitions and validation of the MRI lesions of the SIJ for patients with SpA by the ASAS MRI WG.[13] With the present paper, our group has completed a set of updated definitions for all the relevant axial MRI lesions in SpA.

The present analysis is based on the evaluation of MRIs from the ASAS classification cohort by international experts, who are all full ASAS members with extensive experience in the reading of such images. The group of experts agreed, based on[20] the best possible wording for the definition of all known types of spinal lesions for activity (bone marrow oedema with and without concomitant structural lesions) and structural (fat, erosions, sclerosis, ankylosis) findings. The wording of definition of localisation (central, or lateral, in the vertebral body or in the posterior elements, that is, outside the vertebral bodies), extension (VC vs vertebral endplate) and extent (minimum number of slices needed for identifications of lesions) of these lesions was also agreed on. Overall, in comparison to the previous publication,[2] updated definitions are now provided for both the active and the

Table 2 Single measures intraclass correlation coefficient for absolute agreement of all reader pairs and all nine readers for the different spinal lesions

		Mean (SD) of reader pairs	Range of reader pairs	All 9 readers (95% CI)
Bone marrow oedema	Total BMO	0.69 (0.12)	0.39–0.91	0.68 (0.58 to 0.77)
	Corner lesions	0.66 (0.13)	0.24–0.87	0.63 (0.53 to 0.72)
	Thoracic lateral vertebral lesions	0.59 (0.22)	0.18–0.96	0.58 (0.48 to 0.68)
	Facet lesions	0.08 (0.17)	−0.04–0.66	0.04 (−0.001 to 0.1)
	Posterior lesions	0.34 (0.23)	−0.04–0.90	0.38 (0.29 to 0.49)
	Vertebral endplate lesions	0.23 (0.30)	0.0–0.85	0.34 (0.25 to 0.45)
	Type A lesions	0.70 (0.10)	0.50–0.85	0.67 (0.60 to 0.76)
	Type B lesions	0.36 (0.21)	−0.03–0.87	0.39 (0.29 to 0.51)
Total fat*		0.88 (0.08)	0.69–0.98	0.87 (0.82 to 0.91)
Vertebral corner fat		0.91 (0.06)	0.77–0.99	0.92 (0.89 to 0.94)
Erosion		0.15 (0.21)	−0.04–0.78	0.16 (0.09 to 0.25)
Bone spur		0.61 (0.36)	−0.00–0.99	0.61 (0.51 to 0.70)
Ankylosis	Total	0.67 (0.21)	0.23–0.98	0.58 (0.48 to 0.68)
	Corner	0.41 (0.29)	−0.01–1.0	0.39 (0.29 to 0.50)
	Non-corner (intervertebral)	0.32 (0.44)	0.0–1.0	0.42 (0.32 to 0.53)
	Facet	0.54 (0.31)	0.1–1.0	0.45 (0.35 to 0.56)

*Includes total fat score for VC, lateral vertebral, non-corner and facet lesions.
BMO, bone marrow oedema.

structural spinal lesion types. For the active lesions, the anatomic localisation was considered, now including not only the VC with the different types of appearance (monomorphic and dimorphic) but also the lesions located at the endplate, the lateral vertebral region, the facet joints and the posterior elements. For the structural lesions, an update was felt necessary for erosions, syndesmophytes and ankylosis, leaving the definition of fat lesions unchanged.[2] Finally, for bone spurs, which occur in the longitudinal ligaments and the tissue intimately attached to them, it is well known that they are less well detected on conventional MRI than on conventional radiographs or CT. In addition, especially for the identification on MRI in contrast to the identification on conventional radiographs or CT, the presence of syndesmophytes may not necessarily be interpreted as a reliable sign of spondyloarthritis.[20]

The statistical analysis of the MRI evaluation confirmed that not only inflammatory but also structural lesions are frequently observed in the spine of patients with axSpA. These lesions seem to be most frequently located at the VC area, consisting either of fat or bone marrow oedema. In addition, the VC inflammatory lesion type that was clearly more frequently and more reliably observed was the monomorphic BMO lesion, where the inflammatory signal extends to the VC, in contrast to the dimorphic BMO lesions, where the signal does not cover the whole corner but extends to both the endplate and anterior or posterior border of the vertebra. This is an important finding, since these lesions especially have been reported to be associated with the highest risk for radiographic progression in follow-up examinations of patients with radiographic axSpA.[16 17] The lower frequency of the dimorphic BMO lesion in our analysis compared with previous reports is likely because the ASAS classification cohort is a cohort of patients at an early stage of their disease.[21] In addition, it also needs to be taken into account that dimorphic lesions may also be less reliably detected because of their complex morphology and the fact that the short tau inversion recovery signal is often of low intensity, making it difficult to detect these lesions, which often reflect resolving inflammation. Fat lesions and monomorphic BMO lesions were the lesion types with the highest reliability of detection between experts. This result was independent of the number of experts who had to agree on the presence of these lesions, suggesting a possibly similar accuracy of their detection in daily practice settings.

Interestingly, beyond fat and monomorphic BMO lesions, all other lesions assessed were observed far less frequently, as expected for an inception cohort of patients referred with undiagnosed back pain and suspicion of axSpA. The lower degree of reliability at least partly reflects this lower frequency of detection. This was especially true for lesions in the more posterior parts of the spine (posterior VCs and facet joints). The posterior parts of the spine have been reported to be more frequently affected by inflammatory lesions in patients diagnosed at a young age.[22] An explanation for the differences between these findings and the results reported here may be because, despite the early stage of their disease in the ASAS classification cohort, the mean age of these patients was still not different from other studies with axSpA patients. Such data are also consistent with studies that showed no differences in the mean age at diagnosis independent of the stage of radiographic axSpA.[22]

In summary, this publication provides a consensus-based update of the definitions for spinal MRI lesions of patients referred with undiagnosed back pain and with clinical suspicion of axSpA. The most frequent and reliably detected lesions were fat lesions and monomorphic BMO VC lesions, while posterior elements were much less evident in this cohort of patients with undiagnosed back pain referred to a rheumatologist. These results not only confirm the observation that BMO and fat lesions are important in the identification of pathologic findings when evaluating spinal MRIs but may also be used in ongoing efforts for re-evaluation of the definition of a 'positive' MRI of the spine in the context of axSpA versus non-SpA.

Author affiliations
[1]Rheumazentrum Ruhrgebiet Herne, Ruhr-University Bochum, Bochum, Germany
[2]Copenhagen Center for Arthritis Research, Center for Rheumatology and Spine Diseases, Rigshospitalet, Glostrup and Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark
[3]Department of Radiology and Diagnostic Imaging, University of Alberta, Edmonton, Canada and Medical Imaging Consultants, Edmonton, Alberta, Canada
[4]Department of Diagnostic Imaging, Sheba Medical Center, affiliated to the Sackler school of medicine, Tel-Aviv University, Tel Aviv, Israel
[5]Department of Rheumatology, University College London, London, UK
[6]Centre for Rheumatology and MRC Centre for Neuromuscular Diseases, University College London, London, UK
[7]Department of Rheumatology, Northwick Park Hospital, London North West University Healthcare NHS Trust, London, UK
[8]Practice Buchsbaum, Rheumatology, Schaffhausen, Switzerland
[9]VIB Inflammation Research Center, Ghent University, Ghent, Belgium and Rheumatology Department, Ghent University Hospital, Gent, Belgium
[10]Charité - Universitätsmedizin Berlin and German Rheumatism Research Centre, Berlin, Germany
[11]Klinikum Bielefeld, University of Bielefeld, Bielefeld, Germany
[12]Leiden University Medical Center, Leiden, The Netherlands
[13]Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands, and Atrium Medical Center, Heerlen, The Netherlands
[14]Department of Medicine, University of Alberta, Edmonton, Canada; CARE Arthritis, Edmonton, Alberta, Canada

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Twitter Pedro M Machado @pedrommcmachado

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Participation on Advisory Board: AbbVie, Biocad, Eli Lilly, Gilead, GlaxoSmithKline, Janssen, MSD, Moonlake, Novartis, Pfizer, Samsung Bioepis, and UCB. MR: Consulting fees: Abbvie, Eli Lilly, Novartis, Pfizer, UCB. Payment or honoraria for lectures: Abbvie, BMS, Eli Lilly, Janssen, MSD, Novartis, Pfizer, Roche, UCB. Support for attending meetings: Galapagos, Janssen, Novartis, Abbvie. DvdH: Consulting fees: AbbVie, Bayer, BMS, Cyxone, Eisai, Galapagos, Gilead, Glaxo-Smith-Kline, Janssen, Lilly, Novartis, Pfizer, UCB. Director of Imaging Rheumatology bv. Leadership role: Associate Editor of Annals of Rheumatic Diseases, Editorial Board Member Journal of Rheumatology, Advisory Committee RMD Open. RL: Grants: Research grants from Galapagos, AbbVie, Novartis, UCB (paid to institution). Consulting fees: AbbVie, Bristol-Myers Squibb, Celgene, Eli Lilly, Galapagos, Gilead, GlaxoSmithKline, Janssen, Merck, Novartis, Pfizer, Roche, UCB (paid to own company). Payment or honoraria for lectures: AbbVie, Bristol-Myers Squibb, Celgene, Eli Lilly, Galapagos, Gilead, GlaxoSmithKline, Janssen, Merck, Novartis, Pfizer, Roche, UCB (paid to own company). Participation on Advisory Board: UCB, AbbVie, Galapagos, Gilead, Eli Lilly, Jansen, Novartis, Pfizer (paid to own company). Data safety Monitoring Board: UCB (no compensation). Director of Rheumatology Consultancy. Partner of 'Reumatologie Maatschap Sittard/Heerlen' (paid to own company). Leadership role: Council Member and EULAR's chair of quality of care. Member of Editorial Board of Annals of Rheumatic Diseases. WPM: Grants: Abbvie, Galapagos, Novartis, Pfizer, UCB. Consulting fees: Abbvie, Boehringer Ingelheim, Celgene, Lilly, Novartis, Pfizer, UCB. Payment or honoraria for lectures: Abbvie, Janssen, Lilly, Novartis, Pfizer, UCB. Leadership role: SPARTAN Board of directors. Chief Medical Officer of CARE Arthritis Ltd.

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ORCID iDs
Xenofon Baraliakos http://orcid.org/0000-0002-9475-9362
Iris Eshed http://orcid.org/0000-0002-4655-9606
Pedro M Machado http://orcid.org/0000-0002-8411-7972
Ulrich Weber http://orcid.org/0000-0001-6701-670X
Manouk de Hooge http://orcid.org/0000-0002-0652-9808
Joachim Sieper http://orcid.org/0000-0003-0285-9890
Denis Poddubnyy http://orcid.org/0000-0002-4537-6015
Martin Rudwaleit http://orcid.org/0000-0001-5445-548X
Désirée van der Heijde http://orcid.org/0000-0002-5781-158X
Robert BM Landewé http://orcid.org/0000-0002-0577-6620

REFERENCES

1 Lambert RGW, Bakker PAC, van der Heijde D, *et al*. Defining active sacroiliitis on MRI for classification of axial spondyloarthritis: update by the ASAS MRI Working group. *Ann Rheum Dis* 2016;75:1958–63.
2 Hermann K-GA, Baraliakos X, van der Heijde DMFM, *et al*. Descriptions of spinal MRI lesions and definition of a positive MRI of the spine in axial spondyloarthritis: a consensual approach by the ASAS/OMERACT MRI study Group. *Ann Rheum Dis* 2012;71:1278–88.
3 Sieper J, Rudwaleit M, Baraliakos X, *et al*. The assessment of spondyloarthritis International Society (ASAS) Handbook: a guide to assess spondyloarthritis. *Ann Rheum Dis* 2009;68 Suppl 2:ii1–44.
4 Rudwaleit M, Jurik AG, Hermann K-GA, *et al*. Defining active sacroiliitis on magnetic resonance imaging (MRI) for classification of axial spondyloarthritis: a consensual approach by the ASAS/OMERACT MRI group. *Ann Rheum Dis* 2009;68:1520–7.
5 Rudwaleit M, van der Heijde D, Landewé R, *et al*. The development of assessment of spondyloarthritis International Society classification criteria for axial spondyloarthritis (Part II): validation and final selection. *Ann Rheum Dis* 2009;68:777–83.
6 Sieper J, Poddubnyy D. Axial spondyloarthritis. *The Lancet* 2017;390:73–84.
7 Weber U, Zhao Z, Rufibach K, *et al*. Diagnostic utility of candidate definitions for demonstrating axial spondyloarthritis on magnetic resonance imaging of the spine. *Arthritis Rheumatol* 2015;67:924–33.
8 Ostergaard M, Maksymowych WP, Pedersen SJ, *et al*. Structural Lesions Detected by Magnetic Resonance Imaging in the Spine of Patients with Spondyloarthritis - Definitions, Assessment System, and Reference Image Set. *J Rheumatol Suppl* 2009;84:18–34.
9 Lambert RGW, Pedersen SJ, Maksymowych WP, *et al*. Active Inflammatory Lesions Detected by Magnetic Resonance Imaging in the Spine of Patients with Spondyloarthritis - Definitions, Assessment System, and Reference Image Set. *J Rheumatol Suppl* 2009;84:3–17.
10 Krabbe S, Sørensen IJ, Jensen B, *et al*. Inflammatory and structural changes in vertebral bodies and posterior elements of the spine in axial spondyloarthritis: construct validity, responsiveness and discriminatory ability of the anatomy-based CANDEN scoring system in a randomised placebo-controlled trial. *RMD Open* 2018;4:e000624.
11 Baraliakos X, Richter A, Feldmann D, *et al*. Which factors are associated with bone marrow oedema suspicious of axial spondyloarthritis as detected by MRI in the sacroiliac joints and the spine in the general population? *Ann Rheum Dis* 2021;80:469–74.
13 Maksymowych WP, Lambert RG, Østergaard M, *et al*. Mri lesions in the sacroiliac joints of patients with spondyloarthritis: an update of definitions and validation by the ASAS MRI Working group. *Ann Rheum Dis* 2019;78:1550–8.
14 de Winter J, de Hooge M, van de Sande M, *et al*. Magnetic resonance imaging of the Sacroiliac joints indicating sacroiliitis according to the assessment of spondyloarthritis International Society definition in healthy individuals, runners, and women with postpartum back pain. *Arthritis Rheumatol* 2018;70:1042–8.
15 Baraliakos X, Richter A, Feldmann D, *et al*. Frequency of MRI changes suggestive of axial spondyloarthritis in the axial skeleton in a large population-based cohort of individuals aged <45 years. *Ann Rheum Dis* 2020;79:186–92.
16 Chiowchanwisawakit P, Lambert RGW, Conner-Spady B, *et al*. Focal fat lesions at vertebral corners on magnetic resonance imaging predict the development of new syndesmophytes in ankylosing spondylitis. *Arthritis Rheum* 2011;63:2215–25.
17 Baraliakos X, Heldmann F, Callhoff J, *et al*. Which spinal lesions are associated with new bone formation in patients with ankylosing spondylitis treated with anti-TNF agents? a long-term observational study using MRI and conventional radiography. *Ann Rheum Dis* 2014;73:1819–25.
18 Machado PM, Baraliakos X, van der Heijde D, *et al*. Mri vertebral corner inflammation followed by fat deposition is the strongest contributor to the development of new bone at the same vertebral corner: a multilevel longitudinal analysis in patients with ankylosing spondylitis. *Ann Rheum Dis* 2016;75:1486–93.
19 Diefenbach MN, Ruschke S, Eggers H, *et al*. Improving chemical shift encoding-based water-fat separation based on a detailed consideration of magnetic field contributions. *Magn Reson Med* 2018;80:990–1004.
20 Laloo F, Herregods N, Jaremko JL, *et al*. New bone formation in the intervertebral joint space in spondyloarthritis: an MRI study. *Eur J Radiol* 2018;109:210–7.
21 Sepriano A, Landewé R, van der Heijde D, *et al*. Predictive validity of the ASAS classification criteria for axial and peripheral spondyloarthritis after follow-up in the ASAS cohort: a final analysis. *Ann Rheum Dis* 2016;75:1034–42.
22 Bochkova AG, Levshakova AV, Bunchuk NV, *et al*. Spinal inflammation lesions as detected by magnetic resonance imaging in patients with early ankylosing spondylitis are more often observed in posterior structures of the spine. *Rheumatology* 2010;49:749–55.
23 Kiltz U, Baraliakos X, Karakostas P, *et al*. Do patients with non-radiographic axial spondylarthritis differ from patients with ankylosing spondylitis? *Arthritis Care Res* 2012;64:1415–22.

CLINICAL SCIENCE

Treatment with tumour necrosis factor inhibitors is associated with a time-shifted retardation of radiographic spinal progression in patients with axial spondyloarthritis

Murat Torgutalp ,[1] Valeria Rios Rodriguez ,[1] Ani Dilbaryan,[2] Fabian Proft ,[1] Mikhail Protopopov ,[1] Maryna Verba,[1] Judith Rademacher ,[1,3] Hildrun Haibel,[1] Joachim Sieper ,[1] Martin Rudwaleit ,[4] Denis Poddubnyy [1,5]

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For numbered affiliations see end of article.

Correspondence to
Professor Denis Poddubnyy, Department of Gastroenterology, Infectious Diseases and Rheumatology (including Nutrition Medicine), Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany;
denis.poddubnyy@charite.de

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ABSTRACT

Objective The objective of the current study was to analyse the association between treatment with tumour necrosis factor inhibitors (TNFi) and radiographic spinal progression in patients with axial spondyloarthritis (axSpA) from a long-term inception cohort.

Methods A total of 243 patients with axSpA from the German Spondyloarthritis Inception Cohort with at least two sets of spinal radiographs obtained at least 2 years apart during a 10-year follow-up were included. Spinal radiographs were evaluated by three trained and calibrated readers according to the modified Stoke Ankylosing Spondylitis Spine Score (mSASSS). The association between the current TNFi, previous TNFi and radiographic spinal progression defined as the absolute mSASSS change score over 2 years was analysed using longitudinal generalised estimating equations analysis.

Results TNFi treatment in the current 2-year interval was not associated with retardation of radiographic spinal progression (β=−0.02 (95% CI −0.37 to 0.34) and −0.17 (95% CI −0.54 to 0.20) for any and ≥12 months treatment duration, respectively, adjusted for sex, the Ankylosing Spondylitis Disease Activity Score, smoking, presence of definite radiographic sacroiliitis, mSASSS at baseline and non-steroidal anti-inflammatory drug intake). TNFi treatment in the previous 2-year interval, was, however, significantly associated with reduction of mSASSS progression, which was especially evident in patients who received TNFi in the previous and in the current intervals: β=−0.58 (95% CI −1.02 to −0.13), adjusted for the same variables.

Conclusion TNFi treatment was associated with a time-shifted effect on radiographic spinal progression in axSpA that became evident between years 2 and 4 after treatment initiation.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Observational studies demonstrated that tumour necrosis factor inhibitors (TNFi) may retard radiographic progression in patients with ankylosing spondylitis.

⇒ The minimal duration of TNFi treatment that is needed to observe reduction of radiographic progression and the question if such an effect can also be observed in patients at an earlier disease stage remained uncertain.

WHAT THIS STUDY ADDS

⇒ In this long-term (10 years) inception cohort of patients with axial spondyloarthritis, treatment TNFi was significantly associated with a time-shifted retardation of radiographic spinal progression, which became evident between year 2 and 4 after treatment initiation.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE AND/OR POLICY

⇒ This study suggests that continuous treatment with an effective anti-inflammatory drug such as TNFi has disease-modifying properties in axial spondyloarthritis.

INTRODUCTION

Radiographic spinal progression in axial spondyloarthritis (axSpA) is largely attributable to the process of new bone formation with development of so-called syndesmophytes, which build bridges between vertebral bodies resulting into spinal ankylosis.[1] Also other spinal structures (facet joints, costrovertebral and costotranversal joints) might become damaged and ankylosed in axSpA,[2] but syndesmophyte formation is usually considered as the main proxy in the assessment of structural damage that is referred to as radiographic spinal progression in axSpA if evaluated on conventional radiographs. Disease activity and structural damage in the spine are the two major determinants of spinal mobility and function in axSpA[3 4]; at the advanced disease stage, the contribution of structural damage to the functional impairment might become leading. New bone formation in axSpA is assumed to be preceded by bony inflammation, which induces repair mechanisms with subchondral granulation tissue formation and subsequent stimulation of osteogenesis.[5–7] After first studies had shown that effective anti-inflammatory treatment with tumour necrosis factor inhibitors (TNFi) over 2 years does not inhibit radiographic progression in patients with advanced axSpA (radiographic axSpA - r-axSpA also termed ankylosing spondylitis—AS) as compared with historical cohorts[8–10] it became

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evident that a long-term suppression of inflammation might be necessary to see the effect of anti-inflammatory treatment on structural damage development.[11–18] The minimal duration of TNFi treatment that is needed to observe reduction of radiographic progression and the question if such an effect can also be observed in patients at an earlier disease stage remained uncertain.

The objective of the current study was to analyse the association between the TNFi exposure and radiographic spinal progression in patients with axSpA in a long-term inception cohort.

METHODS

Cohort description and patient selection

The German Spondyloarthritis Inception Cohort (GESPIC) is an ongoing longitudinal study focussing on clinical and radiographic outcomes of patients with SpA. The study design and the inclusion criteria have been reported in detail elsewhere.[19] Briefly, the cohort was initiated in 2000 as a national multicentre study within the German Competence Network Rheumatology programme and comprised four university clinics, five community hospitals and four private practices. The last patient was enrolled in the cohort in 2009. Patients with axSpA were included if they had r-axSpA (AS) fulfilling the modified New York criteria and symptom duration of up to 10 years or non-radiographic axSpA(nr-axSpA) fulfilling the slightly modified European Spondyloarthropathy Study Group criteria and symptom duration of up to 5 years.[19] Classification as r-axSpA or nr-axSpA was performed based on central evaluation of sacroiliac X-rays as described elsewhere[20]; in the absence of central reading results, the local rheumatologist's assessment was used for the classification. There were no restrictions in terms of treatment, but the majority of patients were recruited before introduction of TNFi in daily clinical practice. Patients were investigated at baseline, every 6 months during the first 2 years and annually thereafter up to year 10. Disease activity was assessed by the Bath Ankylosing Spondylitis Disease Activity Index, C reactive protein (CRP) and the patient global assessment of disease activity. Furthermore, the Ankylosing Spondylitis Disease Activity Score (ASDAS) was calculated. Function was evaluated by the Bath Ankylosing Spondylitis Functional Index, spinal mobility—by the Bath Ankylosing Spondylitis Metrology Index (BASMI). Information on treatment was collected at every visit. If TNFi intake was recorded on two consecutive visits, it was assumed that TNFi was taken during the period between those visits. For non-steroidal anti-inflammatory drugs (NSAIDs), the Assessment of Spondyloarthritis International Society NSAIDs intake score[21] was calculated as previously described.[22] Cervical and lumbar radiographs were obtained at baseline and every 2 years thereafter. Radiographs had to be performed in a ±6 months window around the date of the clinical visit. For the purpose of the present analysis, we selected patients who had at least two sets of spinal radiographs (cervical and lumbar spine, lateral views) during the 10-year follow-up period. The mean and median interval lengths between radiographs were 25.2 and 24 (IQR: 22 to 28) months, respectively. A total of 243 patients (130 with nr-axSpA and 113 with r-axSpA) were finally included in the current study; the flowchart of patient selection is presented in online supplemental figure S1.

Patients and the public involvement

Patients and the public were not involved in the design, conduct, reporting or dissemination plans of the current research.

Reading of radiographs

Three trained and calibrated readers (AD, VRR, MT) scored spinal radiographs (up to six time points per patient: baseline, year 2, year 4, year 6, year 8 and year 10) according to the modified Stoke Ankylosing Spondylitis Spine Score (mSASSS) system. The readers were blinded for all clinical information but knew the chronology of the images.

Statistical analyses

A total mSASSS ranging from 0 to 72 was calculated for each reader. The final mSASSS was calculated as a mean of three reader score for per patient and time point. We allowed for up to six missing scores of single vertebral corners per time point and up to three for the anatomical region (cervical or lumbar spine).

Table 1 Baseline characteristics of the patients with axial spondyloarthritis in GESPIC who included and excluded from the present study

Parameter	All patients in GESPIC (n=525)	Included patients (n=243)	Excluded patients (n=282)
Age, years, mean±SD	35.7±10.3	36.2±10.2	35.2±10.4
Male sex, n (%)	286 (54.5)	120 (49.4)	166 (58.9)
Symptom duration, years, mean±SD	3.9±2.7	4.0±2.5	3.9±2.8
Smoking current, n (%)	132 (25.1)	67 (27.6)	65 (23.1)
HLA-B27 positivity, n (%)	406 (77.9)	191 (79.3)	215 (76.8)
Positive family history for SpA, n (%)	159 (30.3)	85 (35.1)	74 (26.2)
Peripheral arthritis, current, n (%)	77 (14.7)	28 (11.5)	49 (17.4)
Enthesitis, current, n (%)	105 (20.0)	46 (18.9)	59 (20.9)
Dactylitis, current, n (%)	27 (5.1)	13 (5.4)	14 (5.0)
Uveitis ever, n (%)	86 (16.4)	45 (18.5)	41 (14.5)
Psoriasis ever, n (%)	53 (10.1)	28 (11.5)	25 (8.9)
IBD ever, n (%)	14 (2.7)	7 (2.9)	7 (2.5)
CRP, mg/litre, mean±SD	11.1±17.5	11.4±18.8	10.9±16.3
ASDAS-CRP, mean±SD	2.6±1.0	2.5±1.0	2.6±0.9
BASDAI (0–10 points NRS), mean±SD	3.9±2.1	3.7±2.1	4.1±2.1
BASFI (0–10 points NRS), mean±SD	2.8±2.4	2.7±2.3	2.8±2.4
BASMI (0–10 points NRS), mean±SD	1.5±1.6	1.6±1.6	1.4±1.7
Treatment with NSAIDs, n (%)	352 (67.1)	163 (67.1)	189 (67.0)
Treatment with csDMARDs, n (%)	121 (23.1)	58 (23.9)	63 (22.3)
Treatment with TNFi, n (%)	13 (2.5)	8 (3.3)	5 (1.8)
Treatment with systemic steroids, n (%)	48 (9.1)	15 (6.2)	33 (11.7)
Patients with r-axSpA, n (%)	249 (47.4)	113 (46.5)	136 (48.2)
mSASSS points, mean±SD	2.6±5.9 N=378	2.6±6.6 N=225	2.7±4.9 N=153
≥1 syndesmophyte, n (%)	66 (17.5) N=378	40 (17.8) N=225	26 (17.0) N=153

ASDAS, Ankylosing Spondylitis Disease Activity Score; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; BASMI, Bath Ankylosing Spondylitis Metrology Index; CRP, C reactive protein; csDMARDs, conventional synthetic disease-modifying antirheumatic drugs; HLA-B27, human leucocyte antigen B27; IBD, inflammatory bowel disease; mSASSS, modified Stroke Ankylosing Spondylitis Spine Score; NRS, numeric rating scale; NSAIDs, non-steroidal anti-inflammatory drugs; SpA, spondyloarthritis; TNFi, tumour necrosis factor alpha inhibitor.

Table 2 The association between progression of the mSASSS over 2 years and TNFi exposure in patients with axial spondyloarthritis from in the longitudinal generalised estimating equation analysis

Variable	Univariable analysis β (95% CI)	Multivariable model 1 β (95% CI)	Multivariable model 2 β (95% CI)	Multivariable model 3 β (95% CI)	Multivariable model 4 β (95% CI)	Multivariable model 5 β (95% CI)
Any TNFi use in the current 2-year interval	0.26 (−0.14 to 0.65)	−0.02 (−0.37 to 0.34)	–	–	–	–
TNFi for ≥12 months in the current 2-year interval	−0.03 (−0.40 to 0.34)	–	−0.17 (−0.54 to 0.20)	–	–	–
Any TNFi use in the previous 2-year interval	−0.31 (−0.61 to 0.00)	–	–	−0.41 (−0.69 to −0.13)	–	–
TNFi for ≥12 months in the previous 2-year interval	−0.41 (−0.69 to −0.13)	–	–	–	−0.54 (−0.93 to −0.14)	–
TNFi for ≥12 months in the previous and ≥12 months in the current 2-year interval	−0.43 (−0.73 to −0.14)	–	–	–	–	−0.58 (−1.02 to −0.13)
Male sex	0.52 (0.19 to 0.84)	0.27 (0.01 to 0.53)	0.27 (0.01 to 0.53)	0.27 (0.01 to 0.53)	0.26 (0.00 to 0.52)	0.27 (0.01 to 0.53)
Symptom duration, years	0.04 (0.00 to 0.08)	0.00 (−0.03 to 0.03)	0.01 (−0.03 to 0.04)	0.01 (−0.02 to 0.04)	0.01 (−0.02 to 0.05)	0.01 (−0.02 to 0.05)
Time-averaged ASDAS, points	0.24 (0.00 to 0.47)	0.20 (−0.03 to 0.42)	0.19 (−0.03 to 0.41)	0.18 (−0.04 to 0.40)	0.18 (−0.04 to 0.39)	0.18 (−0.03 to 0.40)
Current smoking	0.52 (0.15 to 0.89)	0.37 (0.08 to 0.65)	0.36 (0.08 to 0.64)	0.37 (0.09 to 0.65)	0.38 (0.10 to 0.66)	0.38 (0.10 to 0.66)
Classification as r-axSpA	0.61 (0.27 to 0.94)	0.24 (−0.02 to 0.50)	0.25 (−0.01 to 0.51)	0.24 (−0.02 to 0.50)	0.23 (−0.03 to 0.49)	0.23 (−0.03 to 0.49)
mSASSS, points	0.10 (0.07 to 0.13)	0.09 (0.06 to 0.12)	0.09 (0.06 to 0.12)	0.09 (0.06 to 0.12)	0.09 (0.06 to 0.12)	0.09 (0.06 to 0.12)
NSAID score, per 10 points	0.02 (−0.03 to 0.06)	−0.04 (−0.09 to −0.00)	−0.04 (−0.09 to −0.00)	−0.05 (−0.09 to −0.00)	−0.05 (−0.09 to −0.00)	−0.05 (−0.09 to −0.00)

ASDAS, Ankylosing Spondylitis Disease Activity Score; axSpA, axial spondyloarthritis; mSASSS, modified Stroke Ankylosing Spondylitis Spine Score; NSAID, non-steroidal anti-inflammatory drugs; r-axSpA, radiographic axSpA; TNFi, tumour necrosis factor alpha inhibitor.

Figure 1 The multivariable longitudinal generalised estimating equation analysis* for the association between progression in the mSASSS over 2 years and TNFi use in patients with axial spondyloarthritis. *Parameter estimates from the multivariable models adjusted for sex, symptom duration at the beginning of the current 2-year interval, time-averaged ASDAS in the current 2-year interval, smoking in the current 2-year interval, classification as radiographic axSpA, mSASSS at the beginning of the current 2-year interval and NSAID intake score. ASDAS, Ankylosing Spondylitis Disease Activity Score; axSpA, axial spondyloarthritis; mSASSS, modified Stroke Anlylosing Spondylitis Spine Score; NSAID, non-steroidal anti-inflammatory drug; TNFi, tumour necrosis factor alpha inhibitor.

Missing value of a single vertebral corner was replaced by the values of the same vertebral corner obtained at the next available time point (previous for the last one) or by 0 if all were missing. Furthermore, we imputed missing time points if the previous and the next available time points had the same mSASSS. No further imputations were performed.

The reliability of assessment was evaluated by the intraclass correlation coefficient (ICC) for the mSASSS status and change scores between the readers.

The primary outcome used in this analysis was the absolute change in mSASSS in a 2-year interval. The secondary outcomes included progression in the mSASSS by ≥2 points over 2 years and formation of ≥1 new syndesmophyte (as recorded by at least two out of three readers) over 2 years.

The TNFi exposure was defined as follows:

1. any TNFi use in the current 2-year interval.
2. TNFi for ≥12 months in the current 2-year interval,
3. Any TNFi use in the previous 2-year interval.
4. TNFi for ≥12 months in the previous 2-year interval.
5. TNFi for ≥12 months in the previous and ≥12 months in the current 2-year interval.

The longitudinal association between TNFi treatment and radiographic spinal progression was evaluated using linear and binomial generalised estimating equations (GEE). All relevant interactions between mSASSS, TNFi exposure and other covariates were tested and revealed no significant interaction ($p>0.15$). An autoregressive correlation structure was used for the models. In choosing the best correlation structure, we followed the guide suggested by Hardin and Hilbe.[23] Briefly, since our data contained missings and were collected over time, we chose the autoregressive structure. This provides a better understanding of the true longitudinal relationship because the cross-sectional effects (within-participant) are removed and the value of the outcome at a given time point is predicted by the outcome variable at the previous time point ('autoregression'). In addition, we tested the correlation structures based on Quasilikelihood under the Independence model Criterion. Univariable and multivariable GEE analyses were performed for primary and secondary

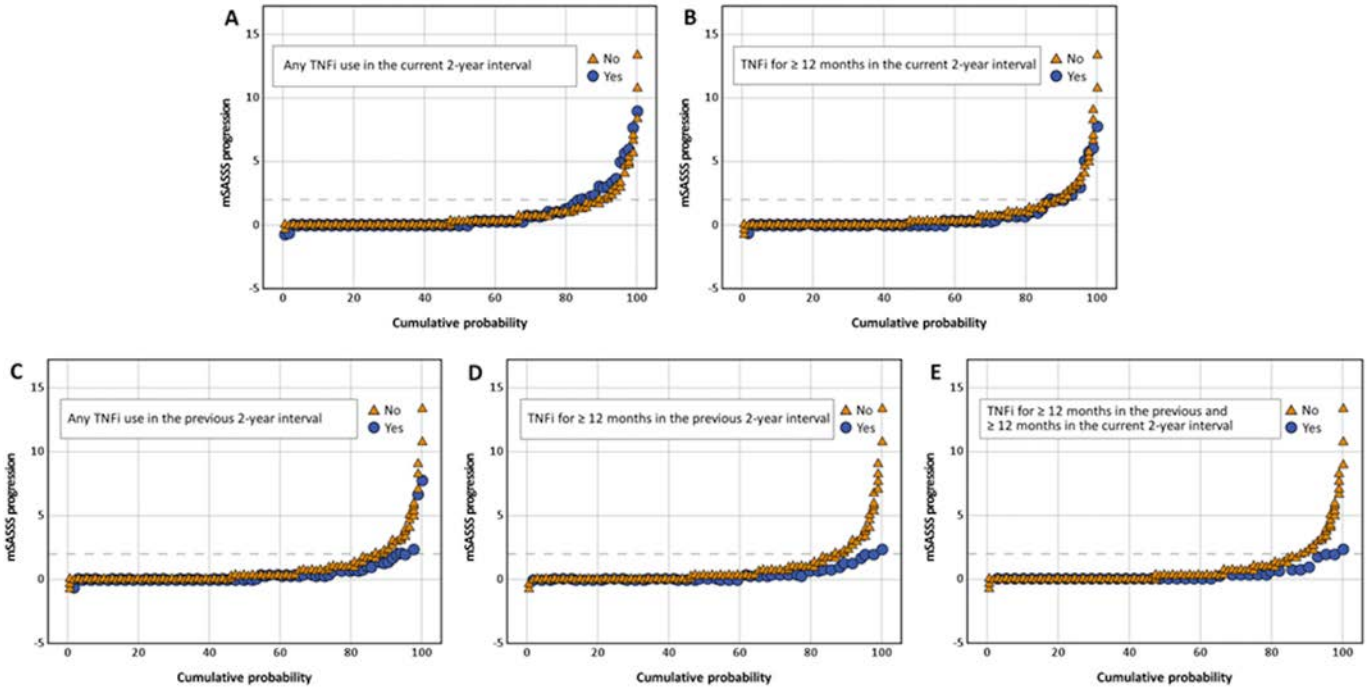

Figure 2 Cumulative probability plot of the 2-year mSASSS change scores stratified by TNFi exposure status. (A) Any TNFi in the current interval, (B) at least 12 months TNFi in the current interval, (C) any TNFi in the previous interval, (D) at least 12 months TNFi in the previous interval and (E) TNFi for ≥12 months in the previous and ≥12 months in the current 2-year intervals. mSASSS, the modified Stoke Ankylosing Spondylitis Spinal Score; TNFi, tumour necrosis factor alpha inhibitor.

outcomes. All multivariable GEE models with different TNFi definitions were adjusted for the following variables: mSASSS at the beginning of the 2-year interval, classification status (radiographic or non-radiographic), sex (male vs female), symptom duration, current smoking status (yes vs no), time-averaged ASDAS and the NSAID intake score. In addition, we evaluated direct and indirect (mediated by disease activity—ASDAS) effects of TNFi on mSASSS progression as described by Hayes,[24] and described in online supplemental figure S2. This graph depicts the conceptional causal framework between TNFi exposure (X), mediator (ASDAS (M)), and outcome (progression in mSASSS (Y)). While '*a*b*' represents the indirect effect via the mediator, *c*' represents the direct effect of TNFi exposure on progression. Parameter estimates (β/OR—OR, where appropriate) with 95% CIs were calculated.

RESULTS

The baseline characteristics of 243 included patients are shown in table 1. In comparison to patients excluded (n=282) due to missing radiographs which precluded the assessment of radiographic progression, included patients were less frequently male (49.4% vs 58.9%), had more frequently a family history of SpA (35.1% vs 26.2%) and more often a history of psoriasis (11.5% vs 8.9%). Included patients had similar ASDAS (2.5 vs 2.6) and BASMI (1.6 vs 1.4), and received less frequently systemic steroids (6.2% vs 11.7%). In addition, baseline mSASSS was slightly lower (2.6 vs 2.7) in the included patients, but there was no difference in the proportion of patients with baseline syndesmophytes. Among included patients, only eight (3.3%) patients were under TNFi treatment at baseline, while 70 (28.8%) patients received TNFi during follow-up. The included patients contributed a total of 531 2-year radiographic intervals, with 1, 2, 3, 4 and 5 intervals obtained from 114, 41, 44, 17 and 27 patients, respectively. Of these, 103 (19.4%) and 78 (14.7%) intervals were covered by TNFi treatment of any duration and TNFi treatment of at least 12 months, respectively. The distributions of the intervals and number of patients with respect to each definition of the TNFi exposure are shown in online supplemental figure S3.

The interobserver reliability between the three readers with regards to the mSASSS status score was good to excellent at all time points with ICC ranging from 0.84 to 0.97 (online supplemental table S1). The reliability of the mSASSS change score was poor to good with ICC ranging from 0.31 to 0.84 (online supplemental table S2).

Longitudinal association between TNFi exposure and mSASSS change

In the univariable analyses, TNFi exposure for ≥12 months in the previous 2-year interval was significantly associated with lower mSASSS progression (table 2). This was confirmed in the multivariable analyses: any TNFi exposure in the previous 2-year interval, exposure ≥12 months in the previous 2-year interval and same exposure that continued in the current interval were associated with reduction of the mSASSS progression by 0.41, 0.54, 0.58 mSASSS points, respectively, having TNFi unexposed patients as a reference (table 2 and figure 1). Of note, exposure to TNFi in the current 2-year interval was not associated with a reduction of the mSASSS progression. Cumulative probability plots (figure 2A–E) reflect the mSASSS progression in TNFi exposed and unexposed patients according to the different definitions across all available 2-year intervals. Interestingly, NSAIDs intake in the current 2-year interval was consistently associated with reduction of radiographic progression in the same interval in all multivariable models (table 2).

Table 3 The effects of TNFi on spinal radiographic progression in mediation analyses

	β	SE	95% CI
Any TNFi use in the current 2-year interval*			
TNF → ASDAS	−0.20	0.09	−0.38 to −0.02
ASDAS → mSASSS progression	0.18	0.08	0.02 to 0.34
Indirect effect	−0.04	0.03	−0.11 to 0.01
Direct effect (TNF → mSASSS progression)	−0.05	0.16	−0.37 to 0.28
Total (direct and indirect) effect	−0.08	0.16	−0.40 to 0.24
TNFi for ≥12 months in the current 2-year interval*			
TNF → ASDAS	−0.36	0.10	−0.57 to −0.16
ASDAS → mSASSS progression	0.17	0.08	0.01 to 0.33
Indirect effect	−0.06	0.05	−0.18 to 0.01
Direct effect (TNF → mSASSS progression)	−0.19	0.19	−0.56 to 0.18
Total (direct and indirect) effect	−0.25	0.19	−0.62 to 0.11
Any TNFi use in the previous 2-year interval*			
TNF → ASDAS	−0.25	0.11	−0.46 to −0.04
ASDAS → mSASSS progression	0.16	0.08	0.00 to 0.33
Indirect effect	−0.04	0.04	−0.14 to 0.01
Direct effect (TNF → mSASSS progression)	−0.40	0.19	−0.77 to −0.04
Total (direct and indirect) effect	−0.45	0.19	−0.81 to −0.08
TNFi for ≥12 months in the previous 2-year interval*			
TNF → ASDAS	−0.34	0.13	−0.59 to −0.08
ASDAS → mSASSS progression	0.16	0.08	0.00 to 0.32
Indirect effect	−0.05	0.05	−0.18 to 0.02
Direct effect (TNF → mSASSS progression)	−0.55	0.23	−0.99 to −0.10
Total (direct and indirect) effect	−0.60	0.23	−1.05 to −0.15
TNFi for ≥12 months in the previous and ≥12 months in the current 2-year interval*			
TNF → ASDAS	−0.27	0.14	−0.55 to 0.01
ASDAS → mSASSS progression	0.17	0.08	0.01 to 0.33
Indirect effect	−0.04	0.05	−0.17 to 0.02
Direct effect (TNF → mSASSS progression)	−0.57	0.25	−1.17 to −0.08
Total (direct and indirect) effect	−0.62	0.25	−1.11 to −0.13

The table represents the direct, indirect and total effects of TNFi on mSASSS progression in patients with axSpA. These effects are described in the diagram in online supplemental figure S2 in detail.
*Parameter estimates from all multivariable models with different TNFi exposure definitions were adjusted for sex, symptom duration at the beginning of the current 2-year interval, smoking in the current 2-year interval, classification as radiographic axSpA, mSASSS at the beginning of the current 2-year interval,and NSAID intake score.
ASDAS, Ankylosing Spondylitis Disease Activity Score; axSpA, axial spondyloarthritis; mSASSS, modified Stroke Anlylosing Spondylitis Spine Score; NSAID, non-steroidal anti-inflammatory drugs; SE, standard error; TNFi, tumour necrosis factor alpha inhibitor.

The analysis of the direct and indirect (mediated by reduction in disease activity as reflected by ASDAS) effects of TNFi on radiographic spinal progression is presented in table 3 and described in online supplemental figure S2. In the current 2-year interval, neither indirect (via reduction in ASDAS) nor direct effect of TNFi on mSASSS change was significant. However, in the models that included TNFi exposure in the previous 2-year radiographic interval, we observed significant direct effects of TNFi on radiographic progression in the current 2-year interval (β values were −0.40, −0.55, and −0.57, for any TNFi use in the previous 2-year interval, TNFi for ≥12 months in the previous 2-year interval and TNFi for ≥12 months in the previous and ≥12 months in the current 2-year interval, respectively—table 3).

Online supplemental table 3 presents the changes in mSASSS in the whole axSpA group and in the subgroups according to ASDAS-CRP categories (derived from the time-averaged ASDAS in the current 2 year interval) based on different definitions of TNFi exposure. The progression rate was highest in patients with very high disease activity who received no TNFi. Overall, the effect of TNFi on radiographic spinal progression was largely consistent across all subgroups.

Longitudinal association between TNFi exposure and binary outcomes

The results for the binary definitions of progression (progression ≥2 mSASSS points over 2 years, formation of ≥1 new syndesmophyte over 2 years) were in line with the analyses that used the continuous mSASSS change score as an outcome, although the precision of the effect estimation was lower as reflected by large 95% CIs (table 4). In general, TNFi exposure in the previous 2-year interval was associated with lower odds for progression in the current one; for example, any TNFi exposure in the previous 2-year interval was associated with a 69% reduction of the odds of formation of new syndesmophytes in the current interval, OR 0.31, 95% CI 0.10 to 0.95 (table 4).

DISCUSSION

In the present study, we could demonstrate that TNFi treatment is associated with reduction of radiographic spinal progression in patients with axSpA. Importantly, the effect could not be observed immediately after treatment initiation (in the first 2 years) but became evident after 4 years of observation. The effect was clinically relevant if considered in the context of natural radiographic progression in axSpA with a mean of 1–2 mSASSS points per 2 years[25 26]: TNFi treatment was associated with reduction of radiographic progression by 0.5–0.6 mSASSS points in 2 years as compared with patients not treated with TNFi.

We hope that our data will contribute to current knowledge in the field that evolved after publication of first long-term extension studies with TNFi in AS. These studies showed that TNFi treatment over 2 years was not associated with retardation of radiographic spinal progression as compared with historical controls.[8–10] Subsequent works indicated, however, that such a retardation might be possible, especially if treatment is applied long-term, which is in line with our results, although there are some differences in terms of the study design, patient characteristics and definition of TNFi exposure in the intervals.[11–18] Indeed, it seems that the effect of anti-inflammatory treatment with TNFi cannot be observed immediately after treatment initiation—at least not with radiographs as the method of structural damage assessment. This is related to the fact that inflammation in the vertebral body is followed by the process of repair characterised by the replacement of the inflammatory-affected bone marrow by fibrous repair tissue that gives raise to new bone formation (syndesmophytes) later on.[5 27] This sequel has been confirmed by recent data correlating both, MRI and histological data[7 28] and MRI and radiographic data.[6 29] It can be, therefore, expected that in the first 2 years after TNFi initiation, we observe the process of new bone formation that has started already before or just after ('TNF-brake') release[30] treatment initiation that slows down radiographic progression between year 2 and year 4. This means that effective and continuous (and ideally early) control of inflammation is necessary to modify the natural course of structural damage progression in axSpA, which is also in line with our analysis, where we demonstrated a similar

Table 4 The association between progression ≥2 mSASSS points and formation of ≥1 new syndesmophyte over 2 years and TNFi exposure in patients with axial spondyloarthritis in a binomial generalised estimating equation analysis

Model*	TNFi exposure definition	Reference	Progression ≥2 mSASSS points OR (95% CI)	Formation of ≥1 new syndesmophyte OR (95% CI)
1	Any TNFi use in the current 2-year interval	No TNFi use in the current 2-ear interval	1.39 (0.64 to 3.01)	1.18 (0.50 to 2.79)
2	TNFi for ≥12 months in the current 2-year interval	No TNFi for ≥12 months in the current 2-year interval	0.96 (0.35 to 2.66)	0.75 (0.25 to 2.28)
3	Any TNFi use in the previous 2-year interval	No TNFi use in the previous 2-year interval	0.30 (0.08 to 1.20)	0.31 (0.10 to 0.95)
4	TNFi for ≥12 months in the previous 2-year interval	No TNFi for ≥12 months in the previous 2-year interval	0.21 (0.02 to 3.08)	0.36 (0.12 to 1.07)
5	TNFi for ≥12 months in the previous and ≥12 months in the current 2-year interval	No TNFi for ≥12 months in the previous and ≥12 months in the current 2-year interval	0.29 (0.02 to 4.86)	0.43 (0.12 to 1.55)

*Parameter estimates from the multivariable models adjusted for sex, symptom duration at the beginning of the current 2-year interval, time-averaged ASDAS in the current 2-year interval, smoking in the current 2-year interval, classification as radiographic axSpA, mSASSS at the beginning of the current 2-year interval and NSAID intake score.
ASDAS, Ankylosing Spondylitis Disease Activity Score; axSpA, axial Spondyloarthritis; mSASSS, modified Stroke Anlylosing Spondylitis Spine Score; NSAID, non-steroidal anti-inflammatory drugs; TNFi, tumour necrosis factor alpha inhibitor.

effect of TNFi on radiographic sacroiliitis progression.[20] The observed direct effect of TNFi exposure—especially when used in the previous interval—on radiographic progression, suggests that either the inflammatory burden is not fully captured by ASDAS or CRP (we think this is the most likely explanation since we have not captured the presence and extent of local inflammation in the spine) or that TNFi might also have additional effects on new bone formation independent of anti-inflammatory properties.[31 32] Indeed, earlier studies indicated that TNF might stimulate new bone formation (especially at the early stages of endochondral ossification) via upregulation of osteogenic mediators such as bone morphogenic proteins.[33 34]

It is important to mention that the natural course of structural progression is very heterogeneous and that not all patients with axSpA develop clinically relevant (in terms of irreversible reduction of function and spinal mobility) damage in the spine.[25] Therefore, 'early' is a term that is not well defined in the context of axSpA since duration of symptoms correlates only to some extent with the presence of the structural damage in the spine. The same imprecision holds also true for the so-called 'window of opportunity'—in some patients, the window is rather small (high-risk patients with early syndemophytes, high inflammatory activity as reflected by elevated CRP and spinal inflammation on MRI), but in other cases, the window remains open many years, sometimes life long. In any case, a treatment strategy focussing on symptom and inflammation control seems to be beneficial for all patients; patients with a high risk of structural damage development might need, however, special attention with a tight-control strategy.

Is there a possibility to stop structural damage in axSpA immediately on treatment initiation? This question remains unsolved until now. Such a treatment modality would need to have a direct inhibitory effect on osteoblasts participating in the process of new bone formation in the spine. NSAIDs showed some promising results in an earlier study (with patients treated mainly with a selective cyclooxygenase (COX)−2 drug celecoxib),[35] while a subsequent study (with non-selective COX-inhibitor diclofenac as an investigational drug) could not demonstrate an inhibitory effect of a continuous versus on-demand intake on radiographic spinal progression in axSpA.[36] Interestingly, in our present analysis, higher NSAID intake was associated with reduction of radiographic spinal progression in the current 2-year interval. Similar effect of NSAIDs was observed in the previous work that analysed 2-year data from GESPIC.[22] An ongoing prospective controlled study comparing a TNFi monotherapy with a combination of TNFi plus celecoxib should clarify the question about the potential role of NSAIDs in reducing structural damage progression in axSpA.[37]

There is an ongoing discussion, whether IL-17 blockade is able to retard structural damage progression in axSpA not only through inhibition of inflammation but also through a direct inhibition of osteoblastic activity as suggested by some preclinical data.[38] A currently ongoing head-to-head comparison of an IL-17 inhibitor secukinumab with the TNFi adalimumab focussing on radiographic spinal progression[39] should demonstrate if there is a clinically relevant difference between these drug classes. In GESPIC, the 10-year follow-up visit was completed for most patients before IL-17 inhibitors' approval; therefore, we could not investigate the effect of this drug class on radiographic spinal progression.

There are some other limitations of the current study we need to acknowledge. First, with conventional spinal radiographs, we capture only a relatively small part of the structural damage occurring in the spine of patients with axSpA. CT might be able to substitute radiographs in the future for a comprehensive assessment of structural damage and a better sensitivity to change.[40 41] Second, MRI scans of the spine were not performed in GESPIC, thus, the presence and extent of spinal inflammation could only be captured indirectly by CRP. Third, unblinded reading of radiographs might lead to an overestimation of progression. Nevertheless, this method was chosen due to its higher sensitivity to change and potential reduction of 'background noise' not related to true structural changes especially in a setting with multiple time points.[42] Although the interobserver reliability of the mSASSS was good to excellent for the status scores across all time points, the poor to good ICCs concerning the change scores may be considered as a further limitation. Finally, we had to exclude a substantial number of patients who had no complete sets of spinal radiographs. Although the included and the excluded groups were largely comparable, the risk of attrition bias cannot be completely excluded.

In conclusion, in the present study, we could demonstrate retardation of radiographic spinal progression associated with TNFi treatment in patients with axSpA. This effect was time shifted and observed between 2 and 4 years after treatment initiation.

Author affiliations
[1]Department of Gastroenterology, Infectious Diseases and Rheumatology (Including Nutrition Medicine), Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany
[2]Department of Internal Medicine, Moscow City Clinical Hospital, Moscow, Russia
[3]Berlin Institute of Health at Charite, Berlin, Germany

[4]Department of Internal Medicine and Rheumatology, Klinikum Bielefeld, Bielefeld University, Bielefeld, Nordrhein-Westfalen, Germany
[5]Epidemiology Unit, German Rheumatism Research Center, Berlin, Germany

Twitter Mikhail Protopopov @MProtopopov

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ORCID iDs
Murat Torgutalp http://orcid.org/0000-0003-4600-9484
Valeria Rios Rodriguez http://orcid.org/0000-0001-5612-043X
Fabian Proft http://orcid.org/0000-0003-4306-033X
Mikhail Protopopov http://orcid.org/0000-0003-4840-5069
Judith Rademacher http://orcid.org/0000-0001-7442-2570
Joachim Sieper http://orcid.org/0000-0003-0285-9890
Martin Rudwaleit http://orcid.org/0000-0001-5445-548X
Denis Poddubnyy http://orcid.org/0000-0002-4537-6015

REFERENCES

1 Sieper J, Poddubnyy D. Axial spondyloarthritis. *The Lancet* 2017;390:73–84.
2 Stal R, van Gaalen F, Sepriano A, *et al*. Facet joint ankylosis in r-axSpA: detection and 2-year progression on whole spine low-dose CT and comparison with syndesmophyte progression. *Rheumatology* 2020;59:3776–83.
3 Machado P, Landewé R, Braun J, *et al*. Both structural damage and inflammation of the spine contribute to impairment of spinal mobility in patients with ankylosing spondylitis. *Ann Rheum Dis* 2010;69:1465–70.
4 Poddubnyy D, Listing J, Haibel H, *et al*. Functional relevance of radiographic spinal progression in axial spondyloarthritis: results from the German spondyloarthritis inception cohort. *Rheumatology* 2018;57:703–11.
5 Poddubnyy D, Sieper J. Mechanism of new bone formation in axial spondyloarthritis. *Curr Rheumatol Rep* 2017;199:55.
6 Machado PM, Baraliakos X, van der Heijde D, *et al*. Mri vertebral corner inflammation followed by fat deposition is the strongest contributor to the development of new bone at the same vertebral corner: a multilevel longitudinal analysis in patients with ankylosing spondylitis. *Ann Rheum Dis* 2016;75:1486–93.
7 Bleil J, Maier R, Hempfing A, *et al*. Granulation tissue Eroding the Subchondral bone also promotes new bone formation in ankylosing spondylitis. *Arthritis Rheumatol* 2016;68:2456–65.
8 van der Heijde D, Salonen D, Weissman BN, *et al*. Assessment of radiographic progression in the spines of patients with ankylosing spondylitis treated with adalimumab for up to 2 years. *Arthritis Res Ther* 2009;11:R127.
9 van der Heijde D, Landewé R, Einstein S, *et al*. Radiographic progression of ankylosing spondylitis after up to two years of treatment with etanercept. *Arthritis Rheum* 2008;58:1324–31.
10 van der Heijde D, Landewé R, Baraliakos X, *et al*. Radiographic findings following two years of infliximab therapy in patients with ankylosing spondylitis. *Arthritis Rheum* 2008;58:3063–70.
11 Haroon N, Inman RD, Learch TJ. The impact of tumor necrosis factor alpha inhibitors on radiographic progression in ankylosing spondylitis. *Arthritis Rheum* 2013;6510:2645–54.
12 Baraliakos X, Haibel H, Listing J, *et al*. Continuous long-term anti-TNF therapy does not lead to an increase in the rate of new bone formation over 8 years in patients with ankylosing spondylitis. *Ann Rheum Dis* 2014;73:710–5.
13 Sepriano A, Ramiro S, Wichuk S, *et al*. Tumor necrosis factor inhibitors reduce spinal radiographic progression in patients with radiographic axial spondyloarthritis: a longitudinal analysis from the Alberta prospective cohort. *Arthritis Rheumatol* 2021;73:1211–9.
14 Maas F, Arends S, Wink FR, *et al*. Ankylosing spondylitis patients at risk of poor radiographic outcome show diminishing spinal radiographic progression during long-term treatment with TNF-α inhibitors. *PLoS One* 2017;12:e0177231.
15 Koo BS, Oh JS, Park SY, *et al*. Tumour necrosis factor inhibitors slow radiographic progression in patients with ankylosing spondylitis: 18-year real-world evidence. *Ann Rheum Dis* 2020;79:1327–32.
16 Park JW, Kim MJ, Lee JS, *et al*. Impact of tumor necrosis factor inhibitor versus nonsteroidal antiinflammatory drug treatment on radiographic progression in early ankylosing spondylitis: its relationship to inflammation control during treatment. *Arthritis Rheumatol* 2019;71:82–90.
17 Molnar C, Scherer A, Baraliakos X, *et al*. Tnf blockers inhibit spinal radiographic progression in ankylosing spondylitis by reducing disease activity: results from the Swiss clinical quality management cohort. *Ann Rheum Dis* 2018;77:63–9.
18 Hebeisen M, Micheroli R, Scherer A, *et al*. Spinal radiographic progression in axial spondyloarthritis and the impact of classification as nonradiographic versus radiographic disease: data from the Swiss clinical quality management cohort. *PLoS One* 2020;15:e0230268.
19 Rudwaleit M, Haibel H, Baraliakos X, *et al*. The early disease stage in axial spondylarthritis: results from the German spondyloarthritis inception cohort. *Arthritis Rheum* 2009;60:717–27.
20 Torgutalp M, Rios Rodriguez V, Proft F, *et al*. Treatment with Tumour Necrosis Factor Inhibitors is Associated with a Time-Shifted Retardation of Radiographic Sacroiliitis Progression in Patients with Axial Spondyloarthritis: 10-year Results from the German Spondyloarthritis Inception Cohort. *Arthritis & Rheumatology* 2022.

21 Dougados M, Paternotte S, Braun J, *et al*. ASAS recommendations for collecting, analysing and reporting NSAID intake in clinical trials/epidemiological studies in axial spondyloarthritis. *Ann Rheum Dis* 2011;70:249–51.
22 Poddubnyy D, Rudwaleit M, Haibel H, *et al*. Effect of non-steroidal anti-inflammatory drugs on radiographic spinal progression in patients with axial spondyloarthritis: results from the German spondyloarthritis inception cohort. *Ann Rheum Dis* 2012;71:1616–22.
23 Hardin J, Hilbe J. *Generalized estimating equations*. 2 ed. CRC Press. Taylor & Francis Group. Chapman and Hall book, 2012.
24 Hayes AF. *Introduction to mediation, moderation, and conditional process analysis: a regression-based approach*. 2 ed. The Guilford Press, 2017.
25 Ramiro S, Stolwijk C, van Tubergen A, *et al*. Evolution of radiographic damage in ankylosing spondylitis: a 12 year prospective follow-up of the OASIS study. *Ann Rheum Dis* 2015;74:52–9.
26 Poddubnyy D, Haibel H, Listing J, *et al*. Baseline radiographic damage, elevated acute-phase reactant levels, and cigarette smoking status predict spinal radiographic progression in early axial spondylarthritis. *Arthritis Rheum* 2012;64:1388–98.
27 Sieper J, Appel H, Braun J, *et al*. Critical appraisal of assessment of structural damage in ankylosing spondylitis: implications for treatment outcomes. *Arthritis Rheum* 2008;58:649–56.
28 Baraliakos X, Boehm H, Bahrami R, *et al*. What constitutes the fat signal detected by MRI in the spine of patients with ankylosing spondylitis? A prospective study based on biopsies obtained during planned spinal osteotomy to correct hyperkyphosis or spinal stenosis. *Ann Rheum Dis* 2019;78:1220–5.
29 Baraliakos X, Heldmann F, Callhoff J, *et al*. Which spinal lesions are associated with new bone formation in patients with ankylosing spondylitis treated with anti-TNF agents? a long-term observational study using MRI and conventional radiography. *Ann Rheum Dis* 2014;73:1819–25.
30 Pedersen SJ, Chiowchanwisawakit P, Lambert RGW, *et al*. Resolution of inflammation following treatment of ankylosing spondylitis is associated with new bone formation. *J Rheumatol* 2011;38:1349–54.
31 Sedger LM, McDermott MF, . Tnf and TNF-receptors: from mediators of cell death and inflammation to therapeutic giants – past, present and future. *Cytokine Growth Factor Rev* 2014;25:453–72.
32 Kalliolias GD, Ivashkiv LB. Tnf biology, pathogenic mechanisms and emerging therapeutic strategies. *Nat Rev Rheumatol* 2016;12:49–62.
33 Lories RJ, Haroon N. Bone formation in axial spondyloarthritis. *Best Pract Res Clin Rheumatol* 2014;28:765–77.
34 Carter S, Braem K, Lories RJ. The role of bone morphogenetic proteins in ankylosing spondylitis. *Ther Adv Musculoskelet Dis* 2012;4:293–9.
35 Wanders A, Heijde Dvander, Landewé R, *et al*. Nonsteroidal antiinflammatory drugs reduce radiographic progression in patients with ankylosing spondylitis: a randomized clinical trial. *Arthritis Rheum* 2005;52:1756–65.
36 Sieper J, Listing J, Poddubnyy D, *et al*. Effect of continuous versus on-demand treatment of ankylosing spondylitis with diclofenac over 2 years on radiographic progression of the spine: results from a randomised multicentre trial (ENRADAS). *Ann Rheum Dis* 2016;75:1438–43.
37 Proft F, Muche B, Listing J, *et al*. Study protocol: comparison of the effect of treatment with nonsteroidal anti-inflammatory drugs added to anti-tumour necrosis factor a therapy versus anti-tumour necrosis factor a therapy alone on progression of structural damage in the spine over two years in patients with ankyLosing spondylitis (CONSUL) – an open-label randomized controlled multicenter trial. *BMJ Open* 2017;7:e014591.
38 van Tok MN, van Duivenvoorde LM, Kramer I, *et al*. Interleukin-17A inhibition diminishes inflammation and new bone formation in experimental spondyloarthritis. *Arthritis Rheumatol* 2019;71:612–25.
39 Baraliakos X, Østergaard M, Gensler LS, *et al*. Comparison of the effects of Secukinumab and adalimumab Biosimilar on radiographic progression in patients with ankylosing spondylitis: design of a randomized, phase IIIB study (surpass). *Clin Drug Investig* 2020;40:269–78.
40 Ward MM, Tan S. Better quantification of Syndesmophyte growth in axial spondyloarthritis. *Curr Rheumatol Rep* 2018;20:46.
41 de Bruin F, de Koning A, van den Berg R, *et al*. Development of the CT Syndesmophyte score (CTSS) in patients with ankylosing spondylitis: data from the SIAS cohort. *Ann Rheum Dis* 2018;77:371–7.
42 Wanders A*et al*. Scoring of radiographic progression in randomised clinical trials in ankylosing spondylitis: a preference for paired reading order. *Ann Rheum Dis* 2004;63:1601–4.

EPIDEMIOLOGICAL SCIENCE

Rising incidence and prevalence of systemic lupus erythematosus: a population-based study over four decades

Alí Duarte-García ,[1,2] Mehmet Hocaoglu,[1,3] Maria Valenzuela-Almada,[1] Shirley-Ann Osei-Onomah,[1] Jesse Y Dabit ,[1] Alain Sanchez-Rodriguez,[1] Stephanie Q Duong,[4] Rachel E Giblon,[4] Hannah E Langenfeld,[4] Graciela S Alarcón ,[5,6] Charles G Helmick,[7] Cynthia S Crowson [1,4]

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For numbered affiliations see end of article.

Correspondence to
Dr Alí Duarte-García, Mayo Clinic Division of Rheumatology, Rochester, Minnesota, USA; Duarte.Ali@mayo.edu

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ABSTRACT

Objectives To determine the trends in incidence, prevalence and mortality of systemic lupus erythematosus (SLE) in a US population over four decades.

Methods We identified all the patients with SLE in Olmsted County, Minnesota who fulfilled the European Alliance of Associations for Rheumatology (EULAR)/American College of Rheumatology (ACR) criteria for SLE during 1976–2018. Age-specific and sex-specific incidence and prevalence rates were adjusted to the standard 2000 projected US population. The EULAR/ACR score was used as a proxy for disease severity. Standardised mortality ratio (SMR) was estimated.

Results There were 188 incident SLE cases in 1976–2018 (mean age 46.3±SD 16.9; 83% women). Overall age-adjusted and sex-adjusted annual SLE incidence per 100 000 population was 4.77 (95% CI 4.09 to 5.46). Incidence was higher in women (7.58) than men (1.89). The incidence rate increased from 3.32 during 1976–1988 to 6.44 during 2009–2018. Incidence rates were higher among the racial and ethnic minority populations than non-Hispanic whites. The EULAR/ACR score did not change significantly over time. Overall prevalence increased from 30.6 in 1985 to 97.4 in 2015. During the study period, there was no improvement in SMR over time (p=0.31).

Conclusions The incidence and prevalence of SLE are increasing in this US population. The increase in incidence may be at least partially explained by the rising ethnic/racial diversity of the population. There was no evidence that the severity of SLE has changed over time. The survival gap between SLE and the general population remains unchanged. As the US population grows more diverse, we might continue to see an increase in the incidence of SLE.

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT?

⇒ Prior studies have reported discrepant results regarding the incidence trends of systemic lupus erythematosus (SLE), some reporting an increase while others reporting a decrease in SLE incidence. However, case ascertainment has been conducted in short periods of time, thus not providing long-term trends.

WHAT DOES THIS STUDY ADD?

⇒ Our study shows increasing incidence and prevalence of SLE over the last four decades in this US population. Concomitant with the increased ethnic/racial diversity of the region.
⇒ The severity of SLE at diagnosis has remained the same from 1976 to 2018.
⇒ There have not been improvements in SLE survival in recent decades.

HOW MIGHT THIS IMPACT ON CLINICAL PRACTICE OR FUTURE DEVELOPMENTS?

⇒ As the US population grows more diverse, we might continue to see an increase in the incidence of SLE.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic systemic autoimmune disease with heterogeneous clinical manifestations. Its complex clinical presentation and the pluralistic nature of the US health system have made it difficult to obtain accurate estimates of the incidence, prevalence and mortality of SLE. The Centers for Disease Control and Prevention (CDC) five surveillance registries have provided estimates regarding the incidence and prevalence of SLE across different racial and ethnic groups. These registries have confirmed that SLE is more frequent in racial and ethnic minority populations compared with the white population.[1] Prior studies have reported discrepant results regarding the incidence trends of SLE, some reporting an increase while others reporting a decrease in SLE incidence. However, case ascertainment has been conducted in short periods of time, thus not providing long-term trends. In addition, prior studies have not explored potential explanations for the observed trends.

The aims of this study were to examine the trends in the incidence, prevalence and mortality of SLE in a well-defined geographic area over four decades.

METHODS

Study design

The Lupus Midwest Network (LUMEN) is a population-based study that uses the resources of the Rochester Epidemiology Project (REP), a

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record-linkage system. This epidemiologic study includes residents of Olmsted County, Minnesota, and has a >99% capture of the census population in the REP. This system ensures virtually complete ascertainment of all clinically recognised cases of SLE among the residents of Olmsted County, Minnesota.[2] The characteristics and strengths of the REP, as well as its generalisability have been described elsewhere.[3 4] The population of Olmsted County was 144 248 in 2010, with 74.7% aged ≥18 years and a racial/ethnic distribution of 85.7% white, 4.2% Hispanic, 4.8% black, 5.5% Asian/Native Hawaiian/Pacific Islander and 0.2% American Indian/Alaska Native.[5] Patients and the public were not involved in this study.

Case finding, definitions and ascertainment

We screened potential SLE cases in Olmsted County using (1) the International Classification of Diseases (ICD)-9 and ICD-10 codes for SLE, cutaneous lupus erythematosus, and other associated diseases (online supplemental material) and (2) laboratory measures associated with SLE such as antinuclear antibodies (ANAs) (≥1:80), low complement, anti-double stranded DNA (anti-dsDNA), anti-Smith (anti-Sm), lupus anticoagulant anticardiolipin (IgG, IgM and IgA) and anti-beta 2 glycoprotein 1 (IgG, IgM and IgA) antibodies.

Clinical data for these criteria were thoroughly abstracted through medical record review (online supplemental material). We used the European Alliance of Associations for Rheumatology (EULAR)/American College of Rheumatology (ACR) criteria because it classifies more patients with SLE in population-based studies than the Systemic Lupus Erythematosus International Collaborating Clinics (SLICC) and ACR97 criteria.[6 7] If a disease manifestation could be better explained by a condition other than SLE, it was not counted towards the criteria. The SLE incidence date was defined as the earliest date of criteria fulfilment. Only adults (age 18 and older) were included in this study. A case was considered to be incident if the patient was an Olmsted County resident prior to the SLE incidence date. Data regarding age, sex, self-reported race and ethnicity (Hispanic, and non-Hispanic white, Asian and black), date of first documentation of each manifestation, clinical data from the EULAR/ACR, SLICC and ACR97 criteria and the estimated SLE damage index 2000 (SLEDAI-2K), date of diagnosis, date of last follow-up, vital status, clinical characteristics and laboratory findings were recorded. To be considered a prevalent case, patients needed to reside in Olmsted County and meet the case definition prior to our four dates of point prevalence estimation: 1 January of 1985, 1995, 2005 and 2015. To fully capture the prevalent cases, those subjects who migrated to Olmsted County after diagnosis (and therefore were under treatment) were included in the prevalence estimation (in addition to those identified using the incidence case definition) if they had 7 EULAR/ACR points and a physician diagnosis. The first author performed an independent review of all patients who met the EULAR/ACR criteria to confirm that the disease manifestations were correctly attributed to SLE.

Statistical analysis

Descriptive statistics were used to summarise continuous and categorical data. Age-specific and sex-specific incidence rates were estimated by using the number of incident cases as the numerator and population counts from the REP census as the denominator.[2] Overall incidence rates were age-adjusted or age/sex-adjusted per 100 000 population to the standard 2000 projected US population.[8] To compute 95% CIs for incidence rates, it was assumed that the number of incident cases followed a Poisson distribution. Trends in incidence rates were examined using Poisson regression methods with smoothing splines for age and calendar year. Sensitivity analyses were performed using the ACR97 and SLICC criteria. SLE incidence rates over the 1976–2018 study period were graphically illustrated for seven age groups. The relationship between age at SLE diagnosis and incidence date was also evaluated using linear regression.

To explore the increasing racial/ethnic diversity in the county potentially affecting the incidence of SLE, we estimated the overall incidence rates in the non-Hispanic white and racial and ethnic minority populations during the 1999–2018 timeframe and examined differences in incidence rates between the two groups using Poisson regression methods. Race-specific denominators were available starting in 1999. To investigate if milder cases were being identified over the years, the EULAR/ACR score points up to 1 year after classification were calculated as a proxy for disease severity, and its relationship to the corresponding SLE incidence date was assessed using linear regression and local polynomial regression fitting methods.[9] A sensitivity analysis using a retrospectively estimated SLEDAI-2K at time of classification was performed[10 11] (further methods details in online supplemental material).

The point prevalence of SLE in 1985, 1995, 2005 and 2015 was determined using the number of prevalent cases on 1 January of the respective year as the numerator and the Olmsted County population based on the REP census in the corresponding year as the denominator adjusted to the standard 2000 projected US total population.

Mortality rates following the diagnosis of SLE were estimated using Kaplan-Meier methods and were compared with the expected survival rates in the Minnesota population. The standardised mortality ratio (SMR) was estimated as the ratio of the observed to the expected number of deaths. Trends in SMR over time were examined using Poisson regression models. 95% CIs for the SMR were calculated assuming that the expected rates are fixed, and the observed rates followed a Poisson distribution.

Analyses were performed using SAS software V.9.4 (SAS Institute) and R V.4.0.3 (R Foundation for Statistical Computing).

RESULTS

From 1 January 1976 to 31 December 2018, there were a total of 188 SLE incident cases in Olmsted County. The mean age at diagnosis was 46.3 (SD ±16.9) years, and 83% of the patients were women. Eighty-two per cent of the patients were non-Hispanic white, 5% were non-Hispanic black, 3% were Hispanic and 10% were non-Hispanic Asian. Over the four decades of the study, the percentage of female patients decreased from 93% to 78%. Also, the percentage of non-Hispanic white patients decreased from virtually 100% to 70% in the most recent decade (table 1).

Clinical characteristics of incident SLE

At the time the 188 patients met the EULAR/ACR classification criteria, 100% were ANA positive. The most common clinical manifestations at the time patients met the classification criteria were arthritis (54%), leucopenia (44%) and acute cutaneous lupus (21%). Seven patients (4%) had biopsy proven lupus nephritis and 15 (8%) had proteinuria. Neurologic manifestations were rare at classification time. Most of the patients were positive for anti-dsDNA, anti-Sm or both, 13% were positive for at least one antiphospholipid antibody (aPL) and more than 30% had low complement levels (either C3, C4 or both) (table 2).

Table 1 Demographics of incident systemic lupus erythematosus patients between 1 January 1976 and 31 December 2018, Olmsted County, Minnesota

	Timeframe				
	1976 to 1988 **(n=28)**	1989 to 1998 **(n=31)**	1999 to 2008 **(n=53)**	2009 to 2018 **(n=76)**	Total **(n=188)**
Sex, n (%)					
Female	26 (93)	28 (90)	43 (81)	59 (78)	156 (83)
Male	2 (7)	3 (10)	10 (19)	17 (22)	32 (17)
Age (years)					
Mean (SD)	45.3 (16.7)	41.0 (16.7)	45.6 (16.7)	49.4 (16.8)	46.3 (16.9)
Race/ethnicity, n (%)					
Hispanic	0 (0)	0 (0)	2 (4)	4 (5)	6 (3)
Asian*	0 (0)	2 (7)	4 (8)	12 (16)	18 (10)
Black*	0 (0)	1 (3)	1 (2)	7 (9)	9 (5)
White*	27 (100)	27 (90)	45 (85)	53 (70)	152 (82)
Other*	0 (0)	0 (0)	1 (2)	0 (0)	1 (1)
Missing	1	1	0	0	2

*Non-Hispanic.

Incidence of SLE

The overall age-adjusted and sex-adjusted incidence of SLE from 1976 to 2018 per 100 000 population was 4.77 (95% CI 4.09 to 5.46). The incidence was higher in women (7.58, 95% CI 6.38 to 8.78) than in men (1.89, 95% CI 1.23 to 2.55) (table 3). The overall incidence rates were higher among women for those ages 18–79. Beyond age 80, the incidence rates for men and women were comparable (data not shown).

Table 2 Clinical manifestations at the time of meeting classification among 188 incident EULAR/ACR criteria-defined systemic lupus erythematosus cases, Olmsted County, Minnesota, 1976–2018*

	(n=188)
EULAR/ACR criteria	**n (%)**
ANA positive	188 (100)
Clinical criteria	
Fever	12 (6)
Leucopenia	83 (44)
Thrombocytopenia	32 (17)
Autoimmune haemolysis	4 (2)
Delirium	1 (1)
Psychosis	0 (0)
Seizure	2 (1)
Non-scarring alopecia	5 (3)
Oral ulcers	12 (6)
Subacute cutaneous OR discoid lupus	21 (11)
Acute cutaneous lupus	39 (21)
Pleural or pericardial effusion	17 (9)
Acute pericarditis	10 (5)
Arthritis	102 (54)
Proteinuria	15 (8)
Class II or V lupus nephritis	3 (2)
Class III or IV lupus nephritis	4 (2)
Immunologic criteria	
Antiphospholipid antibodies	24 (13)
Low C3 OR C4	41 (22)
Low C3 AND C4	27 (14)
Anti-dsDNA OR anti-Sm	132 (70)

*Systemic lupus erythematosus (SLE) cases were defined according to the European Alliance of Associations for Rheumatology (EULAR)/American College of Rheumatology (ACR) criteria (met at least 10 points, and at least one clinical criterion and ANA positivity). The clinical manifestation included was at the time the case met classification criteria.
ANA, antinuclear antibodies; anti-dsDNA, anti-double-stranded DNA antibody; anti-Sm, anti-Smith antibody.

The overall incidence increased in each consecutive decade, from 3.32 (95% CI 2.03 to 4.60) per 100 000 during 1976–1988 to 6.44 (95% CI 4.97 to 7.91) per 100 000 during 2009–2018, an increase of 2% per year (95% CI: 1% to 3%, p<0.001) (table 3). These results were similar using the SLICC criteria (177 incident cases, increase of 2% per year (95% CI 0.4% to 3%, p=0.009)), but by the ACR97 criteria did not reach statistical significance (155 incident cases, increase of 1% per year (95% CI −0.3% to 2.4%, p=0.099)). The rise in incidence was observed both in women and men and across age groups (table 3; figure 1). In women, we observed over a 60% increase in the incidence of SLE over four decades (from 5.9 to 9.6/100 000), while in men, we observed almost a sixfold increase (from 0.55 to 3.18 per 100 000). The female:male ratio changed from 11:1 in 1976–1988, to 3:1 in the most recent decade. However, there was no evidence that the increase over calendar time was significantly different between men and women (interaction p=0.27). The mean age at SLE diagnosis increased over time by 1.8 years (95% CI −0.3 to 3.9) per decade (figure 2) (p=0.09).

The overall incidence rate for SLE for the non-Hispanic white and racial and ethnic minority populations during 1999–2018 was 5.42 (95% CI: 4.34 to 6.51) per 100 000 and 8.17 (95% CI: 5.04 to 11.30) per 100 000, respectively; this difference was statistically significant (rate ratio: 1.89, 95% CI: 1.25 to 2.84, p=0.002).

The overall incidence rate of SLE increased 24% from 5.19/100 000 in the 1999–2008 decade to 6.44/100 000 in the 2009–2018 decade, but this increase did not reach statistical significance (rate ratio: 1.25 per decade; 95% CI: 0.88 to 1.78; p=0.20). Among whites, the incidence rate of SLE increased 4% from 5.27/100 000 in the 1999–2008 decade to 5.46/100 000 in the 2009–2018 decade, but this increase did not reach statistical significance (rate ratio: 0.01 per decade; 95% CI: 0.68 to 1.51; p=0.95).

Figure 3 shows the EULAR/ACR score within 1 year of meeting classification criteria among all the incident cases. We observed that for each decade the EULAR/ACR points decreased by 0.9 (95% CI: −1.8 to 0.1; p=0.07). The sensitivity analysis

Table 3 Estimated incidence and prevalence rates (per 100 000 population) and 95% CIs of systemic lupus erythematosus, overall and by sex groups and decade over 43 years, Olmsted County, Minnesota, 1976–2018*

	Rates (95% CI)		
Calendar year	Females†	Males†	Overall‡
Incidence			
1976–1988	5.89 (3.52 to 8.26)	0.55 (0 to 1.33)	3.32 (2.03 to 4.60)
1989–1998	6.22 (3.88 to 8.57)	0.95 (0 to 2.04)	3.58 (2.29 to 4.87)
1999–2008	8.08 (5.65 to 10.51)	2.24 (0.84 to 3.64)	5.19 (3.78 to 6.59)
2009–2018	9.59 (7.10 to 12.09)	3.18 (1.65 to 4.72)	6.44 (4.97 to 7.91)
1976–2018	7.58 (6.38 to 8.78)	1.89 (1.23 to 2.55)	4.77 (4.09 to 5.46)
Prevalence			
1985	51.48 (30.08 to 72.87)	7.93 (0.01 to 15.86)	30.65 (18.86 to 42.44)
1995	72.9 (50.79 to 95.0)	10.79 (1.2 to 20.38)	42.67 (30.41 to 54.94)
2005	99.87 (76.68 to 123.06)	19.14 (8.24 to 30.04)	60.53 (47.51 to 73.55)
2015	158.54 (130.5 to 186.59)	32.83 (19.39 to 46.27)	97.40 (81.61 to 113.19)

*Rates are per 100 000 population. Denominator data are based on the Rochester Epidemiology Project census (see reference in text).
†Age adjusted to the 2000 projected US population.
‡Age-adjusted and sex-adjusted to the 2000 projected US population.

using SLEDAI-2K showed a decrease of 0.3 points per decade (95% CI: −1.2 to 0.6; p=0.36).

Prevalence of SLE

There were 28 prevalent cases with SLE on 1 January 1985 and 153 cases on 1 January 2015. The overall point prevalence per 100 000 increased from 30.65 (95% CI: 18.86 to 42.44) in 1985 to 97.4 (95% CI: 81.61 to 113.19) in 2015. As noted in table 3, the prevalence in women had a threefold increase while the prevalence in men increased fourfold. To assess whether ascertainment bias may have impacted the prevalence rates over time, we examined the length of prior medical history, Olmsted County residence and SLE duration for each prevalence cohort. No trends were found in these assessments.

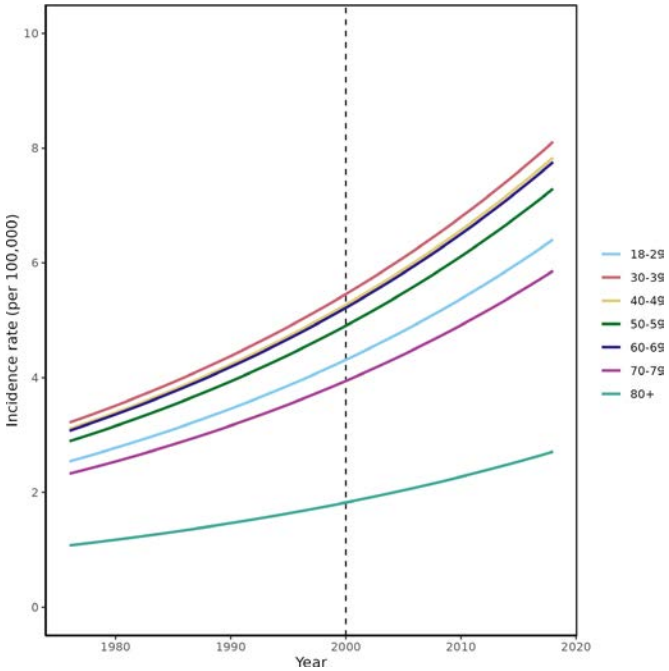

Figure 1 Trends in incidence of systemic lupus erythematosus (SLE) among residents of Olmsted County, Minnesota in 1976–2018 according to age groups. Trends in incidence rates were examined using Poisson regression methods with smoothing splines for age and calendar year. SLE incidence rates over the 1976–2018.

Mortality in SLE

During a median follow-up of 9.7 years, 47 patients with incident SLE died. Survival in our cohort was 93% (95% CI: 89% to 97%) at 5 years, 83% (95% CI: 77% to 89%) at 10 years and 69% (95% CI: 61% to 79%) at 20 years after diagnosis of SLE. The survival of patients with SLE was lower than the general population (figure 4). There were no improvements in the mortality rate over the four decades of the study. The SMR of SLE patients compared with the general population between 1976 and 2018 was 2.2 (95% CI: 1.6 to 3.0) and there was no improvement in the SMR over time (rate ratio: 1.01 per calendar year; 95% CI: 0.99 to 1.04; p=0.31) (table 4).

DISCUSSION

Since the mid-2000s the CDC has funded a number of SLE registries that provided state-of-the-art epidemiologic estimates of SLE across different racial/ethnic populations in the USA among the CDC-funded SLE registries, our study provides estimates regarding the incidence, prevalence and mortality trends over four decades using the recently validated and endorsed EULAR/ACR criteria.

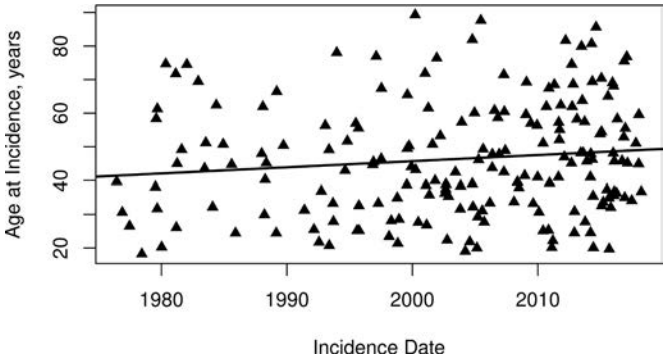

Figure 2 Trends in age at diagnosis of systemic lupus erythematosus (SLE) among residents of Olmsted County, Minnesota in 1976–2018. The mean age at SLE diagnosis increased over time by 1.8 years (95% CI: −0.3 to 3.9) per decade (p=0.09).

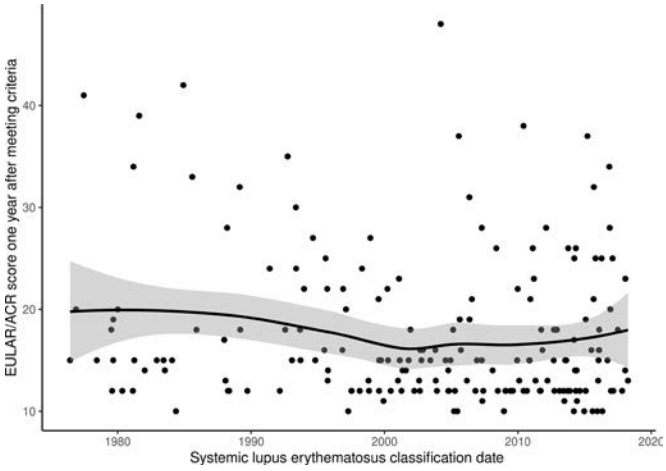

Figure 3 Trends in the systemic lupus erythematosus EULAR/ACR criteria score within 1 year of fulfilling criteria (as a proxy for disease severity) in Olmsted County, Minnesota 1976–2018. Per decade there is a decrease of 0.9 (95% CI: −1.8 to 0.1) in the EULAR/ACR criteria 1 year after classification (p=0.07). ACR American College of Rheumatology; EULAR, European League Against Rheumatism.

In our population-based study, we estimated an SLE incidence of 4.77 per 100 000 over the study period (1976–2018) and a prevalence of 97.4 per 100 000 on 1 January 2015. Our study showed that the incidence and prevalence of SLE have increased over the decades particularly in men. In our analysis of incidence by race/ethnicity (1999–2018), the incidence of SLE was higher in racial and ethnic minority population than non-Hispanic and has increased more rapidly in the general population than in the non-Hispanic white population only. While the latter did not reach statistical significance due to the rarity of SLE, it suggests that the increased incidence of SLE in recent decades may be driven at least in part due to the increased racial and ethnic diversity of the population in the same time period. During the study period, Olmsted County's minority population went from <1% in the 1970s to ~21% in the most recent census.[12 13] There was no evidence that the severity of SLE has decreased over time. Our study also showed that the mortality of SLE has remained persistently elevated, with no improvement in SMR compared with the general population.

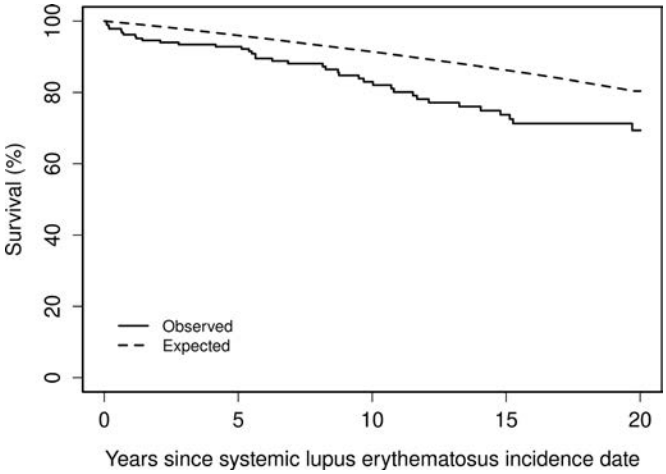

Figure 4 Overall survival among patients diagnosed with systemic lupus erythematosus residing in Olmsted County, Minnesota in 1976–2018.

Earlier reports by the CDC showed an SLE incidence rate of 4.6–7.4/100 000, using the ACR97 criteria.[14–18] Our study using the EULAR/ACR criteria had a similar overall incidence, however, the ascertainment period of the previous CDC registries was 3 years compared with the 43 years of the current study. In addition, the demographics of the populations of each registry are different.

We are aware of only one prior study in the USA that explored long-term SLE epidemiology. This study was performed in Rochester, Minnesota (a city within Olmsted county) and compared the incidence trends from 1980 to 1992 and those from an earlier study (1950–1979). In that study, the incidence increased from 1.51 to 5.56/100 000.[19] The study predates the development of ACR classification criteria, the widespread adoption of lupus erythematosus (LE) cell, ANA and specific autoantibody testing, which may explain the very low incidence in the 1950s. Our study started in 1976 when the use of ANA, complement and anti-ds DNA was already available in clinical practice.

Several studies, predominantly in Europe, have examined SLE incidence trends. Studies performed in the UK using the UK General Practice Research Database have had discrepant results; from 1990 to 1999, there were no changes in the SLE incidence, however, from 1999 to 2012 they did observe a decline of 1.8% per year.[20 21] These studies used different methodologies and the Read codes used for these studies were not validated against SLE classification criteria or physician diagnosis. Studies done in Denmark and Norway showed stable incidence over 8 and 10 years, respectively.[22 23] Southern European countries have had discrepant results. While a study from Crete, Greece reported an increase in SLE from 1999 to 2010 and then stabilisation from 2010 to 2013, studies from Lugo, Spain showed that from 1987 to 2006 there was an initial increase in the incidence in the earlier years of the study and decrease in the latter portion, without an overall trend.[24 25] A report from South Korea showed a decrease in incidence from 2005 to 2015.[26]

Our study showed that over a period of 43 years, the incidence of SLE increased 2% per year. Furthermore, this increase was observed both in women and men and across age groups. In contrast to prior studies, our study was able to capture long-term trends. By reviewing more than four decades of data from a population with access to exhaustive medical records (including autopsies), we were able to fully capture all SLE cases occurring during this period. Our finding agrees with what has been noted in the other registries, that the incidence of SLE is higher in racial and ethnic minorities than in non-Hispanic whites, suggesting that the increasing racial and ethnic diversity in Olmsted County which became more pronounced in the last two decades is responsible at least in part for the increase in the incidence of SLE. This last point is of relevance in the USA. The most recent census showed that from 2010 to 2020 the proportion of non-Hispanic whites decreased from 63.7% to 57.8% in 2020.[27] This trend has persisted for decades and is likely going to continue, since the population under age 15 is even more diverse than the general population.[28] Thus, it is possible that as the US population becomes more diverse, the incidence of SLE will continue increasing.

We observed an increase in SLE prevalence from 30.65 in 1985 to 97.4/100 000 in 2015. Our study agrees with multiple prior reports showing that the prevalence of SLE is rising.[29] This can be explained at least in part due to improved survival. While our study did not show improvement in mortality rates, perhaps due to the small number of events, a meta-analysis of SLE survival studies showed improvement in mortality up until the mid-1990s, these gains in survival in recent decades might be

Table 4 Mortality rate and standardised mortality ratios (SMRs) for 188 patients with incident systemic lupus erythematosus (SLE), by decade and by age group, Olmsted County, Minnesota (1976–2018)

	Number of deaths	Expected number of deaths	SMR (95% CI)	10-year mortality rate (%) (95% CI)
Decade				
1976–1988	15	8.38	1.79 (1.0 to 2.95)	12.2 (0.0 to 24.3)
1989–1998	10	3.33	3.00 (1.44 to 5.52)	16.9 (2.2 to 29.5)
1999–2008	11	5.67	1.94 (0.97 to 3.47)	13.7 (3.7 to 22.6)
2009–2018	11	3.80	2.89 (1.44 to 5.18)	24.4 (7.5 to 38.3)
Total (1976–2018)	47	21.18	2.22 (1.63 to 2.96)	17.0 (10.7 to 22.9)
Age				
<40	7	2.02	3.46 (1.39 to 7.14)	4.6 (0.0 to 9.7)
≥40	40	19.16	2.09 (1.49 to 2.84)	25.3 (15.5 to 34)

driving the rise in prevalence of SLE.[30] We did not find evidence of ascertainment bias that could explain the increases in SLE prevalence. Our data show that the gains in survival in SLE have not been enough to keep up with the gains in the general population or to close the survival gap.

Our study has several strengths. We ascertained cases for over 40 years, allowing us to provide a unique description of how the epidemiology of SLE is changing in the long-term. Through the record-linkage system of the REP, we identified all cases of SLE in a dynamic population that has been extensively characterised since 1966. We ascertained SLE cases using the newly endorsed EULAR/ACR classification criteria through detailed medical record review, thus minimising misclassification.

Our study also has limitations. First, being a retrospective study, we relied on the completeness of medical record documentation and workup to ascertain SLE cases. Second, aPL started to be tested in the 1980s. aPL only contributes two points of the total EULAR/ACR score and the impact in the incidence and prevalent estimate is minimal. We minimised the lack of aPL testing in the earlier decades by attributing the potential two points related to aPL to those patients who had a false positive serologic test for syphilis. Third, the EULAR/ACR score requires that the patients have a positive ANA, potentially missing ANA-negative lupus; however, in a prior study we showed that the EULAR/ACR criteria performed better in epidemiologic studies than other criteria.[6] In addition, in the SLICC cohort only 4% of their patients were ANA negative at a titre of 1:80, therefore is unlikely this would have an impact on our estimates.[31] Fourth, our data are based on a single US county, so differences in population distributions by age and race/ethnicity may not be generalisable, particularly to US regions that are highly diverse. Extrapolation of our results to other populations should be done with caution. Fifth, our study may be underpowered to detect subtle improvements in mortality trends.

In conclusion, the results of this population-based study showed that in this US population the incidence and prevalence of SLE is increasing. The increase in incidence may be at least partially explained by the increased ethnic and racial diversity in the population. The survival gap between SLE and the general population remains unchanged. As the US population grows more diverse, we might continue to see an increase in the incidence of SLE.

Author affiliations
[1]Division of Rheumatology, Mayo Clinic, Rochester, Minnesota, USA
[2]Robert D. and Patricia E. Kern Center for the Science of Health Care Delivery, Mayo Clinic, Rochester, Minnesota, USA
[3]Department of Medicine, University of Maryland Medical Center Midtown Campus, Baltimore, Maryland, USA
[4]Department of Quantitative Health Sciences, Mayo Clinic, Rochester, New York, USA
[5]Division of Clinical Immunology and Rheumatology, Department of Medicine, University of Alabama at Birmingham, Birmingham, Alabama, USA
[6]School of Medicine, Universidad Peruana Cayetano Heredia, Lima, Peru
[7]Centers for Disease Control and Prevention, Atlanta, Georgia, USA

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Contributors All authors were involved in drafting the article or revising it critically for important intellectual content and all authors approved the final version to be submitted for publication. AD-G and CSC had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. AD-G, CSC contributed to study conception and design. MH, MV-A, S-AO-O, JD, AS-R contributed to acquisition of data. AD-G, MH, SQD, REG, HL, GSA, CGH, CSC contributed to analysis and interpretation of data. AD-G and CSC are guarantors.

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Patient consent for publication Not applicable.

Ethics approval The study was approved by the institutional review boards of the Mayo Clinic (20-006485) and Olmsted Medical Center (036-OMC-20).

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ORCID iDs
Alí Duarte-García http://orcid.org/0000-0003-1749-5719
Jesse Y Dabit http://orcid.org/0000-0003-3150-9603
Graciela S Alarcón http://orcid.org/0000-0001-5190-9175
Cynthia S Crowson http://orcid.org/0000-0001-5847-7475

REFERENCES

1 Izmirly PM, Parton H, Wang L, *et al*. Prevalence of systemic lupus erythematosus in the United States: estimates from a meta-analysis of the centers for disease control and prevention national lupus registries. *Arthritis Rheumatol* 2021;73:991–6.

2 St Sauver JL, Grossardt BR, Yawn BP, *et al*. Use of a medical records linkage system to enumerate a dynamic population over time: the Rochester epidemiology project. *Am J Epidemiol* 2011;173:1059–68.
3 St Sauver JL, Grossardt BR, Yawn BP, *et al*. Data resource profile: the Rochester epidemiology project (Rep) medical records-linkage system. *Int J Epidemiol* 2012;41:1614–24.
4 St Sauver JL, Grossardt BR, Leibson CL, *et al*. Generalizability of epidemiological findings and public health decisions: an illustration from the Rochester epidemiology project. *Mayo Clin Proc* 2012;87:151–60.
5 Population of Olmsted County, Minnesota. Census 2010 and 2000 interactive map, demographics, statistics, graphs, quick facts. Available: http://censusviewer.com/county/MN/Olmsted
6 Duarte-García A, Hocaoglu M, Osei-Onomah S-A, *et al*. Population-Based incidence and time to classification of systemic lupus erythematosus by three different classification criteria. *Rheumatology* 2021. doi:10.1093/rheumatology/keab807. [Epub ahead of print: 29 Oct 2021].
7 Aringer M, Costenbader K, Daikh D, *et al*. 2019 European League against Rheumatism/American College of rheumatology classification criteria for systemic lupus erythematosus. *Arthritis Rheumatol* 2019;71:1400–12.
8 Klein RJ, Schoenborn CA. Age adjustment using the 2000 projected U.S. population. *Healthy People 2010 Stat Notes* 2001:1–10.
9 Whittall Garcia LP, Gladman DD, Urowitz M, *et al*. New EULAR/ACR 2019 SLE classification criteria: defining ominosity in SLE. *Ann Rheum Dis* 2021;80:767–74.
10 Gladman DD, Ibañez D, Urowitz MB. Systemic lupus erythematosus disease activity index 2000. *J Rheumatol* 2002;29:288–91.
11 FitzGerald JD, Grossman JM. Validity and reliability of retrospective assessment of disease activity and flare in observational cohorts of lupus patients. *Lupus* 1999;8:638–44.
12 United States census bureau. Available: https://www.census.gov/quickfacts/olmstedcountyminnesota [Accessed 26 Jan 2022].
13 Rochester epidemiology project population overview. Available: https://rochesterproject.org/for-researchers/population-overview/ [Accessed 26 Jan 2022].
14 Dall'Era M, Cisternas MG, Snipes K, *et al*. The incidence and prevalence of systemic lupus erythematosus in San Francisco County, California: the California lupus surveillance project. *Arthritis Rheumatol* 2017;69:1996–2005.
15 Ferucci ED, Johnston JM, Gaddy JR, *et al*. Prevalence and incidence of systemic lupus erythematosus in a population-based registry of American Indian and Alaska native people, 2007-2009. *Arthritis Rheumatol* 2014;66:2494–502.
16 Izmirly PM, Wan I, Sahl S, *et al*. The incidence and prevalence of systemic lupus erythematosus in New York County (Manhattan), New York: the Manhattan lupus surveillance program. *Arthritis Rheumatol* 2017;69:2006–17.
17 Lim SS, Bayakly AR, Helmick CG, *et al*. The incidence and prevalence of systemic lupus erythematosus, 2002-2004: the Georgia lupus registry. *Arthritis Rheumatol* 2014;66:357–68.
18 Somers EC, Marder W, Cagnoli P, *et al*. Population-Based incidence and prevalence of systemic lupus erythematosus: the Michigan lupus epidemiology and surveillance program. *Arthritis Rheumatol* 2014;66:369–78.
19 Uramoto KM, Michet CJ, Thumboo J, *et al*. Trends in the incidence and mortality of systemic lupus erythematosus, 1950-1992. *Arthritis Rheum* 1999;42:46–50.
20 Somers EC, Thomas SL, Smeeth L, *et al*. Incidence of systemic lupus erythematosus in the United Kingdom, 1990-1999. *Arthritis Rheum* 2007;57:612–8.
21 Rees F, Doherty M, Grainge M, *et al*. The incidence and prevalence of systemic lupus erythematosus in the UK, 1999-2012. *Ann Rheum Dis* 2016;75:136–41.
22 Laustrup H, Voss A, Green A, *et al*. Occurrence of systemic lupus erythematosus in a Danish community: an 8-year prospective study. *Scand J Rheumatol* 2009;38:128–32.
23 Lerang K, Gilboe I, Garen T, *et al*. High incidence and prevalence of systemic lupus erythematosus in Norway. *Lupus* 2012;21:1362–9.
24 Gergianaki I, Fanouriakis A, Repa A, *et al*. Epidemiology and burden of systemic lupus erythematosus in a southern European population: data from the community-based lupus Registry of Crete, Greece. *Ann Rheum Dis* 2017;76:1992–2000.
25 Alonso MD, Llorca J, Martinez-Vazquez F, *et al*. Systemic lupus erythematosus in northwestern Spain: a 20-year epidemiologic study. *Medicine* 2011;90:350–8.
26 Bae EH, Lim SY, Han K-D, *et al*. Trend of prevalence and incidence of systemic lupus erythematosus in South Korea, 2005 to 2015: a nationwide population-based study. *Korean J Intern Med* 2020;35:652–61.
27 Jensen EJN, Rabe M, Pratt B. The chance that two people chosen at random are of different race or ethnicity groups has increased since, 2010. Available: https://www.census.gov/library/stories/2021/08/2020-united-states-population-more-racially-ethnically-diverse-than-2010.html
28 Fey WH. Declines in white youth population are countered by gains in other racial groups, 2019. Available: https://www.brookings.edu/research/less-than-half-of-us-children-under-15-are-white-census-shows/ [Accessed 24 Sep 2021].
29 Barber MRW, Drenkard C, Falasinnu T, *et al*. Global epidemiology of systemic lupus erythematosus. *Nat Rev Rheumatol* 2021;17:515–32.
30 Tektonidou MG, Lewandowski LB, Hu J, *et al*. Survival in adults and children with systemic lupus erythematosus: a systematic review and Bayesian meta-analysis of studies from 1950 to 2016. *Ann Rheum Dis* 2017;76:2009–16.
31 Choi MY, Clarke AE, St Pierre Y, *et al*. Antinuclear antibody-negative systemic lupus erythematosus in an international inception cohort. *Arthritis Care Res* 2019;71:893–902.

CLINICAL SCIENCE

Mechanism of action of baricitinib and identification of biomarkers and key immune pathways in patients with active systemic lupus erythematosus

Thomas Dörner ,[1] Yoshiya Tanaka ,[2] Ernst R Dow,[3] Alisa E Koch,[3] Maria Silk,[3] Jorge A Ross Terres,[3] Jonathan T Sims,[3] Zhe Sun,[3] Inmaculada de la Torre,[3] Michelle Petri [4]

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[1]Department of Medicine and Department of Rheumatology and Clinical Immunology, Charite Universitatsmedizin Berlin and Deutsches Rheumaforschungszentrum (DRFZ), Berlin, Germany
[2]First Department of Internal Medicine, University of Occupational and Environmental Health, Kitakyushu, Japan
[3]Eli Lilly and Company, Indianapolis, Indiana, USA
[4]Division of Rheumatology, Johns Hopkins University, Baltimore, Maryland, USA

Correspondence to
Professor Thomas Dörner, Charite Universitatsmedizin Berlin, Berlin, Berlin, Germany; thomas.doerner@charite.de

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ABSTRACT

Objectives To elucidate the mechanism of action of baricitinib, a Janus kinase (JAK) 1/2 inhibitor, and describe immunological pathways related to disease activity in adults with systemic lupus erythematosus (SLE) receiving standard background therapy in a phase II trial.

Methods Patients with SLE were treated with baricitinib 2 mg or 4 mg in a phase II randomised, placebo-controlled study. Sera from 239 patients (baricitinib 2 mg: n=88; baricitinib 4 mg: n=82; placebo: n=69) and 49 healthy controls (HCs) were collected at baseline and week 12 and analysed using a proximity extension assay (Target 96 Inflammation Panel (Olink)). Interferon (IFN) scores were determined using an mRNA panel. Analytes were compared in patients with SLE versus HCs and in changes from baseline at week 12 between baricitinib 2 mg, 4 mg and placebo groups using a restricted maximum likelihood-based mixed models for repeated measures. Spearman correlations were computed for analytes and clinical measurements.

Results At baseline, SLE sera had strong cytokine dysregulation relative to HC sera. C-C motif chemokine ligand (CCL) 19, C-X-C motif chemokine ligand (CXCL) 10, tumour necrosis factor alpha (TNF-α), TNF receptor superfamily member (TNFRSF)9/CD137, PD-L1, IL-6 and IL-12β were significantly reduced in patients treated with baricitinib 4 mg versus placebo at week 12. Inflammatory biomarkers indicated correlations/associations with type I IFN (CCL19, CXCL10, TNF-α and PD-L1), anti-double stranded DNA (dsDNA) (TNF-α, CXCL10) and Systemic Lupus Erythematosus Disease Activity Index-2000, tender and swollen joint count and worst joint pain (CCL19, IL-6 and TNFRSF9/CD137).

Conclusion These results suggest that baricitinib 4 mg downregulated key cytokines that are upregulated in patients with SLE and may play a role in a multitargeted mechanism beyond the IFN signature although clinical relevance remains to be further delineated.

Trial registration number NCT02708095.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Systemic lupus erythematosus (SLE) is made complex by various immunological abnormalities contributing to SLE pathogenesis.
⇒ In a phase II study of baricitinib in patients with SLE, daily oral baricitinib 4 mg in conjunction with current standard of care (SOC) was superior to placebo plus SOC in improving SLE disease activity at week 24.

WHAT THIS STUDY ADDS

⇒ Insights into the mechanism of action of baricitinib in SLE.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE AND OR/POLICY

⇒ The analysis presented here advances the understanding of how baricitinib may act on clinically relevant pathways in patients with SLE.
⇒ These results also serve to elucidate potential new biological targets that may impact SLE pathogenesis.

INTRODUCTION

The clinical complexity of patients with systemic lupus erythematosus (SLE) is a reflection of various immunological abnormalities contributing to SLE pathogenesis, including dysregulation of both the innate and adaptive immune responses, leading to the breakdown of tolerance, production of autoantibodies, deposition of immune complexes in tissues, leading to the activation of complement and the accumulation of neutrophils, monocytes and self-reactive T and B-lymphocytes.[1–3] Research into the pathogenesis of SLE offers a nexus of gene expression, cell signalling and cellular responses that can present with different degrees of dysregulation among patients with SLE. Key cytokines in SLE comprise, among others, type I interferon (IFN),[4] type II IFN, IL-6, IL-12/23, IL-17 and B lymphocyte stimulator (BAFF/BlyS)[5] representing the clinical and molecular heterogeneity of SLE. Abnormalities include alterations in the expression of IFN inducible chemokines, such as C-X-C motif chemokine ligand (CXCL) 10 and C-C motif chemokine ligand (CCL) 19 (which have been shown to correlate with disease activity),[6] alterations in B cell receptor signalling and alterations in the expression of cytokines related to leucocyte, neutrophil and macrophage trafficking, such as IL-6 and others.[3 7–9]

In a phase II study of baricitinib in patients with SLE, daily oral baricitinib 4 mg in conjunction with current standard of care (SOC) was superior to placebo plus SOC in improving SLE disease activity

at week 24.[10] Microarray analysis on serum samples in this study cohort found that treatment with baricitinib 4 mg significantly reduced the RNA expression of a network of genes functionally interconnected in SLE (signal transducer and activator of transcription (STAT)1, STAT2 and STAT4-target genes and multiple IFN responsive genes). Furthermore, baricitinib downregulated cytokine signalling associated with SLE pathogenesis and the Janus kinase (JAK)/STAT pathway, such as IL-6 and IL-12.[11] While recent phase III trials have shown inconclusive results for the efficacy of baricitinib to treat moderate to severe SLE adult patients, the objective of this study was to evaluate the pharmacodynamic effect of baricitinib on a broad and highly sensitive array of serum cytokines in patients with SLE and to characterise immune pathways involved in the mechanism of action of baricitinib.

METHODS

Study design

Patient samples were obtained from the double-blind, multicentre, randomised, placebo-controlled, 24-week phase II clinical trial, I4V-MC-JAHH.[10] Eligible patients were aged 18 years or older and had a diagnosis of SLE. At baseline, patients were required to have a positive antinuclear antibody, or a positive anti-dsDNA, arthritis or rash as defined by Systemic Lupus Erythematosus Disease Activity Index-2000 (SLEDAI-2K) and a clinical SLEDAI-2K score of ≥4. Study drug was added to existing stable background SOC therapy, which could include corticosteroids up to 20 mg/day of prednisone or equivalent, a single antimalarial, a single immunosuppressant and/or nonsteroidal anti-inflammatory drugs. Tapering of corticosteroids was permitted from baseline to week 16. Active central nervous system SLE or active severe SLE nephritis were not permitted.

This study was done in accordance with the ethical principles of the Declaration of Helsinki and Good Clinical Practise guidelines. All investigation sites received approval from the appropriate authorised institutional review board or ethics committee. All patients provided written consent before the study-related procedures were carried out.

Randomisation and masking

Patients were allocated (1:1:1) using a computer-generated random sequence to baricitinib 2 mg, baricitinib 4 mg or placebo plus SOC. Patients were stratified according to disease activity (SLEDAI-2K score <10 or ≥10), anti-dsDNA status (positive or negative) and region (USA, Europe, Asia or rest of the world). Investigators and patients were masked to allocation.

Serum cytokine quantification (proximity extension analysis)

Serum samples from 239 patients (baricitinib 2 mg: n=88; baricitinib 4 mg: n=82; placebo: n=69) were analysed with the Olink Inflammation I (95302) multiplex proximity extension assay (PEA) technology (Uppsala, Sweden) according to manufacturer's specifications. The levels of analyte-specific DNA amplicons for 92 soluble analytes were quantified for each patient on the Fluidigm Biomark HD (San Francisco, California) at baseline and week 12. Serum samples from 49 age/sex-matched healthy controls (HCs) were included for comparisons to baseline SLE samples.

Statistical analyses

Comparisons between patients with SLE and HCs as well as those treated either with baricitinib 2 mg, baricitinib 4 mg or placebo plus SOC were made using a mixed-effect repeated measure model with an unstructured variance–covariance matrix and $\log_2$ transformed relative protein expression as the response. The lme4 function in R V.4.0.3 with fixed effect, covariates of sex, batch and corticosteroid use at baseline were used to fit the model.

A total of four different contrasts were tested

1. Patients with SLE compared with HCs at baseline.
2. Changes from baseline to week 12 compared between baricitinib 4 mg versus placebo plus SOC.
3. Changes from baseline to week 12 compared between baricitinib 2 mg versus placebo plus SOC.
4. Changes from baseline to week 12 compared between baricitinib 4 mg treatment versus baricitinib 2 mg treatment.

For within-protein multiplicity adjustment, the glht function[12] was applied to all comparisons. The threshold of adjusted p value generated from within-protein multiplicity control to identify statistically significant proteins was 0.0237. For between-protein multiplicity adjustment, the q value was calculated with the smallest within-protein p value using a false discovery rate threshold set to 0.05. Spearman correlation was applied to key continuous clinical outcomes and protein expression levels with Benjamini-Hochberg multiple comparison adjustment to report the correlation values and adjusted p values. We used 0.05 as the cut-off value to determine the statistical significance of correlation coefficients.

Clinical correlations

IFN signature

Score was assessed previously using a validated mRNA panel.[13]

Anti-dsDNA serum levels

Serum samples were analysed for changes from baseline over time for anti-dsDNA antibodies using INOVA QUANTA Lite SC ELISA (INOVA Diagnostics, San Diego, California).[10]

SLEDAI-2K

The SLEDAI-2K[14] is a validated global disease activity instrument that focuses on high-impact disease manifestations across nine organ systems. It includes 24 clinical and laboratory variables with manifestations graded by the affected organ system.

Worst joint pain

Worst joint pain was measured using the Brief Pain Inventory (short form) (BPI-sf)-modified worst joint pain item, which is a self-administered question developed for the rapid assessment of pain intensity. Worst joint pain item asks patients to rate their pain at its worst over the past 7 days.[15]

28 swollen and tender joint count

The 28 joints examined and assessed as tender or not tender for tender joint count and as swollen or not swollen for swollen joint count include 14 joints on each side of the patient's body.[16]

Patient and public involvement statement

Patients were not involved in the study design.

RESULTS

Analyte abnormalities in patients with SLE at baseline characterise cytokine dysregulation

Cytokine levels were analysed at baseline in patients with SLE and compared with HCs. Of the 92 detectable analytes measured, 17 were significantly upregulated (table 1) and 9

Table 1 Upregulated analytes in patients with SLE versus HCs at baseline

Analytes	Patients with SLE vs HCs at baseline		
	FC	P value	Adjusted p value
CCL19	2.4	<0.001	<0.001
CXCL10	2.1	<0.001	<0.001
CXCL9	1.8	<0.001	<0.001
CCL20	1.7	<0.001	0.001
IL-10	1.7	<0.001	<0.001
TNF-α	1.6	<0.001	<0.001
CXCL11	1.6	0.001	0.003
IL-6	1.5	0.003	0.011
CDCP1	1.4	<0.001	<0.001
CCL3	1.4	0.005	0.016
IL-12β	1.4	0.005	0.018
TNFRSF9	1.3	<0.001	0.001
CCL2	1.3	0.005	0.019
IL-17A	1.3	0.001	0.004
CCL28	1.2	0.001	0.003
PD-L1	1.2	<0.001	0.001
GDNF	1.2	0.002	0.008

An adjusted p value of 0.0237 was used as the cut-off for within-protein significance (see Statistical analyses section in Methods).
CCL2, C-C motif chemokine ligand 2; CCL3, C-C motif chemokine ligand 3; CCL19, C-C motif chemokine ligand 19; CCL20, C-C motif chemokine ligand 20; CCL28, C-C motif chemokine ligand 28; CDCP1, membrane glycoprotein gp140; CXCL9, C-X-C motif chemokine ligand 9; CXCL10, C-X-C motif chemokine ligand 10; CXCL11, C-X-C motif chemokine ligand 11; FC, fold change; GDNF, glial cell derived neurotrophic factor; HC, healthy control; IL-6, interleukin-6; IL-10, interleukin-10; IL-17A, interleukin-17A; IL-12β, interleukin-12 beta chain; PD-L1, PDCD1 ligand 1; SLE, systemic lupus erythematosus; TNFRSF9 (soluble), TNF receptor superfamily member 9; TNF-α, tumour necrosis factor alpha.

were downregulated in patients with SLE (online supplemental table S1). Of note, several chemokines such as CCL19, CXCL10, CXCL9, CCL2 and CCL20 and proinflammatory cytokines, such as IL-6, IL-12, IL-17A, were increased in patients with SLE versus HCs that, in addition to the increased PD-L1 and IL-10, indicate abnormalities of chronic SLE immunity.

Baricitinib modulates disturbances of cytokine networks in SLE

Treatment with baricitinib 4 mg significantly reduced the serum expression levels of 7 of the 17 initially increased analytes relative to placebo plus SOC in patients with SLE at 12 weeks (table 2) among others (online supplemental table S2).

Baricitinib 4 mg treatment specifically and significantly downregulated serum cytokines that mediate lymphocyte and monocyte/macrophage recruitment such as chemokine (C-C motif) ligand 19 (CCL19), IFN-γ-induced proteins such as CXCL10, tumour necrosis factor (TNF) receptor superfamily member 9 (TNFRSF9) and TNF alpha (TNF-α) as well as IL-12β and IL-6 expression levels compared with placebo plus SOC at 12 weeks (table 2). Beyond direction of immune cells towards inflammatory sites, these molecules are relevant for B–T lymphocyte interactions (ie, PD-L1) confirming previous findings using multiplex cytokine panel quantitative (Quanterix) assays.[11]

Less pronounced decreases in similar cytokines were observed with baricitinib 4 mg versus 2 mg (online supplemental table S3). Of note, three analytes not typically associated with IFN signalling, TRANCE/CD254, TNFRSF9 and TNF-α, were reduced in the baricitinib 4 mg treatment group versus 2 mg treatment group.

Table 2 Analytes upregulated in patients with SLE and downregulated by treatment with baricitinib 4 mg relative to placebo plus SOC at week 12

Analytes	Baricitinib 4 mg vs placebo plus SOC at week 12		
	FC	P value	Adjusted p value
CCL19	−1.8	<0.001	<0.001
IL-6	−1.5	<0.001	0.002
TNFRSF9	−1.4	<0.001	<0.001
CXCL10	−1.3	0.003	0.011
IL-12β	−1.3	<0.001	<0.001
TNF-α	−1.2	<0.001	<0.001
PD-L1	−1.2	<0.001	<0.001

An adjusted p value of 0.0237 was used as the cut-off for within-protein significance (see Statistical analyses section in Methods).
CCL19, C-C motif chemokine ligand 19; CXCL10, C-X-C motif chemokine ligand 10; FC, fold change; IL-6, interleukin-6; IL-12β, interleukin-12 beta chain; PD-L1, PDCD1 ligand 1; SLE, systemic lupus erythematosus; SOC, standard of care; TNFRSF9, TNF receptor superfamily member 9; TNF-α, tumour necrosis factor alpha.

On the other hand, only three analytes increased significantly (NT-3, SCF and CXCL5) (online supplemental table S2) under treatment with baricitinib 4 mg versus placebo plus SOC in patients with SLE, but not between the two baricitinib treatment groups (online supplemental table S3).

These results suggest that treatment with baricitinib 4 mg might mediate changes within the inflammatory JAK/STAT cytokine network beyond the IFN signature, considered a key molecular signature in SLE.

Clinical correlates and cytokine changes with baricitinib treatment in SLE

Certain cytokines downregulated by treatment with baricitinib 4 mg correlated with the IFN signature. The most representative ones were CCL19, CXCL10, TNF-α and soluble PD-L1/CD274 (figure 1). Correlation analysis identified a relationship between the observed cytokine/chemokine changes and clinical and serologic measures of SLE activity, including anti-dsDNA production (figure 2). Interestingly, in addition to positive correlations with the IFN signature, there was a significant positive correlation of CCL19 with SLEDAI-2K (figure 2). Significant positive correlations were also found between TNFRSF9 levels and swollen and tender joint counts and between IL-6 levels and worst joint pain (figure 2).

These data indicate that changes in cytokine expression in patients with SLE treated with baricitinib might be relevant to clinical outcome measures. Furthermore, although weak, the positive correlations seen between key cytokines and SLEDAI-2K, joint parameters and anti-dsDNA production suggest that the underlying mechanisms of cytokine modulation can exert an effect on joint pathology in patients with SLE as well as inhibit the B cell activation that results in antibody production. Further clinical evidence will be needed to confirm these observations.

DISCUSSION

The goal of this study was to further understand SLE immunopathogenesis and elucidate how baricitinib might act by identifying key cytokines significantly downregulated after treatment and their potential correlations with clinical outcomes. First, we wanted to investigate any functional regulation of cytokines by baricitinib in patients with SLE, particularly any relating to

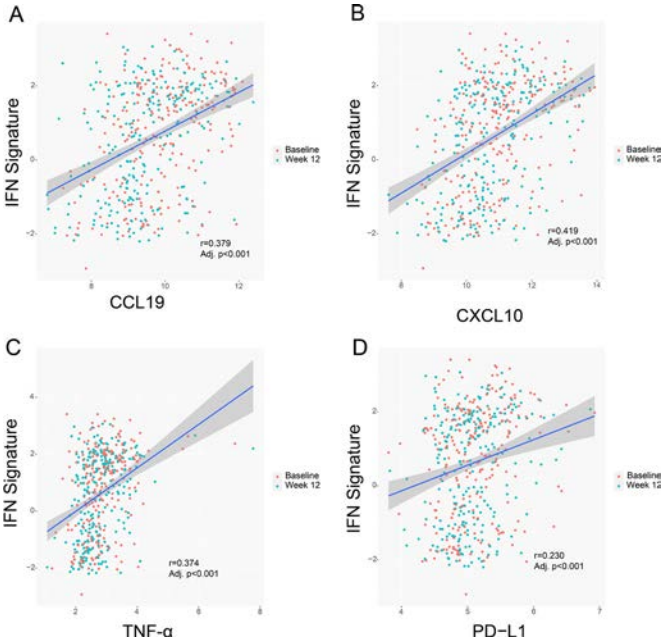

Figure 1 Correlation between key analytes and the IFN signature. Line of regression and confidence intervals are shown for CCL19 (A), CXCL10 (B), TNF-α (C), and PD-L1 (D) and the IFN signature. CCL19, C-C motif chemokine ligand 19; CXCL10, C-X-C motif chemokine ligand 10; IFN, interferon; PD-L1, PDCD1 ligand 1; TNF-α, tumour necrosis factor alpha.

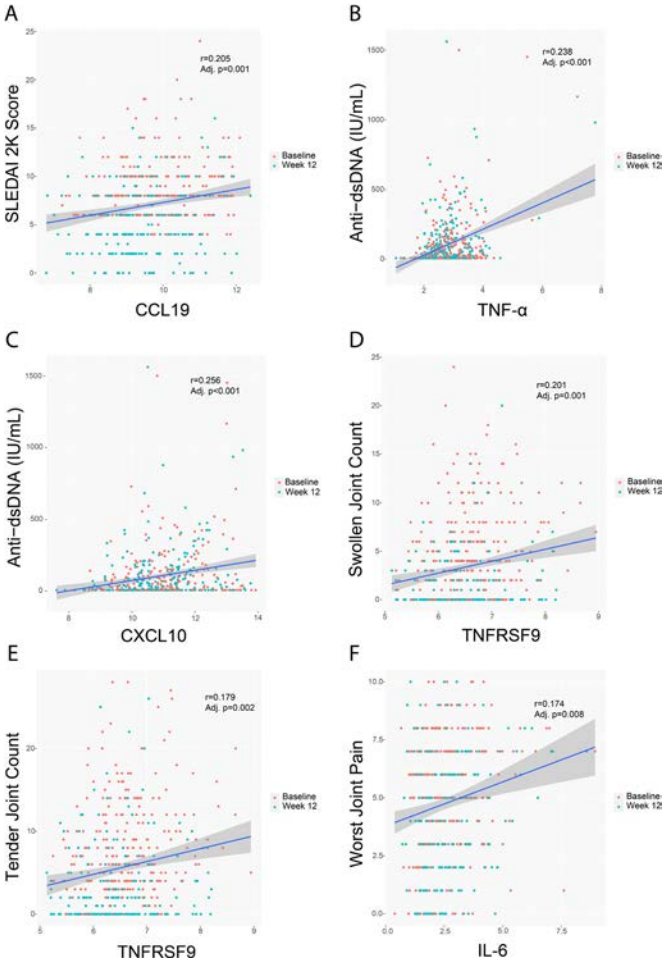

Figure 2 Correlation between key analytes and clinical measurements at baseline and week 12. Line of regression and CIs are shown for CCL19 and SLEDAI-2K (A), TNF-α (B) and CXCL10 (C) and anti-dsDNA expression levels, TNFRSF9 and swollen (D) and tender (E) joint count, and between IL-6 and worst joint pain (F). CCL19, C-C motif chemokine ligand 19; CXCL10, C-X-C motif chemokine ligand 10; TNF-α, tumour necrosis factor alpha; IL-6, interleukin-6; TNFRSF9, TNF receptor superfamily member 9.

previously identified gene associated changes[11] and, second, we wanted to build on the original analysis using a multitargeted inflammatory panel with sensitivity to investigate a wider range of potential therapeutic targets of baricitinib. Such PEAs have been previously used to identify, at the protein level and as such more close to functional consequences than mRNA transcripts, key analytes deregulated and their correlation with clinical outcomes and organ damage in SLE.[17–20]

Specifically, for our study, as previously shown, baricitinib treatment reduced the mRNA expression of functionally interconnected genes involved in SLE including STAT1, STAT2 and STAT4-targeting genes as well as multiple IFN responsive genes. Baricitinib also reduced serum levels of two key cytokines implicated in SLE pathogenesis, IL-6 and IL-12β.[11] Expanding on these preliminary findings, in the new analyses presented here, we detected seven analytes that were significantly elevated at baseline in patients with SLE compared with HCs and significantly reduced at week 12 after treatment with baricitinib 4 mg, including the previously identified IL-6 and IL-12β as well as other cytokines not typically associated with JAK-STAT signalling such as CXCL10, CCL19, TNFRSF9, TNF-α and PD-L1.

A diversity of IFN-regulated cytokines is elevated in the serum of patients with SLE versus HCs, in particular CXCL10 and CCL19, which have been shown to correlate with SLE disease severity and flares.[6] In the analysis presented here, both CXCL10 and CCL19 were significantly downregulated by baricitinib 4 mg and were positively correlated with the IFN signature, anti-dsDNA titre and SLEDAI-2K overall disease activity. These findings, in addition to the effect on TNF superfamily members such as TNFRSF9, imply an indirect inhibition by baricitinib, and a potential role in lymphocyte migration, rather than only cellular activation and differentiation. Overall, these results suggest that lymphocyte recruitment, and migration into lymphoid organs and peripheral tissues, might be a unique potential mechanism of the action of baricitinib.

The patient population used in this analysis was primarily moderately active SLE patients, with a large representation of musculoskeletal and mucocutaneous manifestations and was comparable to other recent phase II–III clinical trials.

While the analysis presented here supports previous findings that baricitinib's mechanism of action is partly mediated by its effects on the IFN signature, the other novel analytes identified are relevant to SLE immunopathogenic pathways such as B–T lymphocyte interactions, macrophage trafficking and signalling pathways linking the innate and adaptive arms of the immune system. The correlation between the expression of some of these molecules, like IL-6 and TNFRSF9, with clinical outcomes, in particular, joint manifestations and pain measures, expands not only our understanding of pathogenic mechanisms in SLE but also of potential response biomarkers.

Arthralgia in patients with SLE is mainly related to tenosynovitis as opposed to the erosion and joint destruction more common in rheumatoid and psoriatic arthritis.[21] The correlation analysis presented here found no apparent relationship with IL-17 (the IL-12/–23 axis) but a potential link to the IL-6 pathway. Prior studies in rheumatoid arthritis analysing changes

in cytokine production by B cells under IL-6R blockade[22] found that B cell cytokine production did not simply suppress the IL-6 inflammatory axis but restored certain cytokines and chemokines under treatment, confirming a significant but weak correlation between IL-6 blockade and the production of macrophage inflammatory proteins and β-nerve growth factor at baseline, both relevant for persistent synovitis and pain sensation in rheumatoid arthritis. There are clear molecular (cytokine) differences related to joint manifestations in rheumatoid arthritis versus SLE, and the pathogenic mechanisms that lead to joint manifestations likely differ between the two diseases and remain to be fully delineated. However, lack of erosive changes and preferential tendon involvement in SLE versus rheumatoid arthritis suggest subtle pathogenic differences. The role of IL-6 in both diseases may also be different in terms of IL-17 induction.

IL-6 was also positively correlated with worst joint pain. It has been shown that IL-6 is an important modulator of pain through mechanisms that influence pain signalling at the central nervous system level. Specifically, for baricitinib, such an impact on pain has been demonstrated to happen beyond its impact on inflammation[23] implying that, at least in part, baricitinib's effect on pain is uncoupled from its anti-inflammatory mechanics.

Here, treatment with baricitinib 4 mg downregulated a potential network of cytokines involved in lymphocyte and monocyte/macrophage recruitment with CCL19, IFN-γ-induced proteins such as CXCL10 as well as TNFRSF9, IL-6 and others (TNF-α, IL-12β, PD-L1). However, mechanistically their impact on disease may differ since only CCL19, CXCL10, TNF-α and PD-L1 correlated with the IFN signature. Although CCL19 was positively correlated with SLEDAI-2K score and CXCL10 with anti-dsDNA titres, IL-6 and TNFRSF9 correlated with joint pain as well as swollen and tender joints. These results confirm previously reported data,[11] and also broaden the evidence using an assay with sensitivity, extending the analysis at the protein level to additional IFN and non-IFN-related cytokines. The significant associations with clinical outcomes (although not at the highest level of correlation probably due to small sample size), in particular, for joint manifestations, pain and B/T cell activity, further expand our understanding of pathogenic mechanisms in SLE and identify potential response biomarkers for both systemic and organ-specific disease activity in patients with SLE.

However, recent inconclusive findings from two phase III clinical trials investigating the efficacy of baricitinib in patients with SLE (NCT03616912 and NCT03616964) pose a challenge to further interpret these biomarkers. A priori, this post hoc phase II analysis was powered for skin and joint manifestations but no other SLE domains (renal, haematology, central nervous system). Independent studies of the biomarkers identified by the current analysis will provide a rich resource to validate their impact comparing phase III responders and non-responders.

The downregulation of key cytokines and the observed correlations with clinical variables presented here indicate that treatment with baricitinib might be particularly effective in the subgroup of patients with high serological and disease activity (especially musculoskeletal manifestations). However, the recent inconclusive data from phase III trials warrant further analyses and consideration.

Of note, our study is limited to a preselected set of analytes (Olink INF I panel) and may have missed important analytes that are not part of the predefined inflammation assay panel. As well, while correlation analysis found the relationship between several analytes and clinical measures to be significant, the small number of patients from the phase II trial and the r values between 0.15 and 0.4 might further limit clinical relevance.

Despite the inconclusive results on the efficacy of baricitinib from the phase III trials, and the broad body of difficult to interpret literature already published on the topic of SLE pathogenesis and cytokine dysregulation, the analysis presented here remains relevant as it contributes to our understanding of the molecular pathways involved in SLE and the impact of baricitinib on immunological/cytokine signatures.

CONCLUSION

The analysis presented here advances the understanding of how baricitinib might act in patients with SLE by modulating multiple disease relevant proteins. However, based on inconclusive findings from the phase III trials, benefit of treatment might be limited. These results also serve to elucidate potential new biological targets that may impact SLE disease activity.

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Patient consent for publication Not applicable.

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Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Lilly provides access to all individual participant data collected during the trial, after anonymisation, with the exception of pharmacokinetic or genetic data. Data are available to request 6 months after the indication studied has been approved in the US and EU and after primary publication acceptance, whichever is later. No expiration date of data requests is currently set once data are made available. Access is provided after a proposal has been approved by an independent review committee identified for this purpose and after receipt of a signed data sharing agreement. Data and documents, including the study protocol, statistical analysis plan, clinical study report, blank or annotated case report forms, will be provided in a secure data sharing environment. For details on submitting a request, see the instructions provided at www.vivli.org.

ORCID iDs
Thomas Dörner http://orcid.org/0000-0002-6478-7725
Yoshiya Tanaka http://orcid.org/0000-0002-0807-7139
Michelle Petri http://orcid.org/0000-0003-1441-5373

REFERENCES

1 Mok CC, Lau CS. Pathogenesis of systemic lupus erythematosus. *J Clin Pathol* 2003;56:481–90.
2 Tsokos GC, Lo MS, Costa Reis P, *et al*. New insights into the immunopathogenesis of systemic lupus erythematosus. *Nat Rev Rheumatol* 2016;12:716–30.
3 Wahren-Herlenius M, Dörner T. Immunopathogenic mechanisms of systemic autoimmune disease. *Lancet* 2013;382:819–31.
4 Rönnblom L, Alm GV. A pivotal role for the natural interferon alpha-producing cells (plasmacytoid dendritic cells) in the pathogenesis of lupus. *J Exp Med* 2001;194:F59–64.
5 Scholz JL, Oropallo MA, Sindhava V, *et al*. The role of B lymphocyte stimulator in B cell biology: implications for the treatment of lupus. *Lupus* 2013;22:350–60.
6 Bauer JW, Petri M, Batliwalla FM, *et al*. Interferon-Regulated chemokines as biomarkers of systemic lupus erythematosus disease activity: a validation study. *Arthritis Rheum* 2009;60:3098–107.
7 Tackey E, Lipsky PE, Illei GG. Rationale for interleukin-6 blockade in systemic lupus erythematosus. *Lupus* 2004;13:339–43.
8 Hoffman RW, Merrill JT, Alarcón-Riquelme MME, *et al*. Gene expression and pharmacodynamic changes in 1,760 systemic lupus erythematosus patients from two phase III trials of BAFF blockade with tabalumab. *Arthritis Rheumatol* 2017;69:643–54.
9 Larosa M, Zen M, Gatto M, *et al*. Il-12 and IL-23/Th17 axis in systemic lupus erythematosus. *Exp Biol Med* 2019;244:42–51.
10 Wallace DJ, Furie RA, Tanaka Y, *et al*. Baricitinib for systemic lupus erythematosus: a double-blind, randomised, placebo-controlled, phase 2 trial. *Lancet* 2018;392:222–31.
11 Dörner T, Tanaka Y, Petri MA, *et al*. Baricitinib-associated changes in global gene expression during a 24-week phase II clinical systemic lupus erythematosus trial implicates a mechanism of action through multiple immune-related pathways. *Lupus Sci Med* 2020;7:e000424.
12 Hothorn T, Bretz F, Westfall P. Simultaneous inference in general parametric models. *Biom J* 2008;50:346–63.
13 Dörner T, Tanaka Y, Petri MA, *et al*. Baricitinib-associated changes in global gene expression during a 24-week phase II clinical systemic lupus erythematosus trial implicates a mechanism of action through multiple immune-related pathways. *Lupus Sci Med* 2020;7:e000424.
14 Gladman DD, Ibañez D, Urowitz MB. Systemic lupus erythematosus disease activity index 2000. *J Rheumatol* 2002;29:288–91.
15 Naegeli AN, Tomaszewski EL, Al Sawah S. Psychometric validation of the brief pain Inventory-Short form in patients with systemic lupus erythematosus in the United States. *Lupus* 2015;24:1377–83.
16 Siemons L, ten Klooster PM, Taal E, *et al*. Validating the 28-tender joint count using item response theory. *J Rheumatol* 2011;38:2557–64.
17 Wigren M, Svenungsson E, Mattisson IY, *et al*. Cardiovascular disease in systemic lupus erythematosus is associated with increased levels of biomarkers reflecting receptor-activated apoptosis. *Atherosclerosis* 2018;270:1–7.
18 Björk A, Da Silva Rodrigues R, Richardsdotter Andersson E, *et al*. Interferon activation status underlies higher antibody response to viral antigens in patients with systemic lupus erythematosus receiving no or light treatment. *Rheumatology* 2021;60:1445–55.
19 Carranza-León DA, Oeser A, Marton A, *et al*. Tissue sodium content in patients with systemic lupus erythematosus: association with disease activity and markers of inflammation. *Lupus* 2020;29:455–62.
20 Petrackova A, Smrzova A, Gajdos P, *et al*. Serum protein pattern associated with organ damage and lupus nephritis in systemic lupus erythematosus revealed by pea immunoassay. *Clin Proteomics* 2017;14:32.
21 Dörner T, Vital EM, Ohrndorf S, *et al*. A narrative literature review comparing the key features of musculoskeletal involvement in rheumatoid arthritis and systemic lupus erythematosus. *Rheumatol Ther* 2022. doi:10.1007/s40744-022-00442-z. [Epub ahead of print: 31 Mar 2022].
22 Fleischer S, Ries S, Shen P, *et al*. Anti-interleukin-6 signalling therapy rebalances the disrupted cytokine production of B cells from patients with active rheumatoid arthritis. *Eur J Immunol* 2018;48:194–203.
23 Taylor PC, Keystone EC, van der Heijde D, *et al*. Baricitinib versus placebo or adalimumab in rheumatoid arthritis. *N Engl J Med* 2017;376:652–62.

TRANSLATIONAL SCIENCE

Biological insights into systemic lupus erythematosus through an immune cell-specific transcriptome-wide association study

Xianyong Yin,[1,2,3,4,5,6] Kwangwoo Kim ,[7] Hiroyuki Suetsugu,[8,9,10] So-Young Bang,[11,12] Leilei Wen,[1,2] Masaru Koido,[9,13] Eunji Ha,[7] Lu Liu,[1,2] Yuma Sakamoto,[8,14] Sungsin Jo,[12] Rui-Xue Leng ,[15] Nao Otomo,[8,9,16] Young-Chang Kwon,[12] Yujun Sheng,[1,2] Nobuhiko Sugano ,[17] Mi Yeong Hwang,[18] Weiran Li,[1,2] Masaya Mukai,[19] Kyungheon Yoon,[18] Minglong Cai,[1,2] Kazuyoshi Ishigaki,[9,20,21,22] Won Tae Chung,[23] He Huang,[1,2] Daisuke Takahashi,[24] Shin-Seok Lee,[25] Mengwei Wang,[1,2] Kohei Karino,[26] Seung-Cheol Shim,[27] Xiaodong Zheng,[1,2] Tomoya Miyamura,[28] Young Mo Kang,[29] Dongqing Ye ,[15] Junichi Nakamura ,[30] Chang-Hee Suh,[31] Yuanjia Tang,[32] Goro Motomura,[10] Yong-Beom Park,[33] Huihua Ding ,[32] Takeshi Kuroda,[34] Jung-Yoon Choe,[35] Chengxu Li,[4] Hiroaki Niiro,[36] Youngho Park,[12] Changbing Shen,[37,38] Takeshi Miyamoto,[39] Ga-Young Ahn,[11] Wenmin Fei,[4] Tsutomu Takeuchi ,[40] Jung-Min Shin,[11] Keke Li,[4] Yasushi Kawaguchi,[41] Yeon-Kyung Lee,[11] Yong-Fei Wang ,[42] Koichi Amano,[43] Dae Jin Park,[11] Wanling Yang ,[42] Yoshifumi Tada,[44] Yu Lung Lau,[42] Ken Yamaji,[45] Zhengwei Zhu,[1,2] Masato Shimizu,[46] Takashi Atsumi,[47] Akari Suzuki,[48] Takayuki Sumida,[49] Yukinori Okada ,[50,51,52] Koichi Matsuda,[53,54] Keitaro Matsuo,[55,56] Yuta Kochi ,[57] Japanese Research Committee on Idiopathic Osteonecrosis of the Femoral Head, Kazuhiko Yamamoto ,[48] Koichiro Ohmura,[58] Tae-Hwan Kim ,[11,12] Sen Yang,[1,2] Takuaki Yamamoto,[59] Bong-Jo Kim,[18] Nan Shen ,[32,60,61] Shiro Ikegawa,[8] Hye-Soon Lee,[11,12] Xuejun Zhang,[1,2,62] Chikashi Terao ,[9,63,64] Yong Cui,[4] Sang-Cheol Bae [11,12]

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For numbered affiliations see end of article.

Correspondence to
Dr Chikashi Terao, Laboratory for Statistical and Translational Genetics Analysis, RIKEN Center for Integrative Medical Sciences, Kanagawa, Japan; chikashi.terao@riken.jp, Professor Yong Cui, Department of Dermatology, China-Japan Friendship Hospital, Beijing, People's Republic of China; wuhucuiyong@vip.163.com and Professor Sang-Cheol Bae, Department of Rheumatology, Hanyang University Hospital for Rheumatic Diseases, Seoul, South Korea; scbae@hanyang.ac.kr

XY, KK and HS contributed equally.

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ABSTRACT

Objective Genome-wide association studies (GWAS) have identified >100 risk loci for systemic lupus erythematosus (SLE), but the disease genes at most loci remain unclear, hampering translation of these genetic discoveries. We aimed to prioritise genes underlying the 110 SLE loci that were identified in the latest East Asian GWAS meta-analysis.

Methods We built gene expression predictive models in blood B cells, $CD4^+$ and $CD8^+$ T cells, monocytes, natural killer cells and peripheral blood cells of 105 Japanese individuals. We performed a transcriptome-wide association study (TWAS) using data from the latest genome-wide association meta-analysis of 208 370 East Asians and searched for candidate genes using TWAS and three data-driven computational approaches.

Results TWAS identified 171 genes for SLE ($p<1.0\times10^{-5}$); 114 (66.7%) showed significance only in a single cell type; 127 (74.3%) were in SLE GWAS loci. TWAS identified a strong association between *CD83* and SLE ($p<7.7\times10^{-8}$). Meta-analysis of genetic associations in the existing 208 370 East Asian and additional 1498 cases and 3330 controls found a novel single-variant

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT?

⇒ Genome-wide association studies have identified >100 risk loci for systemic lupus erythematosus (SLE), but the disease genes at most of these loci remain unclear.

WHAT DOES THIS STUDY ADD?

⇒ We built six immune cell-specific gene expression reference panels based on data from East Asians and performed a transcriptome-wide association study for SLE for the first time.
⇒ We identified 276 candidate disease genes in 110 SLE loci including 104 genes in novel loci.
⇒ We explored allele-specific regulatory mechanisms at *ACAP1* that increase SLE risk.

HOW MIGHT THIS IMPACT CLINICAL PRACTICE OR FUTURE DEVELOPMENTS?

⇒ We identified numerous potential drug targets for SLE.

association at rs72836542 (OR=1.11, p=4.5×10^{-9}) around *CD83*. For the 110 SLE loci, we identified 276 gene candidates, including 104 genes at recently-identified SLE novel loci. We demonstrated in vitro that putative causal variant rs61759532 exhibited an allele-specific regulatory effect on *ACAP1*, and that presence of the SLE risk allele decreased *ACAP1* expression.

Conclusions Cell-level TWAS in six types of immune cells complemented SLE gene discovery and guided the identification of novel genetic associations. The gene findings shed biological insights into SLE genetic associations.

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disorder with genetic predisposition.[1 2] Genome-wide association studies (GWAS) have identified >100 genetic loci robustly associated with SLE.[3–8] The latest effort was a genome-wide association meta-analysis in 208 370 East Asians (hereafter, East Asian meta-analysis), which identified 113 SLE loci.[9] These GWAS have identified SLE genetic determinants and improved our understanding of disease pathogenesis. However, the disease genes through which genetic associations affect SLE remain unclear at most GWAS loci, hindering translation of genetic discoveries into SLE precision health.[10 11]

Experiments have been devoted to identify disease genes at SLE loci,[12 13] but unsurprisingly are time consuming and expensive. Transcriptome-wide association study (TWAS) is an alternative statistical method for identifying candidate genes at GWAS loci.[14] A TWAS usually consists of two steps. In step 1, TWAS learns gene expression predictive models in cohorts with both gene expression and genotype data (hereafter, gene expression reference). In step 2, TWAS uses the predictive models to impute in silico gene expression in cohorts only with genotype or GWAS summary statistics. After that, TWAS tests for associations between imputed gene expression and GWAS traits. TWAS gene expression predictive models use expression quantitative trait loci (eQTL) as predictors. Due to linkage disequilibrium (LD), TWAS can easily implicate hitchhiking genes together with disease genes in GWAS loci. However, methods have been developed to discern disease genes from hitchhikers.[15] TWAS has recently nominated candidate genes for many human diseases,[16 17] providing biological insights into disease associations. However, no TWAS for SLE has been reported.

TWAS requires gene expression references from populations with the same ancestry as in disease GWAS.[18] Most current gene expression references are built from eQTL data sets of European ancestry. Only a few non-European references have been reported,[19] limiting the application of TWAS in non-European samples.

Current TWAS mainly use disease-relevant tissue-level expression references that contain composite expression data from multiple distinct cell types in various cellular states. The heterogeneity in expression reference panels can bias TWAS findings and complicate gene association interpretation. In contrast, cell-level expression reference panels have obvious advantages, but few are available. Immune cells, such as B cells, T cells and monocytes, play key roles in SLE pathogenesis.[20] TWAS using immune cell-level gene expression references might provide a unique opportunity to further our understanding of SLE pathogenesis.

To that end, we created gene expression predictive models from six types of blood immune cells obtained from East Asians. We performed a TWAS using the latest East Asian meta-analysis findings[9] and searched for SLE genes jointly with three other data-driven gene prioritisation approaches. We identified 276 candidate genes, including 104 from recently-identified novel loci. We found that the six cell-level gene expression references complemented SLE gene discovery. We demonstrated that TWAS findings guide the identification of novel genetic associations. Additionally, we explored regulatory mechanisms at *ACAP1* in vitro. Our findings provide biological insight into SLE pathogenesis.

METHODS

Genome-wide association summary statistics

We previously performed the largest genome-wide association meta-analysis of SLE using data from 208 370 individuals of eight East Asian cohorts and identified 113 loci (including 46 novel loci) at p<5.0×10^{-8} (online supplemental table 1).[9] In the present study, we used the index variants for the 110 autosomal loci and genome-wide single-variant association summary statistics at 11 270 530 genetic markers that were available in at least two member cohorts. We excluded the Human Leukocyte Antigens (*HLA*) region in further analyses. This study was carried out in compliance with the Helsinki Declaration.

TWAS and Fine-mapping Of CaUsal gene Sets

To infer gene expression changes in SLE, we performed a TWAS in FUSION[21] using default parameters and eQTL data sets for six blood immune cell types generated from 105 (21 men and 84 women) healthy Japanese individuals with a mean age of 39 years: B cells, CD4$^+$ T cells, CD8$^+$ T cells, monocytes, natural killer (NK) cells and peripheral blood cells.[22] LD was computed from whole-genome sequencing data of 3256 Japanese and 504 East Asians enrolled in the 1000 Genomes Project (1KGP).[23 24] We restricted analysis to protein-coding genes. We defined a significant gene association p value threshold after Bonferroni correction for the number of protein-coding genes tested in each cell type (number of genes: B cells: 5055; CD4$^+$ T cells: 5132; CD8$^+$ T cells: 4988; monocytes: 5546; NK cells: 5239; peripheral blood cells: 5614).

To prioritise genes in genomic regions with ≥2 significant genes in TWAS, we implemented a Bayesian fine-mapping analysis using Fine-mapping Of CaUsal gene Sets (FOCUS) as previously described,[15] which computed a posterior inclusion probability (PIP) for each gene to quantify the probability for being the true disease gene, and then created a 90% credible gene set that contained the putative disease genes with a probability ≥90%. We estimated gene expression weights in the six eQTL data sets and performed FOCUS for each cell type separately. We regarded TWAS significant genes with PIP ≥0.8 as potential disease genes.

Colocalisation analysis

To evaluate whether SLE GWAS associations share the same causal variants with eQTL, we performed colocalisation analysis between SLE GWAS loci and eQTL for genes with significant TWAS associations around the corresponding SLE GWAS index variants. We created ±100 kilobase (kb) genomic regions centring on SLE GWAS index variants and extracted association summary statistics from SLE East Asian meta-analysis[9] and the corresponding eQTL.[22] We restricted analysis to genetic variants with sample size N=208 370 in SLE GWAS meta-analysis. We implemented colocalisation analysis in coloc using default parameters.[25] We defined significant colocalisation with posterior probability (PP_{H4}) ≥0.8.

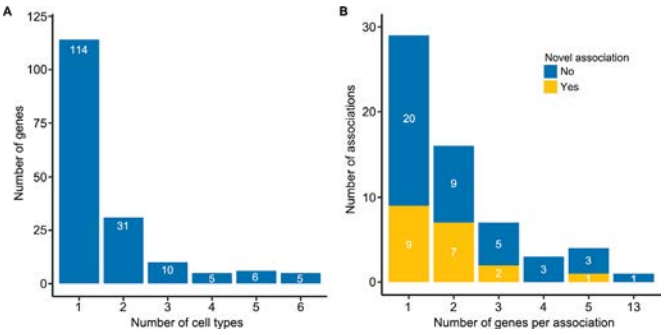

Figure 1 TWAS of East Asian meta-analysis data. (A) Distribution of significant genes across the six types of immune cells. (B) Number of significant TWAS genes per SLE locus. TWAS, transcriptome-wide association study.

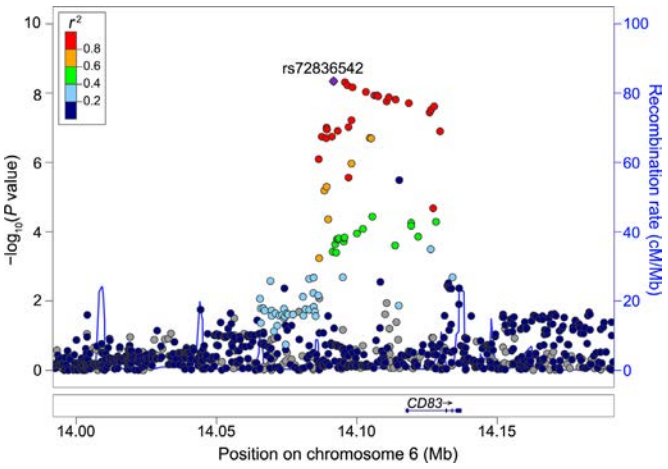

Figure 2 Locuszoom plot for a new single-variant association at the *CD83* gene. Mb, megabase.

Data-driven Expression-Prioritised Integration for Complex Traits

To prioritise SLE genes, we analysed genetic variants with an SLE association $p<5.0\times10^{-8}$ using Data-driven Expression-Prioritised Integration for Complex Traits (DEPICT) V.1 release 194.[26] DEPICT clumped the input variants in a 500 kb region at LD $r^2>0.1$ based on 1KGP East Asian data, yielding 1521 autosomal loci. We identified significant genes using a false discovery rate <5%.

Polygenic Priority Score

To prioritise SLE genes, we applied the Polygenic Priority Score (PoPS) method to East Asian meta-analysis results.[27] First, we computed gene-level association statistics and gene–gene correlations from GWAS summary statistics using MAGMA[28] and LD estimated from the 1KGP East Asian data. Next, we ran enrichment analysis for gene features listed at https://github.com/FinucaneLab/gene_features using MAGMA. We retained features with $p<0.05$ in MAGMA. Finally, we computed PoPS for each gene by fitting a joint model for enrichment of all resulting features. After calculating PoPS for a total of 18 383 protein-coding genes, we kept the top 30% of genes and prioritised those with the highest PoPS in a 1 megabase (Mb) window centred on each of the 110 SLE index variants.

Assay for transposase-accessible chromatin using sequencing in blood CD4$^+$ T and CD19$^+$ B cells

To detect open accessible elements of *ACAP1*, we sorted blood CD4$^+$ T and CD19$^+$ B cells from five healthy Chinese individuals and performed assay for transposase-accessible chromatin using sequencing (ATAC-seq) on the BGISEQ 500 platform as described previously.[29] Each participant provided written informed consent.

Luciferase reporter assay

We previously identified rs61759532 as a likely causal variant at the *ACAP1* locus.[9] To explore the regulatory effects of rs61759532, three identical copies of the 24 bp-element flanking each allele of rs61759532 were subcloned into the luciferase vector, pGL4.26 (luc2/minP/Hydro), between the XhoI and BglII sites upstream of the minimal promoter for the firefly luciferase gene (online supplemental figure 1). The firefly luciferase vector (1 µg) and the normalising *Renilla* luciferase vector (500 ng) were co-transfected into human leukemia monocytic (THP1) cells for 2 days using Lipofectamine 3000 (Thermo Fisher Scientific). Luciferase activity was measured in five independent biological replicates using the Dual-Luciferase Reporter Assay Kit (Promega) according to the manufacturer's instructions. Relative fold-change in firefly luciferase activity was normalised by both transfection efficiency, based on *Renilla* luciferase activity and minimal luciferase activity from the pGL4.26 vector without insert.

Electrophoretic mobility shift assay

Epstein-Barr Virus (EBV)-transformed B or THP1 cells were grown in RPMI 1640 medium including 10% fetal bovine serum and 1% penicillin/streptomycin. Electrophoretic mobility shift assay (EMSA) probes were constructed by annealing biotin-conjugated 30-residue oligonucleotide sequences flanking rs61759532. EMSA was performed using the LightShift Chemiluminescent EMSA Kit (Thermo Fisher Scientific) according to the manufacturer's instructions.

RESULTS

TWAS in the six immune cell types

To identify SLE genes, we performed a TWAS of East Asian meta-analysis data using gene expression references of six types of immune cells. We identified 57, 51, 48, 46, 44 and 40 significant genes in B, NK, peripheral blood, monocytes, CD4$^+$ and CD8$^+$ T cells, respectively (online supplemental table 2), which together comprised 171 genes. The significant genes were enriched in B cells (χ^2 test; $p=5.3\times10^{-3}$). Colocalisation suggested that the same causal variants were shared between 24 SLE loci and 27 of the 171 genes (online supplemental table 3). Notably, only 5 of the 171 genes, namely *B3GALT6*, *ELF1*, *HEATR3*, *TPCN2* and *UHRF1BP1*, attained significance in all six cell types; 114 (66.7%) genes showed significance in only a single cell type (figure 1A). We used *PLD4*, a newly-identified SLE gene,[3] as a positive control. TWAS identified a significant association at *PLD4* only in monocytes ($p=2.6\times10^{-9}$), suggesting that monocytes mediate the effects of *PLD4* on SLE. A previous study reported that PLD4$^{-/-}$ mice developed more blood monocytes[30] and autoimmune phenotypes.[3]

Genes at SLE novel loci

Of the 171 genes, 127 (74.3%) arose within 500 kb from 61 of the 110 SLE index variants.[9] For the majority (n=52; 85.2%) of these 61 SLE loci, TWAS identified ≤3 genes (figure 1B). For 33 loci, TWAS identified the closest protein-coding gene (online supplemental table 2).

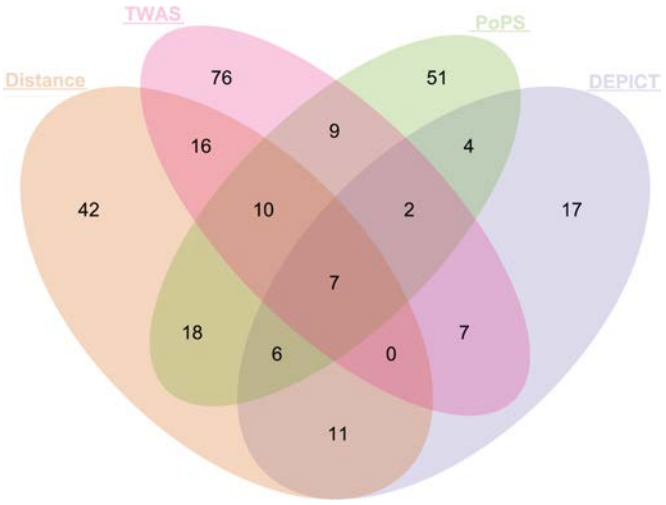

Figure 3 Venn diagram of candidate disease genes at the 110 SLE loci identified using four gene discovery approaches. DEPICT, Data-driven Expression-Prioritised Integration for Complex Traits; PoPS, Polygenic Priority Score; TWAS, transcriptome-wide association study.

Of the 127 genes, 35 came from 19 of the 46 recently-identified novel loci.[9] For example, we identified a novel association at rs3750996 and prioritised rs3750996 as the putative causal variant for this association.[9] TWAS consistently identified *STIM1* gene near rs3750996 in B cells ($p=1.1\times10^{-7}$), $CD4^+$ T cells ($p=4.7\times10^{-9}$), monocytes ($p=5.8\times10^{-9}$) and peripheral blood cells ($p=3.38\times10^{-8}$). Colocalisation suggested that the same causal variant was associated with both SLE risk and *STIM1* expression in all the four types of cells (PP_{H4} >0.97; online supplemental table 3). *STIM1* encodes a calcium channel sensor that regulates type I interferon response[31] and plays an essential role in effector functions of T and B cells.[32–34] Mutations in *STIM1* cause severe immune deficiency in humans.[35] STIM1 is a potential lupus therapeutic target.[36]

As another example, we previously identified a novel association at rs58107865 as an East Asian-specific SLE locus.[9] TWAS identified at this locus the *LEF1* gene ($p=1.3\times10^{-10}$), which encodes a transcription factor that binds to the T-cell receptor-α enhancer site. LEF1 controls the maintenance and functional specification of T_{reg} subsets to prevent autoimmunity.[37] LEF1 antagonist demonstrated tumour inhibition for B-cell chronic lymphocytic leukaemia,[38] suggesting the potential of LEF1 as a drug target.

TWAS-guided identification of novel GWAS association

TWAS identified 44 genes (44=171–127) outside the 110 SLE loci, suggesting that future studies with larger sample sizes might detect novel GWAS associations with SLE around these 44 genes.

TWAS identified a significant association of *CD83* with SLE in B cells ($p=7.7\times10^{-8}$). East Asian meta-analysis only found a borderline significant single-variant association (rs12530098 with the lowest p value; OR=1.10, $p=6.9\times10^{-8}$). We recruited two additional cohorts of 1498 SLE cases and 3330 controls in China[39] and meta-analysed their summary-level associations with East Asian findings in this region. We identified a genome-wide significant association at rs72836542 around *CD83* for the first time (OR=1.11, $p=4.5\times10^{-9}$; LD $r^2=0.93$ with rs12530098; figure 2). SNP rs72836542 regulates *CD83* expression in blood B cells ($\beta=-1.51$, $p=4.2\times10^{-20}$),[22] suggesting that *CD83* might mediate the association with SLE.

CD83 acts as an essential factor during the differentiation of T and B lymphocytes.[40] Soluble human CD83-treated mice showed lower concentrations of anti-histone IgG autoantibodies and significantly delayed onset of anti-dsDNA autoantibody production.[41] These reports suggest that CD83 is a promising drug target for SLE.[41]

Fine-mapping of TWAS genes

Of the 171 genes, 53 arose in TWAS of the same cell types. The flanking regions (±500 kb) for these 53 genes overlapped, suggesting they might arise at the same loci. These 53 genes comprised 17 genomic loci. To prioritise disease genes, we implemented a FOCUS analysis.[15] We identified these 53 genes in the 90% gene credible sets and suggested 23 (43.4%) as likely disease genes (PIP ≥0.8; online supplemental table 4). Among them, *FNIP1*, *HEATR3*, and *CD37* arose at SLE novel loci.

We reported a genome-wide association between SLE and rs11288784 for the first time in our East Asian meta-analysis.[9] At this locus, TWAS identified three genes, *ADCY7*, *BRD7* and *HEATR3* ($p<2\times10^{-8}$; online supplemental table 2). Fine-mapping analysis suggested that *HEATR3*, the closest gene to the association, is most likely the disease gene (PIP >0.998). The eQTL for *HEATR3* was colocalised with the SLE association (PP_{H4} >0.89; online supplemental table 3). *HEATR3* plays a role in NOD2-mediated NF-κB signalling and has been implicated in Crohn's disease.[42]

Complementary gene identification

To complement gene identification at the 110 SLE loci,[9] we implemented three additional gene prioritisation approaches: (1) the nearest protein-coding gene; (2) DEPICT; and (3) PoPS. DEPICT and PoPS identified 54 and 107 protein-coding genes, respectively (online supplemental tables 5-7); 24 (44.4%) and 41 (38.3%) are the closest protein-coding genes to the corresponding SLE associations; 12 and 10 genes arose at SLE novel loci.[9] TWAS and these three gene prioritisation approaches together identified 276 genes within the 110 SLE loci, including 104 genes at novel loci (online supplemental table 7). Notably, only seven genes (*BANK1*, *IRF5*, *BLK*, *NCOA2*, *WDFY4*, *SLC15A4* and *RASGRP1*) were identified by all four methods, of which *NCOA2* arises in the novel SLE locus at rs142937720.[9] Colocalisation analysis using genetic associations with SLE susceptibility and *NCOA2* expression revealed the sharing of causal variant ($PP_{H4}=0.93$; online supplemental table 3). *NCOA2* encodes a transcriptional co-activator of interferon regulatory factor 1[43] that plays a role in SLE.[44]

Regulatory mechanisms at *ACAP1*

One hundred and eighty-six (67.4%) of the 276 genes were identified in only one approach (figure 3). For example, DEPICT identified *ACAP1* at the novel SLE locus around rs61759532.[9] *ACAP1* encodes a key regulator of integrin traffic for cell adhesion and migration.[45] Fine-mapping analysis previously prioritised rs61759532, an intronic variant of *ACAP1*, as the likely causal variant (posterior probability of being causal =0.999; figure 4A). We found that rs61759532 overlaps with an accessible open chromatin region in blood B and T cells (figure 4B). GeneHancer[46] suggested that rs61759532 resides in an enhancer/promoter element of *ACAP1*. Transcriptional reporter assays showed significant allelic differences in the enhancer activity of rs61759532 in THP1 monocyte cell lines (two-sided t-test $p=8.1\times10^{-3}$; figure 4C), consistent with the regulatory effect of the risk allele, *T*, in reducing *ACAP1* expression in whole blood

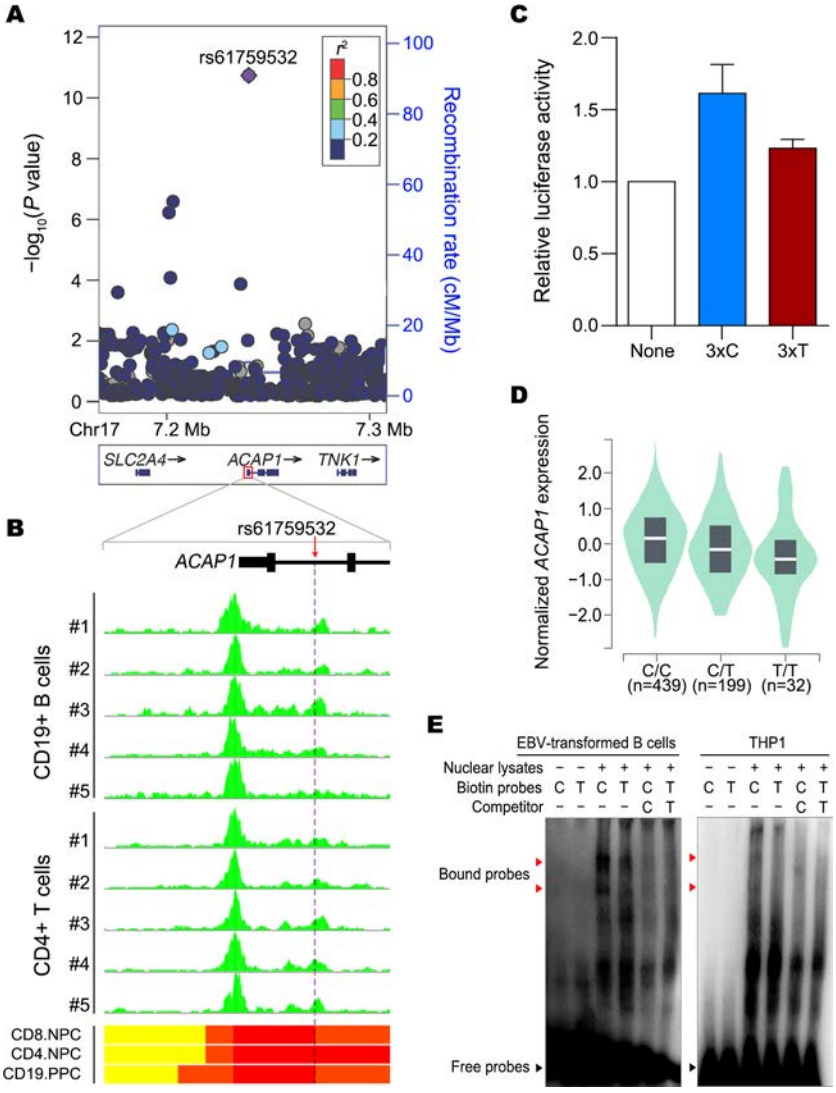

Figure 4 Allele-specific regulatory effect of rs61759532 on *ACAP1*. (A) Regional association plot for the *ACAP1* locus. The lead variant rs61759532 is labelled as a purple diamond. Linkage disequilibrium was estimated using data from 7021 Chinese individuals. (B) Location of rs61759532 within an assay for transposase-accessible chromatin using sequencing open chromatin accessible region in $CD19^+$ B and $CD4^+$ T cells (green tracks) and within active ChromHMM chromatin states (bars on the bottom panel) in primary $CD8^+$ T naive cells (CD8.NPC), T helper naive cells (CD4.NPC) and primary B cells (BLD.CD19.PPC). Chromatin states are coloured red (active transcription start site), orange red (flanking active transcription start site), or yellow (enhancers). (C) Allelic differential enhancing activity of rs61759532 in THP1 cells. None, 3×C, and 3×T denote an empty vector containing a minimal promoter, and vectors with the C and T alleles of rs61759532, respectively. Relative luciferase activities, measured in five independent biological replicates, were significantly higher for inserts with the C allele (two-tailed t-test $p=8.1\times10^{-3}$). Error bars indicate SEMs of five independent biological replicates. (D) Association between the risk allele (T) of rs61759532 and decreased expression of *ACAP1* in GTEx v8 whole blood ($p=1.7\times10^{-47}$). The white line in the centre of each box indicates the median expression value, while the box for each genotype represents the IQR of *ACACP1* expression. (E) Allelic differential protein-DNA binding by rs61759532 in EMSAs. Biotin-conjugated 30-nucleotide probes flanking rs61759532 (denoted as C or T, according to the allele) were incubated with nuclear extracts (10 µg) from EBV-transformed B cells or THP1 cells in EMSAs. Shifted bands (indicated by red arrows) had stronger intensities with the biotin-conjugated C allele probes than the T allele probes and were not detected in the presence of excess non-conjugated probes. EBV: Epstein-Barr Virus; EMSA, electrophoretic mobility shift assay; Mb, megabase; THP1: human leukemia monocytic cell line.

($p=1.7\times10^{-47}$; figure 4D).[47] EMSA revealed that allele-specific biotin-labelled probes containing *T* (risk allele) formed fewer nuclear protein-probe complexes than probes with *C* (non-risk allele) in THP1 and EBV-transformed B cell lines (figure 4E).

DISCUSSION

Here, we performed a TWAS for SLE for the first time and identified 171 genes associated with SLE risk. We nominated 276 genes at 110 SLE loci through TWAS and three computational approaches. One hundred and four genes arise at SLE novel loci; multiple show therapeutic potential. These findings provide insights into SLE biology and can guide future functional experiments.

SLE GWAS have identified >100 risk loci, but the disease genes and underlying molecular mechanisms remain largely unknown.[3–8] TWAS is widely used to identify disease genes and determine disease mechanisms.[14] In TWAS, population ancestry, tissue/cell relevance and cell sources of gene expression references are critical.[48 49] Here, we created cell-level gene expression references from six types of immune cells in East Asians, ensuring

that the reference panels were constructed from individuals with the same ancestry as the SLE GWAS. Various immune cells play a role in SLE pathogenesis.[20] Studies suggested that loci identified in SLE GWAS could contribute to the risk of SLE through their effects on immune cells.[50] Our study showed that 66.7% of these significant genes attained significance only in TWAS of one of the six immune cell types. This finding highlights the value of evaluating diverse immune cells in TWAS of SLE.

TWAS identified 44 genes associated with SLE in regions without prior GWAS associations. Among these 44 genes, we identified a genome-wide single-variant association at *CD83* for the first time. CD83 modulates the production of autoantibodies and might have therapeutic effects in SLE.[41] This result demonstrates that TWAS can help guide the identification of novel GWAS associations.

For the 110 SLE loci that we recently identified in our latest East Asian meta-analysis,[9] TWAS and the three data-driven approaches identified a pool of 276 gene candidates, 186 of which were identified using a single approach. These gene findings warrant careful interpretation. We previously identified rs61759532 as a putative causal variant of SLE.[9] In the present study, we demonstrated in vitro the molecular effects of the different alleles of rs61759532 on *ACAP1* expression levels. We showed that rs61759532 resides in an open chromatin region and exhibited enhancing activity on *ACAP1*. The risk allele *T* of rs61759532 reduces the expression of *ACAP1* in whole blood.

This study has several limitations. The modest study sample size in the cell-level gene expression references likely limited the power and precision of TWAS. SLE has various systemic manifestations, suggesting that many tissues/cells contribute to disease pathogenesis in addition to the immune cells that we studied.[20] Increasing the breadth of cell types and cell state resources in gene expression references would increase the precision of TWAS. We only experimentally explored functional mechanisms for one significant SNP (rs61759532) in one gene, *ACAP1*. The role of *ACAP1* and the biological pathways mediating the effects of *ACAP1* on SLE are worthy of further investigation.

In summary, we performed a TWAS for SLE for the first time and identified 276 gene candidates at SLE loci. These findings help elucidate the genetic mechanisms underlying SLE and provide potential SLE therapeutic targets.

Author affiliations
[1]Department of Dermatology and Institute of Dermatology, First Affiliated Hospital, Anhui Medical University, Hefei, Anhui, People's Republic of China
[2]Key Lab of Dermatology, Ministry of Education (Anhui Medical University), Hefei, Anhui, People's Republic of China
[3]Inflammation and Immune Mediated Diseases Laboratory of Anhui Province, Hefei, Anhui, People's Republic of China
[4]Department of Dermatology, China-Japan Friendship Hospital, Beijing, People's Republic of China
[5]Department of Biostatistics and Center for Statistical Genetics, University of Michigan, Ann Arbor, MI, USA
[6]Human Phenome Institute, Fudan University, Shanghai, People's Republic of China
[7]Department of Biology and Department of Life and Nanopharmaceutical Sciences, Kyung Hee University, Seoul, Korea
[8]Laboratory for Bone and Joint Diseases, RIKEN Center for Medical Sciences, Tokyo, Japan
[9]Laboratory for Statistical and Translational Genetics Analysis, RIKEN Center for Integrative Medical Sciences, Yokohama, Japan
[10]Department of Orthopaedic Surgery, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
[11]Department of Rheumatology, Hanyang University Hospital for Rheumatic Diseases, Seoul, South Korea
[12]Hanyang University Institute for Rheumatology Research, Seoul, South Korea
[13]Division of Molecular Pathology, Department of Cancer Biology, Institute of Medical Science, The University of Tokyo, Tokyo, Japan
[14]Koga Hospital 21, Kurume, Japan
[15]Department of Epidemiology and Biostatistics, School of Public Health, Anhui Medical University, Hefei, People's Republic of China
[16]Department of Orthopedic Surgery, Keio University School of Medicine, Tokyo, Japan
[17]Department of Orthopaedic Medical Engineering, Osaka University Graduate School of Medicine, Osaka, Japan
[18]Division of Genome Science, Department of Precision Medicine, National Institute of Health, Cheongju-si, South Korea
[19]Department of Rheumatology & Clinical Immunology, Sapporo City General Hospital, Hokkaido, Japan
[20]Divisions of Genetics and Rheumatology, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA
[21]Center for Data Sciences, Harvard Medical School, Boston, MA, USA
[22]Program in Medical and Population Genetics, Broad Institute of MIT and Harvard, Cambridge, MA, USA
[23]Department of Internal Medicine, Dong-A University Hospital, Busan, South Korea
[24]Department of Orthopaedic Surgery, Faculty of Medicine and Graduate School of Medicine, Hokkaido University, Hokkaido, Japan
[25]Division of Rheumatology, Department of Internal Medicine, Chonnam National University Medical School and Hospital, Gwangju, South Korea
[26]Department of Rheumatology, Endocrinology and Nephrology, Faculty of Medicine and Graduate School of Medicine, Hokkaido University, Hokkaido, Japan
[27]Division of Rheumatology, Department of Internal Medicine, Chungnam National University Hospital, Daejeon, South Korea
[28]Department of Internal Medicine and Rheumatology, National Hospital Organization, Kyushu Medical Center, Fukuoka, Japan
[29]Division of Rheumatology, Department of Internal Medicine, Kyungpook National University Hospital, Daegu, South Korea
[30]Department of Orthopaedic Surgery, Graduate School of Medicine, Chiba University, Chiba, Japan
[31]Department of Rheumatology, Ajou University School of Medicine, Suwon, South Korea
[32]Shanghai Institute of Rheumatology, Renji Hospital, Shanghai Jiao Tong University, School of Medicine (SJTUSM), Shanghai, People's Republic of China
[33]Department of Internal Medicine, Yonsei University College of Medicine, Seoul, South Korea
[34]Niigata University Health Administration Center, Niigata, Japan
[35]Department of Rheumatology, Catholic University of Daegu School of Medicine, Daegu, South Korea
[36]Department of Medical Education, Kyushu University Graduate School of Medical Sciences, Fukuoka, Japan
[37]Department of Dermatology, Peking University Shenzhen Hospital, Shenzhen, Guangdong, People's Republic of China
[38]Shenzhen Key Laboratory for Translational Medicine of Dermatology, Shenzhen Peking University-The Hong Kong University of Science and Technology Medical Center, Shenzhen, Guangdong, People's Republic of China
[39]Department of Orthopaedic Surgery, Faculty of Life Sciences, Kumamoto University, Kumamoto, Japan
[40]Division of Rheumatology, Department of Internal Medicine, Keio University School of Medicine, Tokyo, Japan
[41]Institute of Rheumatology, Tokyo Women's Medical University, Tokyo, Japan
[42]Department of Paediatrics and Adolescent Medicine, The University of Hong Kong, Hong Kong, People's Republic of China
[43]Department of Rheumatology & Clinical Immunology, Saitama Medical Center, Saitama Medical University, Saitama, Japan
[44]Department of Rheumatology, Faculty of Medicine, Saga University, Saga, Japan
[45]Department of Internal Medicine and Rheumatology, Juntendo University School of Medicine, Tokyo, Japan
[46]Hokkaido Medical Center for Rheumatic Diseases, Sapporo, Japan
[47]Department of Orthopaedic Surgery, Showa University School of Medicine, Tokyo, Japan
[48]Laboratory for Autoimmune Diseases, RIKEN Center for Integrative Medical Sciences, Kanagawa, Japan
[49]Department of Internal Medicine, Faculty of Medicine, University of Tsukuba, Ibaraki, Japan
[50]Department of Statistical Genetics, Osaka University Graduate School of Medicine, Osaka, Japan
[51]Department of Genome Informatics, Graduate School of Medicine, University of Tokyo, Tokyo, Japan
[52]Laboratory for Systems Genetics, RIKEN Center for Integrative Medical Sciences, Kanagawa, Japan
[53]Laboratory of Genome Technology, Human Genome Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan
[54]Laboratory of Clinical Genome Sequencing, Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, University of Tokyo, Tokyo, Japan
[55]Division of Cancer Epidemiology and Prevention, Aichi Cancer Center Research Institute, Nagoya, Japan

[56]Department of Epidemiology, Nagoya University Graduate School of Medicine, Nagoya, Japan
[57]Department of Genomic Function and Diversity, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan
[58]Department of Rheumatology and Clinical Immunology, Kyoto University Graduate School of Medicine, Kyoto, Japan
[59]Department of Orthopaedic Surgery, Faculty of Medicine, Fukuoka University, Fukuoka, Japan
[60]State Key Laboratory of Oncogenes and Related Genes, Shanghai Cancer Institute, Renji Hospital, Shanghai Jiao Tong University School of Medicine (SJTUSM), Shanghai, People's Republic of China
[61]Center for Autoimmune Genomics and Etiology (CAGE), Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA
[62]Department of Dermatology, Institute of Dermatology, Huashan Hospital, Fudan University, Shanghai, People's Republic of China
[63]Clinical Research Center, Shizuoka General Hospital, Shizuoka, Japan
[64]Department of Applied Genetics, School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan

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Data availability statement Data are available upon reasonable request. The ATAC-seq data for human blood B and T cells have been deposited with the China National Genomics Data Center (https://bigd.big.ac.cn/gsa-human/browse) under accession no. HRA000271. The expression quantitative trait loci summary-level data in blood immune cells are publicly available from our website (JENGER; http://jenger.riken.jp/en/). All the other data relevant to the study are included in the article or uploaded as supplementary information. The meta-analysis summary association statistics in the current study are available from the corresponding author on reasonable request.

ORCID iDs
Kwangwoo Kim http://orcid.org/0000-0001-8926-6216
Rui-Xue Leng http://orcid.org/0000-0002-2453-5865
Nobuhiko Sugano http://orcid.org/0000-0003-4035-3989
Dongqing Ye http://orcid.org/0000-0001-6604-9614
Junichi Nakamura http://orcid.org/0000-0003-4005-8832
Huihua Ding http://orcid.org/0000-0002-7530-8953
Tsutomu Takeuchi http://orcid.org/0000-0003-1111-8218
Yong-Fei Wang http://orcid.org/0000-0002-1260-6291
Wanling Yang http://orcid.org/0000-0003-0063-6327
Yukinori Okada http://orcid.org/0000-0002-0311-8472
Yuta Kochi http://orcid.org/0000-0002-8012-5078
Kazuhiko Yamamoto http://orcid.org/0000-0001-9037-3625
Tae-Hwan Kim http://orcid.org/0000-0002-3542-2276
Nan Shen http://orcid.org/0000-0002-5875-4417
Chikashi Terao http://orcid.org/0000-0002-6452-4095
Sang-Cheol Bae http://orcid.org/0000-0003-4658-1093

REFERENCES

1 Carter EE, Barr SG, Clarke AE. The global burden of SLE: prevalence, health disparities and socioeconomic impact. *Nat Rev Rheumatol* 2016;12:605–20.
2 Guerra SG, Vyse TJ, Cunninghame Graham DS. The genetics of lupus: a functional perspective. *Arthritis Res Ther* 2012;14:211.
3 Akizuki S, Ishigaki K, Kochi Y, *et al*. PLD4 is a genetic determinant to systemic lupus erythematosus and involved in murine autoimmune phenotypes. *Ann Rheum Dis* 2019;78:509–18.
4 Cunninghame Graham DS, Morris DL, Bhangale TR, *et al*. Association of NCF2, IKZF1, IRF8, IFIH1, and Tyk2 with systemic lupus erythematosus. *PLoS Genet* 2011;7:e1002341.
5 Gateva V, Sandling JK, Hom G, *et al*. A large-scale replication study identifies TNIP1, PRDM1, Jazf1, UHRF1BP1 and IL10 as risk loci for systemic lupus erythematosus. *Nat Genet* 2009;41:1228–33.
6 Han J-W, Zheng H-F, Cui Y, *et al*. Genome-Wide association study in a Chinese Han population identifies nine new susceptibility loci for systemic lupus erythematosus. *Nat Genet* 2009;41:1234–7.
7 Kim K, Bang S-Y, Lee H-S, *et al*. The HLA-DRβ1 amino acid positions 11-13-26 explain the majority of SLE-MHC associations. *Nat Commun* 2014;5:5902.
8 Okada Y, Shimane K, Kochi Y, *et al*. A genome-wide association study identified AFF1 as a susceptibility locus for systemic lupus eyrthematosus in Japanese. *PLoS Genet* 2012;8:e1002455.
9 Yin X, Kim K, Suetsugu H, *et al*. Meta-Analysis of 208370 East Asians identifies 113 susceptibility loci for systemic lupus erythematosus. *Ann Rheum Dis* 2021;80:632–40.
10 Cano-Gamez E, Trynka G. From GWAS to function: using functional genomics to identify the mechanisms underlying complex diseases. *Front Genet* 2020;11:424.
11 Catalina MD, Owen KA, Labonte AC, *et al*. The pathogenesis of systemic lupus erythematosus: harnessing big data to understand the molecular basis of lupus. *J Autoimmun* 2020;110:102359.
12 Owen KA, Price A, Ainsworth H, *et al*. Analysis of Trans-Ancestral SLE risk loci identifies unique biologic networks and drug targets in African and European ancestries. *Am J Hum Genet* 2020;107:864–81.

13 Su C, Johnson ME, Torres A, *et al*. Mapping effector genes at lupus GWAS loci using promoter Capture-C in follicular helper T cells. *Nat Commun* 2020;11:3294.
14 Li B, Ritchie MD. From GWAS to gene: transcriptome-wide association studies and other methods to functionally understand GWAS discoveries. *Front Genet* 2021;12:713230.
15 Mancuso N, Freund MK, Johnson R, *et al*. Probabilistic fine-mapping of transcriptome-wide association studies. *Nat Genet* 2019;51:675–82.
16 Mancuso N, Gayther S, Gusev A, *et al*. Large-Scale transcriptome-wide association study identifies new prostate cancer risk regions. *Nat Commun* 2018;9:4079.
17 Gusev A, Mancuso N, Won H, *et al*. Transcriptome-Wide association study of schizophrenia and chromatin activity yields mechanistic disease insights. *Nat Genet* 2018;50:538–48.
18 Geoffroy E, Gregga I, Wheeler HE. Population-Matched transcriptome prediction increases TWAS discovery and replication rate. *iScience* 2020;23:101850.
19 Wen J, Xie M, Rowland B, *et al*. Transcriptome-Wide association study of blood cell traits in African ancestry and Hispanic/Latino populations. *Genes* 2021;12:1049.
20 Kaul A, Gordon C, Crow MK, *et al*. Systemic lupus erythematosus. *Nat Rev Dis Primers* 2016;2:16039.
21 Gusev A, Ko A, Shi H, *et al*. Integrative approaches for large-scale transcriptome-wide association studies. *Nat Genet* 2016;48:245–52.
22 Ishigaki K, Kochi Y, Suzuki A, *et al*. Polygenic burdens on cell-specific pathways underlie the risk of rheumatoid arthritis. *Nat Genet* 2017;49:1120–5.
23 Hikino K, Koido M, Otomo N, *et al*. Genome-Wide association study of colorectal polyps identified highly overlapping polygenic architecture with colorectal cancer. *J Hum Genet* 2022;67:149–56.
24 Auton A, Abecasis GR, Altshuler DM, *et al*. A global reference for human genetic variation. *Nature* 2015;526:68–74.
25 Wallace C. A more accurate method for colocalisation analysis allowing for multiple causal variants. *PLoS Genet* 2021;17:e1009440.
26 Pers TH, Karjalainen JM, Chan Y, *et al*. Biological interpretation of genome-wide association studies using predicted gene functions. *Nat Commun* 2015;6:5890.
27 Weeks EM, Ulirsch JC, Cheng NY. Leveraging polygenic enrichments of gene features to predict genes underlying complex traits and diseases. *medRxiv*2020.
28 de Leeuw CA, Mooij JM, Heskes T, *et al*. MAGMA: generalized gene-set analysis of GWAS data. *PLoS Comput Biol* 2015;11:e1004219.
29 Buenrostro JD, Giresi PG, Zaba LC, *et al*. Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position. *Nat Methods* 2013;10:1213–8.
30 Gavin AL, Huang D, Huber C, *et al*. PLD3 and PLD4 are single-stranded acid exonucleases that regulate endosomal nucleic-acid sensing. *Nat Immunol* 2018;19:942–53.
31 Srikanth S, Woo JS, Wu B, *et al*. The Ca^{2+} sensor STIM1 regulates the type I interferon response by retaining the signaling adaptor STING at the endoplasmic reticulum. *Nat Immunol* 2019;20:152–62.
32 Feske S, Skolnik EY, Prakriya M. Ion channels and transporters in lymphocyte function and immunity. *Nat Rev Immunol* 2012;12:532–47.
33 Baba Y, Kurosaki T. Role of calcium signaling in B cell activation and biology. *Curr Top Microbiol Immunol* 2016;393:143–74.
34 Srikanth S, Woo JS, Sun Z, *et al*. Immunological Disorders: Regulation of Ca^{2+} Signaling in T Lymphocytes. *Adv Exp Med Biol* 2017;993:397–424.
35 Lacruz RS, Feske S. Diseases caused by mutations in Orai1 and STIM1. *Ann N Y Acad Sci* 2015;1356:45–79.
36 Prabakaran T, Troldborg A, Kumpunya S, *et al*. A sting antagonist modulating the interaction with STIM1 blocks ER-to-Golgi trafficking and inhibits lupus pathology. *EBioMedicine* 2021;66:103314.
37 Yang B-H, Wang K, Wan S, *et al*. Tcf1 and LEF1 control Treg competitive survival and Tfr development to prevent autoimmune diseases. *Cell Rep* 2019;27:3629–45.
38 Gandhirajan RK, Staib PA, Minke K, *et al*. Small molecule inhibitors of Wnt/beta-catenin/Lef-1 signaling induces apoptosis in chronic lymphocytic leukemia cells in vitro and in vivo. *Neoplasia* 2010;12:326–35.
39 Song Q, Lei Y, Shao L, *et al*. Genome-Wide association study on Northern Chinese identifies KLF2, DOT1L and STAB2 associated with systemic lupus erythematosus. *Rheumatology* 2021;60:4407–17.
40 Grosche L, Knippertz I, König C, *et al*. The CD83 Molecule - An Important Immune Checkpoint. *Front Immunol* 2020;11:721.
41 Starke C, Steinkasserer A, Voll RE, *et al*. Soluble human CD83 ameliorates lupus in NZB/W F1 mice. *Immunobiology* 2013;218:1411–5.
42 Zhang W, Hui KY, Gusev A, *et al*. Extended haplotype association study in Crohn's disease identifies a novel, Ashkenazi Jewish-specific missense mutation in the NF-κB pathway gene, HEATR3. *Genes Immun* 2013;14:310–6.
43 Bhandare R, Damera G, Banerjee A, *et al*. Glucocorticoid receptor interacting protein-1 restores glucocorticoid responsiveness in steroid-resistant airway structural cells. *Am J Respir Cell Mol Biol* 2010;42:9–15.
44 Tsokos GC, Lo MS, Costa Reis P, *et al*. New insights into the immunopathogenesis of systemic lupus erythematosus. *Nat Rev Rheumatol* 2016;12:716–30.
45 Chen P-W, Luo R, Jian X, *et al*. The ARF6 GTPase-activating proteins ARAP2 and ACAP1 define distinct endosomal compartments that regulate integrin α5β1 traffic. *J Biol Chem* 2014;289:30237–48.
46 Fishilevich S, Nudel R, Rappaport N, *et al*. GeneHancer: genome-wide integration of enhancers and target genes in GeneCards. *Database* 2017;2017:bax028.
47 GTEx Consortium. Human genomics. The Genotype-Tissue expression (GTEx) pilot analysis: multitissue gene regulation in humans. *Science* 2015;348:648–60.
48 Wainberg M, Sinnott-Armstrong N, Mancuso N, *et al*. Opportunities and challenges for transcriptome-wide association studies. *Nat Genet* 2019;51:592–9.
49 Bhattacharya A, García-Closas M, Olshan AF, *et al*. A framework for transcriptome-wide association studies in breast cancer in diverse study populations. *Genome Biol* 2020;21:42.
50 Liu L, Yin X, Wen L, *et al*. Several critical cell types, tissues, and pathways are implicated in genome-wide association studies for systemic lupus erythematosus. *G3* 2016;6:1503–11.

TRANSLATIONAL SCIENCE

Induced antibodies directed to the angiotensin receptor type 1 provoke skin and lung inflammation, dermal fibrosis and act species overarching

Xiaoyang Yue ,[1] Junping Yin,[1] Xiaoqing Wang ,[1] Harald Heidecke,[2] Alexander Maximilian Hackel,[3] Xiaoru Dong,[4] Brigitte Kasper,[1] Lifang Wen,[4] Liang Zhang,[1] Kai Schulze-Forster,[2] Juliane Junker,[2] Hanna Grasshoff ,[3] Antje Müller,[5] Gerd Wallukat,[6] Ingolf Schimke,[6] Julian Zeiner,[7] Lisa Marie Deckstein,[7] Nicole Mertens,[7] Anja Kerstein-Staehle,[8] Jennifer Elisabeth Hundt,[9] Evi Kostenis,[7] Xinhua Yu,[1] Gabriela Riemekasten ,[1,3] Frank Petersen[1]

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For numbered affiliations see end of article.

Correspondence to
Professor Gabriela Riemekasten, Department of Rheumatology and Clinical Immunology, University Medical Center Schleswig Holstein Lübeck Campus, Lubeck, Schleswig-Holstein, Germany; gabriela.riemekasten@uksh.de and Professor Frank Petersen, Priority Area Asthma & Allergy, Research Center Borstel, 23845, Borstel, Germany; fpetersen@fz-borstel.de

XY, JY and XW contributed equally.

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ABSTRACT

Objective To determine contributions and functions of autoantibodies (Abs) directed to the angiotensin receptor type 1 (AT1R), which are suggested to be involved in the pathogenesis of AT1R Abs-related diseases such as systemic sclerosis (SSc).

Methods C57BL/6J mice were immunised with membrane-embedded human AT1R or empty membrane as control. Mice deficient for $CD4^+$ or $CD8^+$ T cells and B cells were immunised with membrane-embedded AT1R or an AT1R peptide proposed to be a dominant T cell epitope. A monoclonal (m)AT1R Ab was generated by hybridoma technique and transferred into C57BL/6J and AT1Ra/b knockout mice. The induced phenotype was examined by histology, immunohistochemistry, immunofluorescence, apoptosis assay and ELISA. In vitro, Abs responses towards AT1R were measured in cells of different origins and species.

Results AT1R-immunised mice developed perivascular skin and lung inflammation, lymphocytic alveolitis, weak lung endothelial apoptosis and skin fibrosis accompanied by Smad2/3 signalling, not present in controls or mice deficient for $CD4^+$ T and B cells. The AT1R peptide 149–172 provoked lung inflammation. Application of the mAT1R Ab induced skin and lung inflammation, not observed in AT1Ra/b knockout mice. In vitro, AT1R Abs activated rat cardiomyocytes and human monocytes, enhanced angiotensin II-mediated AT1R activation in AT1R-transfected HEK293 cells via AT1R binding and mAT1R Ab-activated monocytes mediated the induction of profibrotic markers in dermal fibroblasts.

Conclusion Our immunisation strategy successfully induced AT1R Abs, contributing to inflammation and, possibly, to fibrosis via activation of AT1R. Therefore, AT1R Abs are valuable targets for future therapies of SSc and other AT1R Ab-related diseases.

Key messages

What is already known about this subject?

⇒ Angiotensin receptor type 1 (AT1R) autoantibodies (Abs) are suggested to contribute to pathologies found in systemic sclerosis as well as in other conditions with high values of AT1R Abs.

What does this study add?

⇒ AT1R Abs have the potential to induce lung and skin inflammation, dermal fibrosis and endothelial apoptosis.

⇒ Our immunisation strategy to induce functionally active Abs can serve as model to generate Abs against other complex transmembrane proteins.

⇒ AT1R Abs can act agonistic and allosteric in combination with the orthosteric ligand angiotensin II.

How might this impact on clinical practice or future developments?

⇒ As an important ligand of AT1R, AT1R Abs are a novel target for future therapies in diseases associated with high values of AT1R Abs.

INTRODUCTION

Angiotensin receptor type 1 (AT1R, AGTR1) is a G protein-coupled receptor (GPCR) centrally involved in the regulation of vascular tone, proliferation of vascular smooth muscle cells, extracellular matrix generation and inflammation.[1–3] AT1R binds its endogenous ligand angiotensin II (Ang II) in a saturable manner with high structural specificity and affinity, thereby mediating most of the physiological actions of Ang II.[4 5] Physiologically, AT1R is expressed in vascular tissues, including skin and lung.[1 6–8] Human and the two subtypes of rodent AT1R, AT1Ra and AT1Rb, respectively, share a high degree of homology.[9] Notably, increased levels of circulating autoantibodies targeting AT1R (AT1R Abs), partially accompanied by functional activity, have been found in patients with renal transplant rejection, glomerulosclerosis, preeclampsia and autoimmune diseases such as systemic sclerosis (SSc), a severe inflammatory, vascular and fibrotic disease.[10–14] In the latter, high serum levels of AT1R Abs predicted mortality, pulmonary arterial hypertension and digital ulcers.[11 15 16] In vitro, SSc patient-derived IgG containing high levels of AT1R

Abs induced gene expression of transforming growth factor ß (TGFβ), expression of adhesion molecules and chemokines in endothelial cells, release of interleukin 8 (IL-8) and CC-chemokine ligand 18 (CCL18) in leukocytes and production of collagen type I in fibroblasts.[11 17–19] Blockade by AT1R antagonists indicated specificity for AT1R.[17 18] However, no in vivo model exists, demonstrating appearance of SSc manifestations by induction of high AT1R Ab levels. Thus, the study attempted to find out if immunisation of C57BL/6J mice with membrane extracts (ME) of cells overexpressing human AT1R induces AT1R Abs, pathologies and the relevance of T and B cells. Further, a monoclonal (m)AT1R Ab as well as AT1Ra/b knockout mice were applied to validate AT1R Ab binding in vivo.[20] Finally, rodent and human AT1R-expressing cells were stimulated in vitro with the mAT1R Ab or murine IgG containing AT1R Abs. AT1R Ab binding and activation of AT1R were measured by label-free dynamic mass redistribution (DMR) technology in AT1R-transfected HEK293 cells.[21 22] Altogether, AT1R immunisation of C57BL/6J mice led to interstitial lung disease (ILD) and skin fibrosis, accompanied by high levels of AT1R Abs, whose generation most likely depends on $CD4^+$ T cells and B cells. The local application of the mAT1R Ab also led to signs of skin and lung inflammation in C57BL/6J mice, which were diminished in the AT1Ra/b knockout mice. Further, the in vitro results suggest that interactions between rodent or human AT1R-expressing cells and murine IgG containing AT1R Abs or the mAT1R Ab, either alone or in combination with Ang II, promote activation of AT1R and/or subsequent functional responses.

MATERIALS AND METHODS

Detailed experimental procedures are reported in the online supplemental materials and methods (see online supplemental materials and methods).

RESULTS

Immunisation with AT1R induces pulmonary disease and AT1R Abs in vivo

Following two immunisations with AT1R ME, the C57BL/6J mice developed symptoms of ILD as indicated by lymphocytic alveolitis in bronchoalveolar lavage fluid (BALF) which is not observed in the control mice (figure 1A). Histopathological analyses showed a profound inflammation in intra-alveolar areas of the lung, not found in the control mice (figure 1B). Accordingly, the inflammatory score, reflecting number and size of infiltrates in the lung, was higher in the AT1R-immunised mice than in the corresponding controls (figure 1C, $p<0.01$). Immunohistochemical staining revealed a dominant presence of T and B cells in the

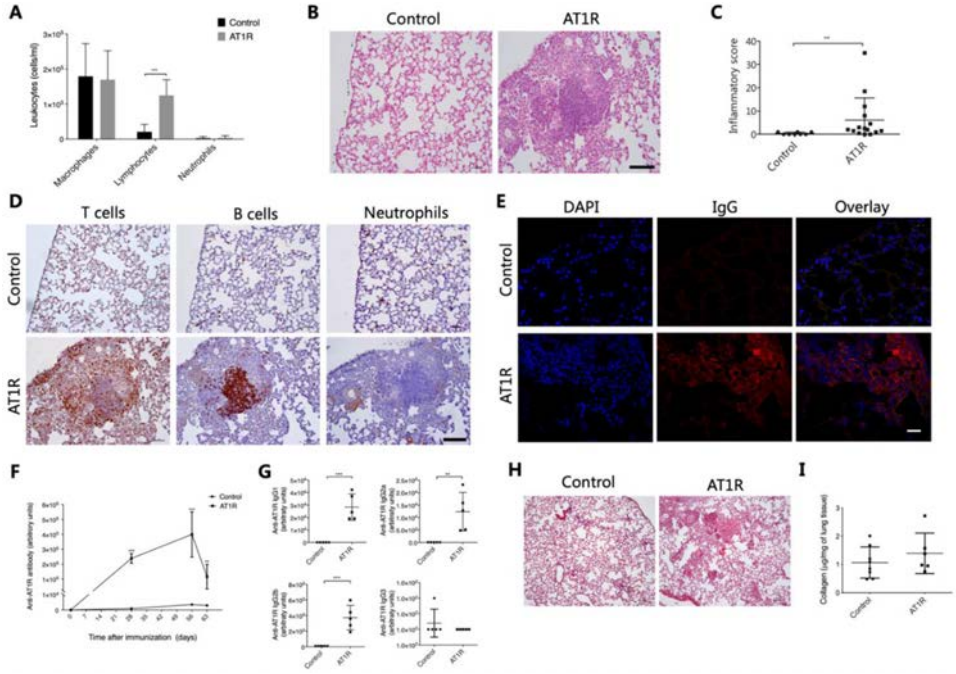

Figure 1 Immunisationwith AT1R induces signs of lung inflammation. (A) Quantitative analysis of leucocyte populations in bronchoalveolar lavage fluid obtained from controls and hAT1R-immunised mice 9 weeks after the first immunisation. (B) Histology of the lungs of control and hAT1R-immunised mice (200×, scale bar=100 µm). (C) Analysis of lung inflammation quantified by scoring the sizes and numbers of immune infiltrates in intra-alveolar areas in a double-blinded fashion. The results are presented as mean±SD. Statistical analyses were performed using the Mann-Whitney U test (**p<0.01). (D) Cellular composition of the infiltrates in the mouse lungs (200×, scale bar=100 µm) as detected by immunohistochemistry (IHC). Both intra-alveolar and perivascular infiltrations were evaluated. Representative micrographs of H&E staining are shown. (E) Immunofluorescence staining of lung cryosections from hAT1R-immunised mice (630×, scale bar=25 µm) featured murine immunoglobuline G (IgG) deposition (red) and nuclei were stained with 4',6-Diaminodino-2-phenylindol (DAPI, blue). Total anti-AT1R IgG (F) and subclasses of anti-hAT1R IgG (G) were detected by ELISA using plates coated with membrane extracts from CHO cells overexpressing hAT1R and the appropriate detection antibodies. The titre of anti-AT1R antibodies was defined as the dilution at which the optical density (OD) value reached half of the maximal OD values of the curve. The results are presented as mean±SD, and statistical analysis was performed using Student's t-test (**p<0.01, ***p<0.001). (H) Representative lung sections from control and hAT1R-immunised mice stained with Masson trichrome (100×, scale bar=100 µm). (I) Collagen content in the lungs of control and hAT1R-immunised mice. The collagen content was determined using a Sircol collagen detection kit and is expressed as µg per mg of lung tissue. Data are presented as mean±SD, and statistical analysis was performed using Student's t-test. AT1R, angiotensin receptor type 1.

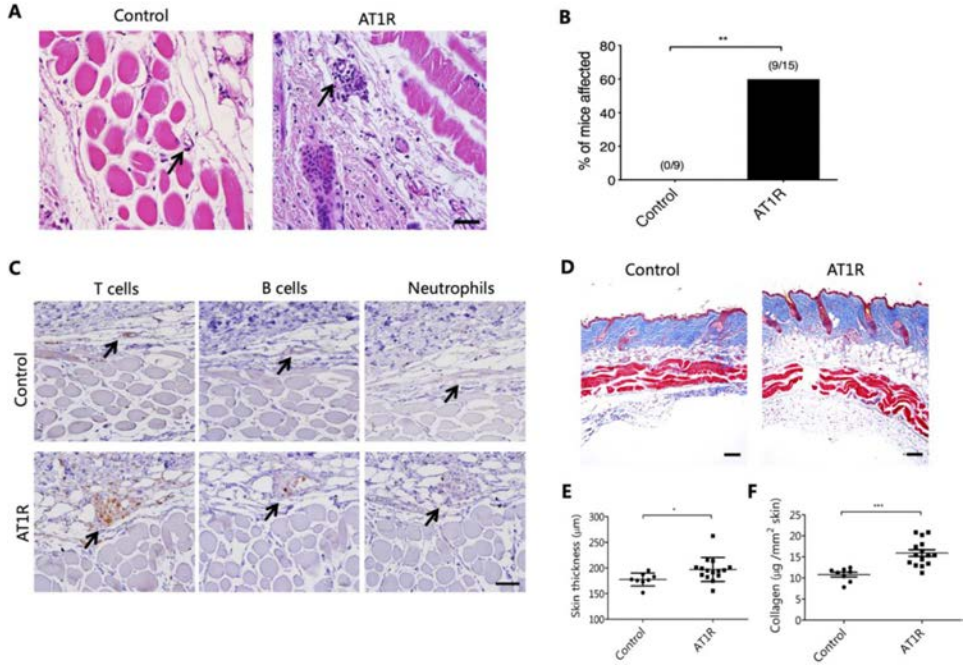

Figure 2 Immunisationwith AT1R induces signs of skin inflammation and fibrosis. (A) Representative H&E staining of skin sections from control and hAT1R-immunised mice (400×, scale bar=50 µm). Black arrows indicate blood vessels in the dermis. (B) Incidence of perivascular inflammatory infiltrates in the skin of control and hAT1R-immunised mice. The numbers on top of the bars show the ratios between the number of mice with perivascular inflammation and the total number of evaluated mice. Statistical analysis was performed using Fisher's exact test (**p<0.01). (C) Cellular composition of the perivascular infiltrates in the skin of controls vs AT1R-immunised mice (400×, scale bar=50 µm) as detected by IHC. (D) Representative dorsal skin sections after Masson trichrome staining of control or hAT1R-immunised mice (100×, scale bar=100 µm). (E) Quantitative analysis of the thickness of the collagen layer as indicated by the blue area in sections stained with Masson trichrome. (F) Collagen content of the skin in control and hAT1R-immunised mice determined by Sircol collagen detection kit and expressed as µg per mm^2 of skin. Data are presented as mean±SD, and statistical analyses were performed using the Mann-Whitney U test or Student's t-test (*p<0.05, **p < 0.01, ***p<0.001). AT1R, angiotensin receptor type 1.

lung (figure 1D). Of note, immunisation with AT1R also induced perivascular inflammation in the lung, again mainly consisting of T and B cells (online supplemental figure 1A). Moreover, IgG deposition indicative of an immune reaction was observed in lung tissue of the AT1R-immunised mice and not present in the animals used as controls (figure 1E). The AT1R-immunised mice displayed high levels of circulating AT1R Abs, peaking at day 56 after the first immunisation, and not detected in control mice (figure 1F). The AT1R-reactive Abs belonged to the IgG1, IgG2a and IgG2b subclasses, while the IgG3 subclass was not observed (figure 1G). In addition, the percentage of apoptotic endothelial cells was higher in the AT1R-immunised mice compared with the control mice (online supplemental figure 1B,C, p<0.01). However, vascular remodelling, a key feature of vasculopathy, as well as lung fibrosis was not observed in the AT1R-immunised mice as investigated by histological and biochemical analyses (figure 1H,I).

Immunisation with AT1R provokes SSc-like skin inflammation and fibrosis in vivo

At day 63 after the first immunisation, 60% of the AT1R-immunised mice showed dermal perivascular infiltrates, which were not present in the controls (figure 2A,B). These infiltrates were dominated by T cells, while B cells and neutrophils were less prominent or absent (figure 2C). In addition, AT1R immunisation resulted in increased skin thickness, which was not seen in the control mice (figure 2D,E, p<0.05). The signs of skin fibrosis observed histologically were validated by quantitative assessment of skin collagen using Sircol reagent. Our results revealed an increased expression of collagen by 48% in the AT1R-immunised mice compared with the control mice (figure 2F, p<0.001), indicating development of fibrosis in this organ. Further, immunofluorescence staining showed an increased number of alpha-smooth muscle actin (α-SMA)+myofibroblasts and cells positive for Smad2/3 phosphorylation in the AT1R-immunised mice compared with controls (online supplemental figure 2), supporting a role of myofibroblasts and TGFβ signalling in the fibrotic process. However, obliterative vasculopathy was not observed in the AT1R-immunised mice. Furthermore, histological analyses of the kidney, heart, intestine and oesophagus did not show any abnormalities (online supplemental figure 3), indicating that immunisation with AT1R preferentially affects murine lung and skin.

$CD4^+$ T and B cells are indispensable for lung and skin pathology following immunisation with AT1R

To clarify whether lymphocytes are required for development of AT1R-induced pathologies, mice deficient in $CD4^+$ T cells, $CD8^+$ T cells and B cells were chosen for AT1R immunisation. In the absence of $CD4^+$ T and B cells, mice were unable to generate AT1R Abs in contrast to mice deficient for $CD8^+$ T cells or wild-type controls. Notably, B cell-deficient mice displayed decreased serum IL-6, supporting a role of B cell-derived IL-6 in the inflammatory response towards AT1R immunisation (online supplemental figure 4). Compared with wild-type controls, development of pulmonary inflammation was dramatically reduced in mice deficient for $CD4^+$ T cells and B cells, but not in the absence of $CD8^+$ T cells

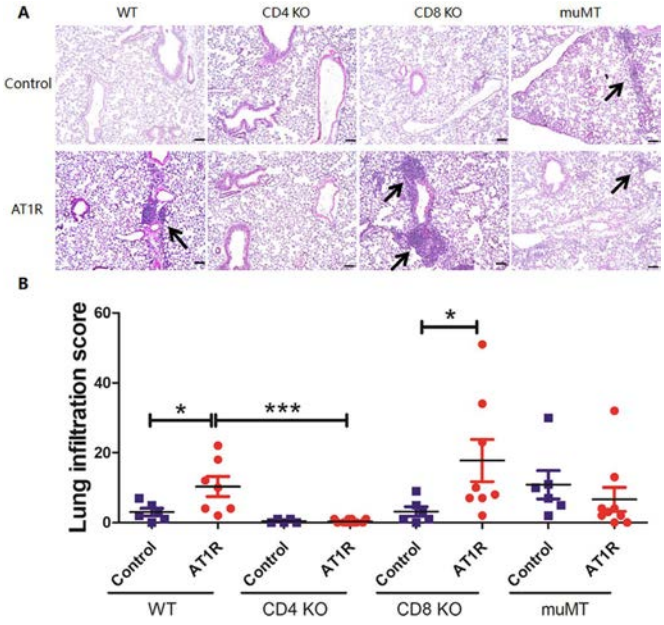

Figure 3 CD4$^+$ T and B cells are indispensable for lung pathology following immunisation with AT1R. (A) Representative micrographs of H&E-stained lung sections of wild-type (WT), CD4$^+$ T cell-deficient (CD4 KO), CD8$^+$ T cell-deficient (CD8 KO) and B cell-deficient (muMT) mice immunised with control ME or AT1R-ME. Black arrows indicate inflammatory cell infiltrates in the lung. Scale bar=100 µm. (B) Quantitative analysis of pulmonary inflammation in mice assessed by size and number of infiltrates scored in a double-blinded fashion. Statistical analyses were performed using Mann-Whitney U test or Student's t-test depending on the normal distribution of data. *p<0.05, ***p<0.001. AT1R, angiotensin receptor type 1.

(figure 3A,B). In line with this, AT1R immunisation induced perivascular skin inflammation in 5 of 7 (71%) wild-type mice, in 50% (4 out of 8 mice) of the CD8$^+$ T cell-deficient mice, but in none of the 11 mice deficient for CD4$^+$ T cells and only in 11.1% (1 out of 9 mice) of the B cell-deficient mice (figure 4A,B). Moreover, AT1R-induced skin thickening and quantitative collagen expression reflecting fibrosis were observed in wild-type and CD8$^+$ cell-deficient mice, but not in CD4$^+$ T cell-deficient or B cell-deficient mice (figure 4C–E). Of note, the number of macrophages in the BALF was not different between the various groups of mice (data not shown). Overall, the data suggest that the generation of AT1R Abs and inflammation require interactions between CD4$^+$ T and B cells. Given the essential role of CD4$^+$ T cells in this novel mouse model, we next investigated the pathogenicity of AT1R-specific CD4$^+$ T cells. By using NetMHCIIpan 3.1 software, AT1R 149–172 peptide was predicted to harbour a potential CD4$^+$ T cell epitope showing strong binding affinity to a murine MHC class II molecule. Immunisation with the AT1R 149–172 peptide induced strong lung inflammation in C57BL/6 mice, while such inflammation was not observed in solvent controls. In addition, no inflammation was observed in skin, heart or kidney in either group of mice (online supplemental figure 5).

Administration of a monoclonal AT1R Ab induces SSc-like signs of skin and lung inflammation in vivo

A single intradermal injection of IgG derived from the AT1R-immunised mice into the ear of naive C57BL/6J mice induced more inflammation compared with IgG injection derived from control mice (online supplemental figure 6), suggesting a possible role of AT1R Abs in the induction of local inflammation. To examine if AT1R Abs contribute to local inflammation, the monoclonal Ab 5.2a, which exhibits specificity to human AT1R by ELISA and western blot (data not shown) was studied in more detail. Repetitive intradermal injections of this mAT1R Ab into the ears of C57BL/6J mice, but not of the IgG isotype control, induced cellular infiltrations into the skin (figure 5A, p<0.01) as well as perivascular lung inflammation in 5 out of 11 mice, which was not observed in mice treated with IgG isotype control (figure 5B, p<0.01). In the skin, infiltrations consisted of neutrophils and to a much lesser extent of B and T cells, whereas lung infiltrates contained mainly T cells, but no neutrophils (figure 5C). To validate a potential role of an Ab-mediated interaction with AT1R for the induction of inflammatory mechanisms, the mAT1R Ab was administered to AT1R-knockout mice, deficient for both murine AT1Ra and AT1Rb. The mAT1R Ab-induced ear infiltration was reduced in the AT1R-deficient mice when compared with wild-type mice (figure 5D). In contrast, the isotype control IgG did not induce ear infiltration in the AT1R-deficient and wild-type mice. In terms of lung inflammation, the number of the available mice and the results obtained so far were too low or too few to yield significant differences between the AT1R-deficient and wild-type mice (data not shown).

Monoclonal AT1R Ab and murine IgG containing AT1R Abs specifically interact with AT1R-expressing cells from different species in vitro

Four different cell types expressing AT1R were employed to examine interactions between AT1R and AT1R Abs in vitro.[23] First, AT1R-expressing rat cardiomyocytes were treated with both mAT1R Ab as well as IgG derived from the AT1R-immunised mice as described before.[24] While the mAT1R Ab increased the spontaneous cardiomyocyte beating frequency to on average 5 beats/minute compared with the isotype control, the stimulatory effect was completely abrogated by losartan, an AT1R antagonist (figure 6A, p<0.001). Similarly, IgG derived from AT1R-immunised mice increased the spontaneous cardiomyocyte beating frequency to on average 16 beats/minute compared with IgG from control mice (figure 6B). Again, this effect was completely inhibited by losartan (figure 6B, p<0.001), indicating specificity for AT1R. Further, following treatment of human epithelial (HEp-2) cells with the mAT1R Ab and IgG derived from AT1R-immunised mice, deposition of IgG onto cell membrane was observed in comparison to isotype control or IgG from control-immunised mice (figure 6C,D). To further validate the mAT1R Ab-induced effects, AT1R-transfected and naive human embryonic kidney (HEK293) cells were used in a label-free optical whole-cell biosensing assay based on detection of DMR.[21 22] This technology platform captures morphological changes that occur in living cells as a consequence of ligand receptor interaction.[21 22] Here, the mAT1R Ab as well as the isotype control did not show an effect when used alone (figure 6E). However, in contrast to the isotype control, the DMR response by Ang II was elevated in the presence of the mAT1R Ab. All DMR responses were completely abrogated by AT1R blockade through losartan and were undetectable in cells without exogenous enrichment of AT1R (figure 6E). The results indicate enhancement of Ang II effects by AT1R Abs in a strictly AT1R-dependent manner. Finally, in blood

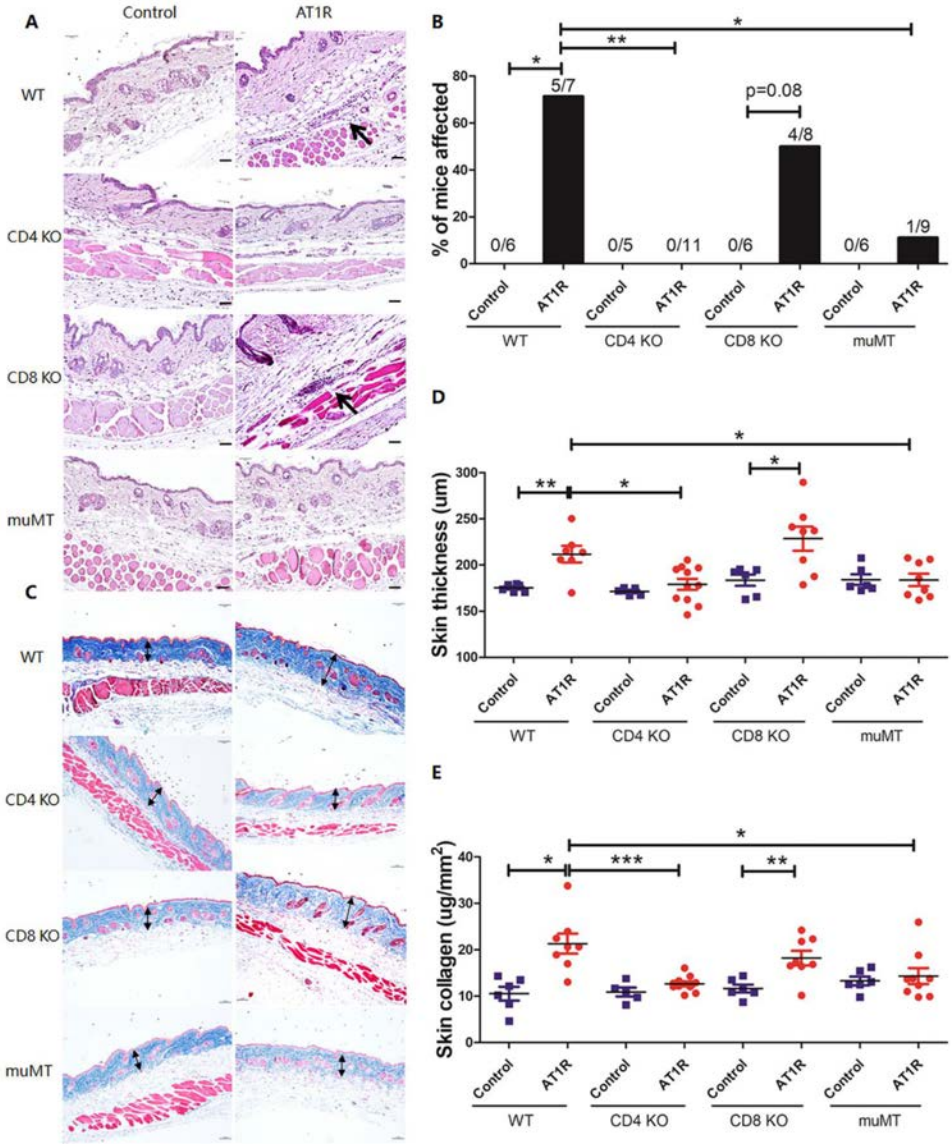

Figure 4 CD4$^+$ T and B cells are indispensable for skin pathology following immunisation with AT1R. (A) Representative micrographs of H&E-stained skin sections of wild-type (WT), CD4$^+$ T cell-deficient (CD4 KO), CD8$^+$ T cell-deficient (CD8 KO) and B cell-deficient (muMT) mice immunised with control ME or AT1R-ME. Black arrows indicate inflammatory cell infiltrates around blood vessels. Scale bar=50 µm. (B) Incidence of skin inflammation as indicated by orange colours. P values were calculated by using Fisher's exact test (*p<0.05, **p<0.01, ***p<0.001). (C) Representative micrographs of Masson trichrome-stained skin sections. Double-headed arrows indicate the collagen layer of the skin. Scale bar=100 µm. (D) Quantitative analysis of collagen layer thickness stained in blue from the Masson trichrome staining. (E) Quantitative analysis of collagen content determined by Sircol collagen detection kit and expressed as µg per mm^2 of the skin. Statistical analysis was performed using Mann-Whitney *U* test or Student's t-test depending on the normal distribution of data (*p<0.05, **p<0.01, ***p<0.001). AT1R, angiotensin receptor type 1.

monocytes from healthy donors, the mAT1R Ab induced CCL18, but not the isotype control (figure 6F). The induction of CCL18 by the mAT1R Ab alone and in combination with Ang II was higher compared with Ang II alone. The effect was abrogated by the AT1R antagonist telmisartan (figure 6G). Conditioned supernatant of mAT1R Ab-stimulated monocytes induced higher expression of α-SMA, connective tissue growth factor (CTGF) and collagen I in primary dermal fibroblast compared with the isotype control (figure 6H, online supplemental figure 7) or compared with the stimulation by the mAT1R Ab alone (figure 6I, online supplemental figure 7), pointing towards a pivotal role of monocytes and the mAT1R Ab/AT1R axis for fibrosis. Taken together, the data indicate that in vitro AT1R Abs interact with AT1R expressed by rodent and human cells. Moreover, AT1R Abs activate AT1R or act in agonistic fashion together with Ang II. These interactions turn into altered functions such as increased heartbeat or elevated chemokine release.

DISCUSSION

Interactions between AT1R Abs and AT1R expressed by tissue-resident and/or circulating immune cells are suspected to contribute to vascular, fibrotic and inflammatory processes, which are also key features in SSc.[11 15 25 26] Here, immunisation of C57BL/6J mice with ME of human AT1R-overexpressing CHO cells induced high levels of AT1R Abs accompanied by lung and skin inflammation. Lymphocytic alveolitis, indicative of ILD, elevated Smad2/3 signalling and collagen expression, indicative of skin fibrosis, as well as mild endothelial apoptosis were

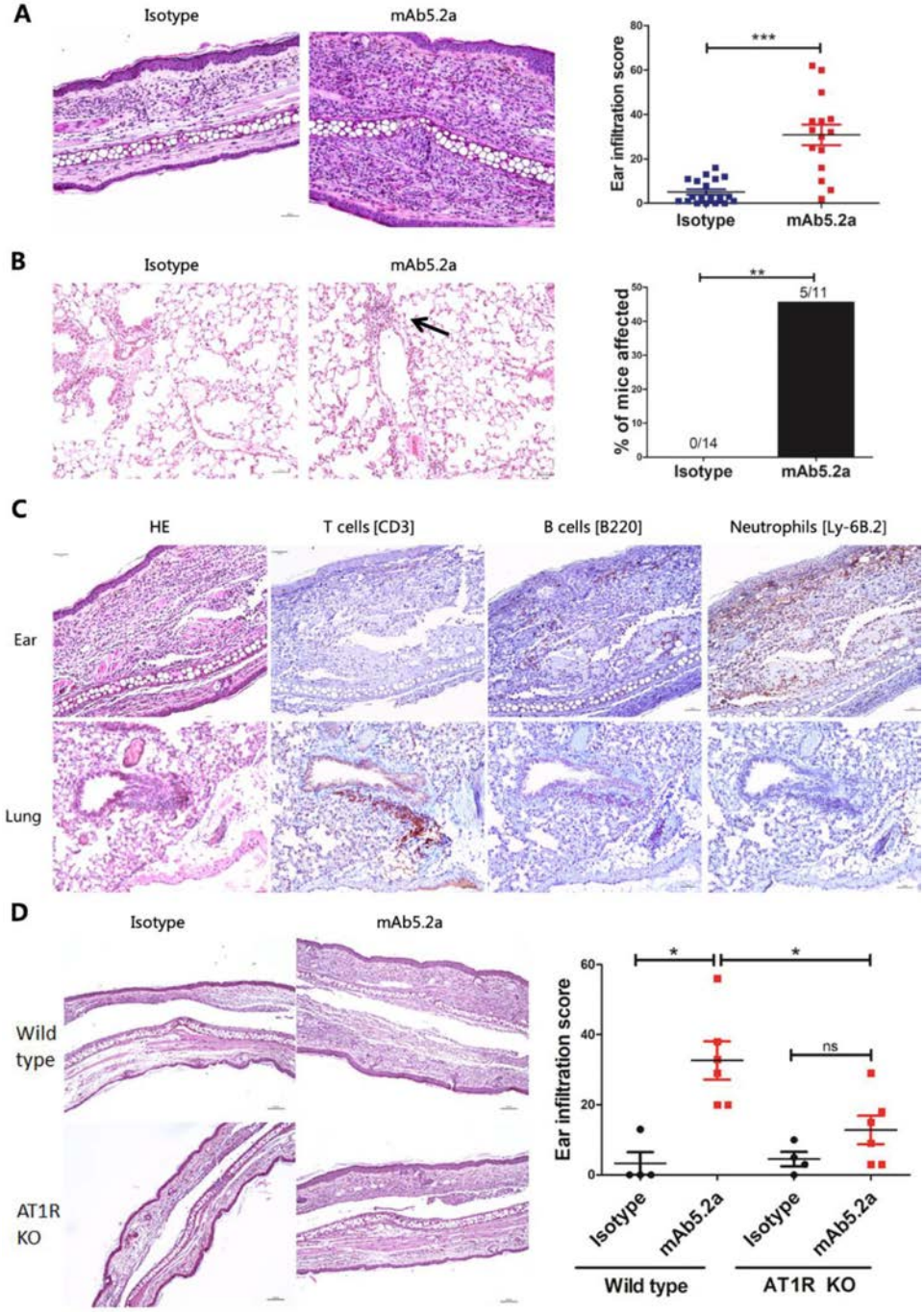

Figure 5 Treatment with a monoclonal AT1R Ab induces skin and lung inflammation in vivo. Each mouse was injected intradermally into the ear repeatedly every other day from day 0 to day 12 (100 µg of anti-AT1R or isotype control IgG/injection). On day 14, the mice were sacrificed, and inflammation in the antibody-treated ear and in the lungs was evaluated histologically. (A) Representative histology of ears injected with mAT1R AB (right) or isotype IgG (IgG2a, left) (200×, scale bar=50 µm). Skin inflammatory scores of ears treated with mAT1R Ab or isotype IgG. The results are presented as mean±SD, and statistical analysis was performed using Student's t-test (***p<0.001). (B) Representative micrographs of H&E-stained lung sections of mice treated with mAT1R AB (right) or isotype IgG (left). Incidence of pulmonary inflammation in mice injected with mAT1R Ab or isotype IgG (**p<0.01, Fisher exact test). (C) Characterisation of inflammatory infiltrations of ears and lungs of mice treated with mAT1R AB. T cells, B cells and neutrophils were detected by IHC. Representative pictures are shown. Scale bar=50 µm. (D) Representative micrographs of H&E-stained ear sections from wild-type and AT1R-deficient mice, which received mAb5.2a or murine IgG2a isotype control. Scale bar=100 µm. Quantified analysis of the severity of inflammation in the ear skin. Statistical analysis was performed using Student's t test or Mann-Whitney U test. *p<0.05. AT1R, angiotensin receptor type 1.

detected. These manifestations were not observed in control mice receiving ME without AT1R overexpression. $CD4^+$ T cells and B cells are indispensable for the AT1R-induced phenotype and immunisation with an AT1R peptide predicted to bind best to MHC class II also induced a phenotype. Moreover, local and repeated immunisation of C57BL/6J mice with an mAT1R Ab generated based on AT1R immunisation also induced skin and lung inflammation. Of note, when AT1Ra/b KO mice were

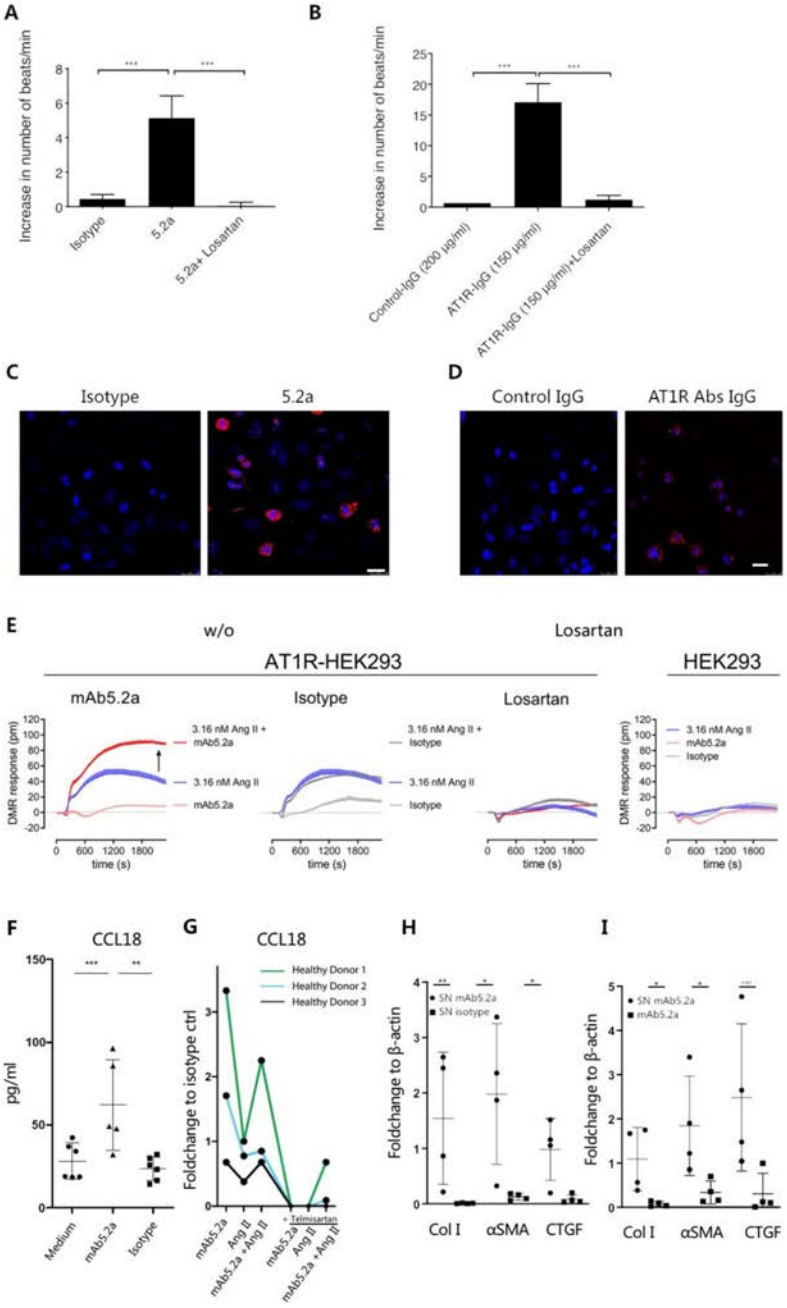

Figure 6 AT1R Abs interact with AT1R-expressing rodent and human cells in vitro. (A) Increase of the beating frequency of cardiomyocyte induced by the mAT1R ab or (B) by IgG isolated from AT1R-immunised mice and blockade by 1 µM losartan. The results are presented as mean±SD, statistical analyses were performed using one-way analysis of variance and Bonferroni's multiple comparisons test (***p<0.001). (C) Uptake of an mAT1R Ab (clone 5.2a) or (D) of IgG isolated from AT1R immunised mice by human epithelial type 2 (HEp-2) cells (630×, scale bar=25 µm) detected by IF showing mouse IgG deposits in red and nuclei (DAPI) in blue. (E) Label-free biosensing of human AT1R-overexpressing HEK293 cells exposed to mAb5.2a or IgG2a isotype control (50 µg/mL) either alone or with agonist Ang II measured by DMR. Arrow in the left panel indicates enhancement of Ang II signal by mAb5.2a. AT1R specificity is shown by pretreatment with 10 µM AT1R antagonist losartan and by stimulating cells without exogenous AT1R enrichment showing no Ab response. Representative kinetic recordings are shown as mean+SEM of a triplicate determination. (F) Human peripheral monocytes were stimulated with recombinant AT1R monoclonal antibody 5.2 a, IgG2a isotype control and unconditioned medium control or (G) with recombinant AT1R mAb 5.2a, Ang II or both with or without telmisartan. CCL18 levels in supernatants (SN) were measured. (H, I) Densitometric analysis of Western blots show collagen 1, α-SMA and CTGF expression of monocyte SN-treated or mAT1R Ab-treated dermal FBS (n=4) relative to β-actin (image Studio Lite). For (H) conditioned SN from mAb 5.2a-stimulated or isotype-stimulated human blood monocytes or for (I) FB stimulated with conditioned SN from mAb 5.2a or with the mAb5.2a alone. Ratio t-test was used to test statistical significance. All data are shown as mean±SD.

immunised with the mAT1R Ab, skin inflammation was reduced when compared with wild type, suggesting interaction between AT1R Abs and AT1R. These data support former in vitro studies showing a correlation between AT1R ab levels and lymphocyte migration.[17] The leucocyte infiltration observed in lung and skin following AT1R immunisation or local application of an mAT1R Ab for the first time implicates that anti-GPCR Abs contribute to immune cell trafficking in vivo. Therefore, our study supports

the idea that AT1R Abs and possibly other anti-GPCR Abs play a role in the regulation of immune cell migration to specific organs.[27]

As mentioned above, following immunisation with AT1R, increased collagen expression and skin thickening were also observed. Interestingly, infusion of Ang II in the skin of C57BL/6J mice results in local inflammation and fibrosis.[28] Taking into account that AT1R Abs have agonistic and synergistic effects to Ang II, such AT1R Abs could indeed augment the effect of Ang II to develop skin fibrosis.[29] Accordingly, supernatants from mAT1R Ab-stimulated monocytes induced α-SMA, CTGF and collagen I in primary dermal fibroblasts.

Here, ME from CHO cells overexpressing AT1R were used for immunisation in order to mimic the natural structure of a transmembrane protein. Applying this approach, functionally active AT1R Abs were induced in vivo. However, in patients with SSc, high AT1R Ab levels were shown to be associated with vascular complications.[11 15] Herein, although perivascular inflammation and endothelial apoptosis were observed, we did not detect signs of obliterative vasculopathy. In addition, the degree of skin fibrosis does not correlate with the Ab levels, which suggests additional mechanisms. Both obliterative vasculopathy and fibrosis are often associated with the presence of Th2 cytokines, which were shown to be predominant in SSc.[30 31] These pathologies also require a bidirectional cross-talk between immune and stromal cells.[32] When using complete/incomplete Freund's adjuvant (CFA/IFA) like herein, a strong Th1 immune response will be induced.[33] Therefore, we cannot exclude that a specific cytokine microenvironment or pathogenic factors inducing Th2 cytokines are required to develop obliterative vasculopathy or more robust fibrosis. In the future, the mouse model established herein could be employed to analyse potentially important further pathways such as the specific role of T cells, for example, by adoptive transfer experiments, or of specific cytokines. Accordingly, as suggested by first experiments on Th2 background, our immunisation strategy could pave the way to establish animal models, for example, for SSc with a more robust phenotype for fibrosis and vasculopathy. The results presented here fit to our novel humanised mouse model, where transfer of peripheral blood mononuclear cells (PBMC) derived from patients with SSc, but not from rituximab-treated patients with SSc, induced inflammatory lung disease indicating the role of B cells and Abs in the early inflammatory phase of SSc.[34] Moreover, as shown in our recent study, AT1R Abs were among the best to identify severe COVID-19 infection, which indicates their relevance in lung inflammation also due to other diseases.[35]

In vitro studies using rat cardiomyocytes, HEp-2 cells and human monocytes yielded that the interaction with both the murine mAT1R Ab and the model-derived IgG containing polyclonal AT1R Abs induced cellular responses which can be blocked by AT1R antagonists.[36] Moreover, the murine mAT1R Ab exhibited agonistic effects alone and in combination with Ang II depending on the cellular function as indicated by stimulation of cardiomyocytes, primary monocytes or by measuring morphological changes of the AT1R-transfected HEK293 cells via DMR.[37] In the latter, induction of a signal by stimulation with both Ang II and mAT1R Abs, but not by mAT1R Ab alone, may fit to the idea that GPCR Abs act allosteric and modulate the effect of an orthosteric ligand such as Ang II towards a prolonged stimulation of the receptor.[14 29] Besides, it has been reported that AT1R Abs from patients with preeclampsia limit the AT1R internalisation.[38] Thus, future experiments could be envisioned to determine if AT1R Abs affect AT1R internalisation by various cells as well and if or how this influences G protein signalling. Overall, interactions between Ang II, AT1R Abs and AT1R need to be investigated in more detail. As recently demonstrated, AT1R Abs as well as other GPCR Abs feature disease-specific interactions with other proteins and receptors that are very likely to affect their specific signalling. Accordingly, a strong cross-talk of AT1R Abs to the endothelin receptor type-1 has been described.[15] Additional work is necessary to find out whether those cross-talks can become a target of therapeutic intervention.[27]

Taken together, induction of AT1R Abs went along with signs of skin and lung inflammation, in particular lymphocytic alveolitis, perivascular infiltrations, endothelial apoptosis and skin fibrosis. Moreover, the induction of skin and lung inflammation following immunisation with the mAT1R Ab indicates that AT1R Abs can indeed directly contribute to the development of symptoms found in SSc. AT1R Abs stimulated AT1R across species. Thus, our mouse model offers a new perspective to examine the role of GPCR Abs in vivo alone and in combination with other ligands or factors. AT1R Abs should come into the focus to develop new therapies for diseases associated with high levels of AT1R Abs.

Author affiliations
[1]Priority Area Asthma & Allergy, Research Center Borstel, Airway Research Center North (ARCN), Members of the German Center for Lung Research (DZL), Borstel, Germany
[2]CellTrend GmbH, Luckenwalde, Germany
[3]Rheumatology and Clinical Immunology, University Medical Center Schleswig Holstein Lübeck Campus, Lubeck, Germany
[4]Xiamen University Faculty of Medicine and Life Sciences, Xiamen, Fujian, China
[5]Rheumatology, University Medical Center Schleswig Holstein Lübeck Campus, Lubeck, Germany
[6]Berlin Cures GmbH, Berlin, Germany
[7]Section Molecular, Cellular and Pharmacobiology, Institute for Pharmaceutical Biology, University of Bonn, Bonn, Germany
[8]Department of Rheumatology and Clinical Immunology, University of Lübeck, Lubeck, Germany
[9]Lübeck Institute of Experimental Dermatology, Luebeck, Germany

Correction notice This article has been corrected since it published Online First. The affiliations and equal contributor statement have both been updated and Prof Petersen has been added as corresponding author.

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Competing interests HH is the owner and GR is an advisor of the company CellTrend GmbH, which produced the ME and the tests for the detection of AT1R Abs. IS and GW are shareholders of Berlin Cures GmbH, where the rat cardiomyocyte assay was performed.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and for donation of fibroblasts, each individual donor submitted an informed written consent and the ethics committee of the Medical Faculty of the University of Lübeck approved the study (21-191). Participants gave informed consent to participate in the study before taking part.

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ORCID iDs
Xiaoyang Yue http://orcid.org/0000-0002-8571-7048
Xiaoqing Wang http://orcid.org/0000-0002-4399-2746
Hanna Grasshoff http://orcid.org/0000-0002-9451-7662
Gabriela Riemekasten http://orcid.org/0000-0002-5406-2464

REFERENCES

1 Murphy TJ, Alexander RW, Griendling KK, *et al*. Isolation of a cDNA encoding the vascular type-1 angiotensin II receptor. *Nature* 1991;351:233–6.
2 Pei F, Wang X, Yue R, *et al*. Differential expression and DNA methylation of angiotensin type 1A receptors in vascular tissues during genetic hypertension development. *Mol Cell Biochem* 2015;402:1–8.
3 Zhang H, Unal H, Gati C, *et al*. Structure of the angiotensin receptor revealed by serial femtosecond crystallography. *Cell* 2015;161:833–44.
4 Goodfriend TL, Elliott ME, Catt KJ. Angiotensin receptors and their antagonists. *N Engl J Med* 1996;334:1649–55.
5 Forrester SJ, Booz GW, Sigmund CD, *et al*. Angiotensin II signal transduction: an update on mechanisms of physiology and pathophysiology. *Physiol Rev* 2018;98:1627–738.
6 Sasaki K, Yamano Y, Bardhan S, *et al*. Cloning and expression of a complementary DNA encoding a bovine adrenal angiotensin II type-1 receptor. *Nature* 1991;351:230–3.
7 Allen AM, Zhuo J, Mendelsohn FA. Localization and function of angiotensin AT1 receptors. *Am J Hypertens* 2000;13:S31–8.
8 Yu L, Shao C, Gao L. Developmental expression patterns for angiotensin receptors in mouse skin and brain. *J Renin Angiotensin Aldosterone Syst* 2014;15:139–49.
9 Yoshida H, Kakuchi J, Guo DF, *et al*. Analysis of the evolution of angiotensin II type 1 receptor gene in mammals (mouse, rat, bovine and human). *Biochem Biophys Res Commun* 1992;186:1042–9.
10 Dragun D, Müller DN, Bräsen JH, *et al*. Angiotensin II type 1-receptor activating antibodies in renal-allograft rejection. *N Engl J Med* 2005;352:558–69.
11 Riemekasten G, Philippe A, Näther M, *et al*. Involvement of functional autoantibodies against vascular receptors in systemic sclerosis. *Ann Rheum Dis* 2011;70:530–6.
12 Xia Y, Kellems RE. Angiotensin receptor agonistic autoantibodies and hypertension: preeclampsia and beyond. *Circ Res* 2013;113:78–87.
13 Lefaucheur C, Viglietti D, Bouatou Y, *et al*. Non-HLA agonistic anti-angiotensin II type 1 receptor antibodies induce a distinctive phenotype of antibody-mediated rejection in kidney transplant recipients. *Kidney Int* 2019;96:189–201.
14 Skiba MA, Kruse AC. Autoantibodies as endogenous modulators of GPCR signaling. *Trends Pharmacol Sci* 2021;42:135–50.
15 Becker MO, Kill A, Kutsche M, *et al*. Vascular receptor autoantibodies in pulmonary arterial hypertension associated with systemic sclerosis. *Am J Respir Crit Care Med* 2014;190:808–17.
16 Avouac J, Riemekasten G, Meune C, *et al*. Autoantibodies against endothelin 1 type A receptor are strong predictors of digital ulcers in systemic sclerosis. *J Rheumatol* 2015;42:1801–7.
17 Günther J, Kill A, Becker MO, *et al*. Angiotensin receptor type 1 and endothelin receptor type A on immune cells mediate migration and the expression of IL-8 and CCL18 when stimulated by autoantibodies from systemic sclerosis patients. *Arthritis Res Ther* 2014;16:R65.
18 Kill A, Tabeling C, Undeutsch R, *et al*. Autoantibodies to angiotensin and endothelin receptors in systemic sclerosis induce cellular and systemic events associated with disease pathogenesis. *Arthritis Res Ther* 2014;16:R29.
19 Rademacher J, Kill A, Mattat K, *et al*. Monocytic angiotensin and endothelin receptor imbalance modulate secretion of the profibrotic chemokine ligand 18. *J Rheumatol* 2016;43:587–91.
20 van Esch JHM, Gembardt F, Sterner-Kock A, *et al*. Cardiac phenotype and angiotensin II levels in AT1A, AT1B, and AT2 receptor single, double, and triple knockouts. *Cardiovasc Res* 2010;86:401–9.
21 Schröder R, Janssen N, Schmidt J, *et al*. Deconvolution of complex G protein-coupled receptor signaling in live cells using dynamic mass redistribution measurements. *Nat Biotechnol* 2010;28:943–9.
22 Schröder R, Schmidt J, Blättermann S, *et al*. Applying label-free dynamic mass redistribution technology to frame signaling of G protein-coupled receptors noninvasively in living cells. *Nat Protoc* 2011;6:1748–60.
23 Marsigliante S, Resta L, Muscella A, *et al*. At1 angiotensin II receptor subtype in the human larynx and squamous laryngeal carcinoma. *Cancer Lett* 1996;110:19–27.
24 Wallukat G, Wollenberger A, Morwinski R, *et al*. Anti-Beta 1-adrenoceptor autoantibodies with chronotropic activity from the serum of patients with dilated cardiomyopathy: mapping of epitopes in the first and second extracellular loops. *J Mol Cell Cardiol* 1995;27:397–406.
25 Cabral-Marques O, Riemekasten G. Functional autoantibodies targeting G protein-coupled receptors in rheumatic diseases. *Nat Rev Rheumatol* 2017;13:648–56.
26 Riemekasten G, Petersen F, Heidecke H. What makes antibodies against G protein-coupled receptors so special? a novel concept to understand chronic diseases. *Front Immunol* 2020;11:1–9.
27 Cabral-Marques O, Marques A, Giil LM, *et al*. GPCR-specific autoantibody signatures are associated with physiological and pathological immune homeostasis. *Nat Commun* 2018;9:5224.
28 Stawski L, Han R, Bujor AM, *et al*. Angiotensin II induces skin fibrosis: a novel mouse model of dermal fibrosis. *Arthritis Res Ther* 2012;14:R194.
29 Sorohan BM, Ismail G, Leca N, *et al*. Angiotensin II type 1 receptor antibodies in kidney transplantation: an evidence-based comprehensive review. *Transplant Rev* 2020;34:100573.
30 Daley E, Emson C, Guignabert C, *et al*. Pulmonary arterial remodeling induced by a Th2 immune response. *J Exp Med* 2008;205:361–72.
31 O'Reilly S, Hügle T, van Laar JM. T cells in systemic sclerosis: a reappraisal. *Rheumatology* 2012;51:1540–9.
32 Worrell JC, O'Reilly S. Bi-Directional communication: conversations between fibroblasts and immune cells in systemic sclerosis. *J Autoimmun* 2020;113:102526.
33 Billiau A, Matthys P. Modes of action of Freund's adjuvants in experimental models of autoimmune diseases. *J Leukoc Biol* 2001;70:849–60.
34 Yue X, Petersen F, Shu Y, *et al*. Transfer of PBMC from SSC patients induces autoantibodies and systemic inflammation in Rag2-/-/IL2rg-/- mice. *Front Immunol* 2021;12:1–12.
35 Cabral-Marques O, Halpert G, Schimke LF, *et al*. Autoantibodies targeting GPCRs and Ras-related molecules associate with COVID-19 severity. *Nat Commun* 2022;13:1220.
36 Karnik SS, Unal H, Kemp JR, *et al*. International Union of Basic and Clinical Pharmacology. XCIX. Angiotensin Receptors: Interpreters of Pathophysiological Angiotensinergic Stimuli [corrected]. *Pharmacol Rev* 2015;67:754–819.
37 Grundmann M, Merten N, Malfacini D, *et al*. Lack of beta-arrestin signaling in the absence of active G proteins. *Nat Commun* 2018;9:1–7.
38 Bian J, Lei J, Yin X, *et al*. Limited AT1 receptor internalization is a novel mechanism underlying sustained vasoconstriction induced by AT1 receptor autoantibody from preeclampsia. *J Am Heart Assoc* 2019;8:e011179.

TRANSLATIONAL SCIENCE

Methylome and transcriptome profiling of giant cell arteritis monocytes reveals novel pathways involved in disease pathogenesis and molecular response to glucocorticoids

Elkyn Estupiñán-Moreno ,[1] Lourdes Ortiz-Fernández ,[1] Tianlu Li,[2] Jose Hernández-Rodríguez,[3] Laura Ciudad,[2] Eduardo Andrés-León,[1] Laura Carmen Terron-Camero,[1] Sergio Prieto-González,[3] Georgina Espígol-Frigolé,[3] Maria Cinta Cid ,[3] Ana Márquez ,[1,4] Esteban Ballestar ,[2] Javier Martín [1]

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For numbered affiliations see end of article.

Correspondence to
Dr Lourdes Ortiz-Fernández and Dr Javier Martín, Institute of Parasitology and Biomedicine López-Neyra, IPBLN, Consejo Superior de Investigaciones Cientificas, CSIC, 18016 Granada, Spain; lourdes@ipb.csic.es, javiermartin@ipb.csic.es and Dr Esteban Ballestar, Epigenetics and Immune Disease Group, Josep Carreras Research Institute (IJC), 08916 Badalona, Barcelona, Spain; eballestar@carrerasresearch.org

EE-M and LO-F contributed equally.

EE-M and LO-F are joint first authors.
AM, EB and JM are joint senior authors.

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ABSTRACT

Objectives Giant cell arteritis (GCA) is a complex systemic vasculitis mediated by the interplay between both genetic and epigenetic factors. Monocytes are crucial players of the inflammation occurring in GCA. Therefore, characterisation of the monocyte methylome and transcriptome in GCA would be helpful to better understand disease pathogenesis.

Methods We performed an integrated epigenome- and transcriptome-wide association study in CD14+ monocytes from 82 patients with GCA, cross-sectionally classified into three different clinical statuses (active, in remission with or without glucocorticoid (GC) treatment), and 31 healthy controls.

Results We identified a global methylation and gene expression dysregulation in GCA monocytes. Specifically, monocytes from active patients showed a more proinflammatory phenotype compared with healthy controls and patients in remission. In addition to inflammatory pathways known to be involved in active GCA, such as response to IL-6 and IL-1, we identified response to IL-11 as a new pathway potentially implicated in GCA. Furthermore, monocytes from patients in remission with treatment showed downregulation of genes involved in inflammatory processes as well as overexpression of GC receptor-target genes. Finally, we identified changes in DNA methylation correlating with alterations in expression levels of genes with a potential role in GCA pathogenesis, such as *ITGA7* and *CD63*, as well as genes mediating the molecular response to GC, including *FKBP5*, *ETS2*, *ZBTB16* and *ADAMTS2*.

Conclusion Our results revealed profound alterations in the methylation and transcriptomic profiles of monocytes from GCA patients, uncovering novel genes and pathways involved in GCA pathogenesis and in the molecular response to GC treatment.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Giant cell arteritis (GCA) is a complex disease mediated by multiple genetic and epigenetic factors, in which CD14+ monocytes play an important role driving the inflammatory processes occurring in this vasculitis.

⇒ The study of the DNA methylation and gene expression profiles of disease-relevant cell types, as well as the integration of omics-datasets, has emerged as a successful approach to better understand the pathogenesis of complex diseases.

WHAT THIS STUDY ADDS

⇒ We evaluate for the first time the DNA methylome and transcriptome landscapes of CD14+ monocytes from patients with GCA in three different states of the disease (patients with active disease, patients in remission with or without treatment), identifying profound alterations that provide evidence of novel genes and pathways potentially involved in GCA pathogenesis.

⇒ The results of this integrative approach allowed the identification of a significant number of CpG-gene expression interactions, including important genes potentially involved in the molecular mechanisms implicated in the active state of the disease, such as *ITGA7* and *CD63*, as well as genes mediating the molecular response to glucocorticoids, including *FKBP5*, *ETS2*, *ZBTB16* and *ADAMTS2*.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE AND/OR POLICY

⇒ A better understanding of the pathogenesis of GCA might result in the identification of potential biomarkers that allow advances in early diagnosis, classification and therapy for GCA.

INTRODUCTION

Giant cell arteritis (GCA) is a systemic vasculitis with complex aetiology, presenting a wide range of clinical manifestations.[1] The most serious complications such as irreversible blindness and stroke can be significantly reduced if patients receive prompt treatment with glucocorticoids (GC).[2] However, early recognition of GCA can be challenging due to its clinical heterogeneity, including the presence of non-specific symptoms, along with the absence of specific biomarkers.[3]

The disease results from dysregulated interactions between the vessel wall and the immune system that lead to inflammation and vascular remodelling of medium and large arteries.[4] Most of our current knowledge has been obtained from examination of temporal artery biopsies, where granulomatous infiltrates consist of both innate and adaptive immune cells.[5] Besides tissue lesions, GCA is characterised by intense systemic inflammation that is driven by IL-6.[6] Although studies focusing on circulating immune cells in GCA are scarce, monocytes are considered major players in the inflammatory process.[7] Indeed, circulating monocytes of GCA patients interact with activated endothelial cells in vasa vasorum and neovessels,[8 9] and develop tissue invasive capabilities by an aberrant production of matrix metalloproteinase (MMP)-9, thus allowing immune cells to access the vascular wall.[10]

It is well established that epigenetic modifications may exert a profound influence on cell function by their capacity to modulate gene expression without altering the DNA sequence.[11] Despite the high relevance of monocytes in GCA, no study has investigated the DNA methylation landscape of this cell type to date. Here, with the aim of shedding light into GCA pathogenesis and identifying molecular mechanisms that might serve as novel biomarkers or potential drug targets, we analysed for the first time the methylome and transcriptome of GCA monocytes as well as the correlation between DNA methylation and gene expression levels.

MATERIAL AND METHODS

Details of the population included in this study, description of the experimental methods, including DNA methylation and RNA sequencing as well as the statistical analyses applied, are provided in online supplemental material.

RESULTS

Dysregulated DNA methylation and gene expression profiles in CD14+ monocytes of patients with GCA

CD14+ monocytes from patients with GCA showed a global hypermethylation pattern

First, the comparison of the DNA methylation landscape of CD14+ monocytes between patients with GCA and controls unveiled the existence of 1371 differentially methylated positions (DMPs), annotated to 1190 unique genes, across the whole genome (figure 1A). Most DMPs were located in intergenic regions (31.8% hypermethylated and 26.6% hypomethylated) and gene bodies (44.3% hypermethylated and 41.4% hypomethylated) and less frequently in promoters, consistent with the findings of studies in other inflammatory conditions,[12 13] which suggests that a substantial part of the methylation aberrations might be located in distal regulatory regions. In addition, DMPs, mainly hypermethylated DMPs, were mostly located in open sea regions, outside CpG island and surrounding areas (figure 1B).

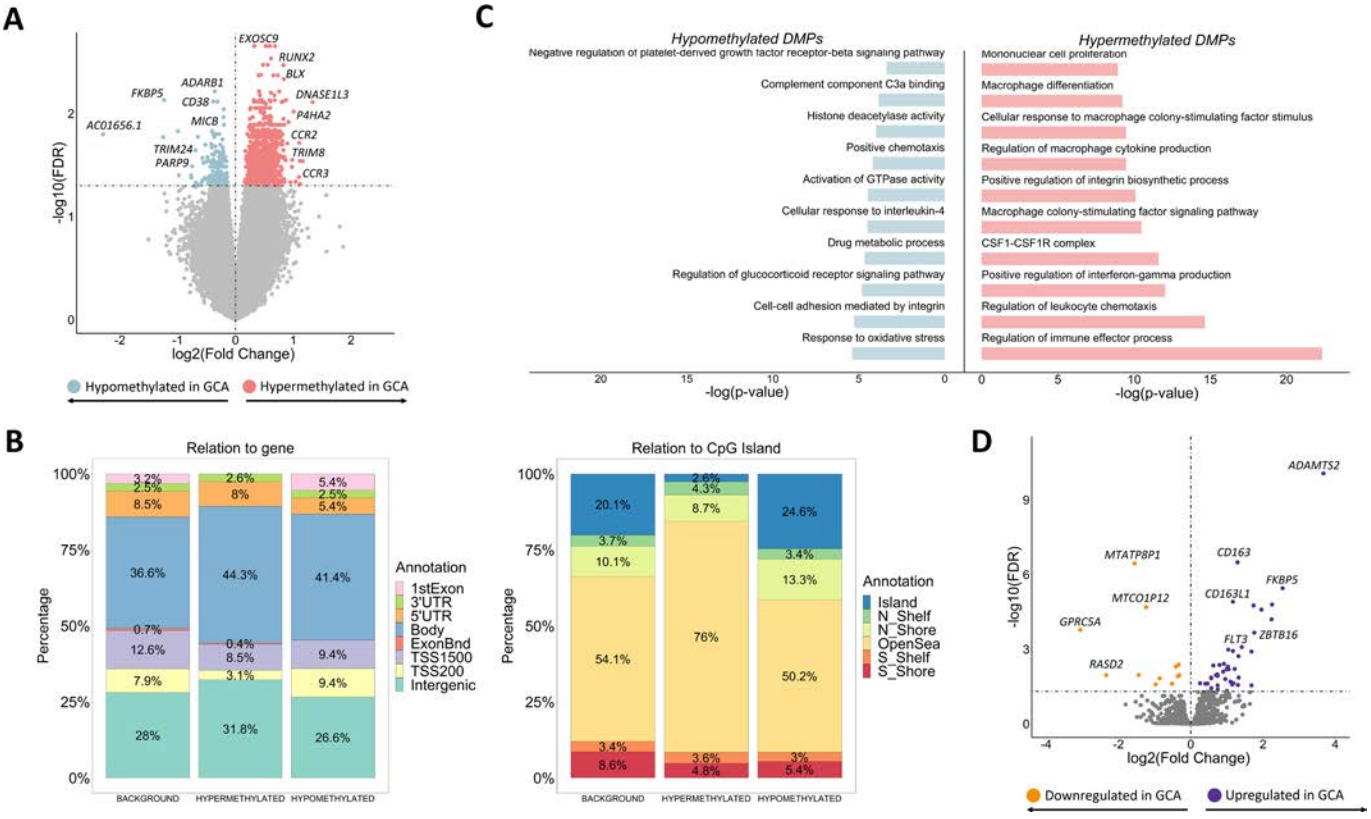

Figure 1 Results from the comparison of both DNA methylation and gene expression patterns of CD14+ monocytes between patients with giant cell arteritis and controls. (A) Volcano plot of the epigenome-wide association study results. False discovery rate (FDR) values are represented on the –log10 scale in the y-axis. Significant threshold (FDR<0.05) is marked by a dashed line. The effect size and direction obtained for each CpG site is depicted in the x-axis. Pink and blue dots represent hypermethylated and hypomethylated differentially methylated positions (DMPs), respectively. (B) Bar plots representing the annotation of the significant hypermethylated and hypomethylated DMPs in relation to CpG island (right panel) and gene location (left panel). (C) Representation of selected gene ontology categories obtained from the DMPs enrichment analysis using the GREAT online tool. (D) Volcano plot of the transcriptome-wide association study results. FDR values are represented on the –log10 scale in the y-axis. Significant threshold (FDR<0.05) is marked by a dashed line. The effect size and direction obtained for each gene is depicted in the x-axis. Purple and orange dots represent upregulated and downregulated differentially expressed genes, respectively. GCA, giant cell arteritis.

Over 85% of the DMPs showed higher DNA methylation levels in patients than in controls. Of note, we identified hypermethylated DMPs located within or close to genes previously associated with immune-mediated diseases, including *P4HA2*, a susceptibility genetic factor for GCA [14] (figure 1A and online supplemental table 1). Through gene ontology analysis, we observed enrichment in functional pathways of the immune response, such as regulation of interferon-gamma (IFN-γ) production, leucocyte chemotaxis and integrin biosynthesis processes. In addition, we detected a significant enrichment in monocyte cell biology pathways, such as the colony stimulating factor 1 (CSF1)-CSF1 receptor complex, differentiation and proliferation of macrophages and cytokine production like macrophage colony-stimulating factor (figure 1C and online supplemental table 2). The hypomethylated DMPs were also mapped to relevant genes in the context of the immune response, like *TRIM24*, *PRDM16*, *PARP9*, *ADARB1*, *CD38* or *MICB* (figure 1A and online supplemental table 3), and were enriched in significant biological processes like cellular response to IL-4, oxidative stress response, positive regulation of chemotaxis, complement component C3a binding and negative regulation of the platelet-derived growth factor-beta receptor (PDGF) signalling pathway (figure 1C, online supplemental table 4).

Global analysis of patients with GCA shows slight alterations of the gene expression profile

Afterwards, we carried out gene expression analysis between CD14+ monocytes from patients with GCA and controls. These results only revealed 54 differentially expressed genes (DEGs), of which 41 were upregulated in GCA patients (figure 1D, online supplemental tables 5-6). In this regard, *ADAMTS2*, *CD163*, *AMPH*, *FLT3* and *IL1R2* were observed to be among the most significantly upregulated DEGs.

Stratified analysis of patients based on clinical status and treatment display specific DNA methylation patterns in CD14+ monocytes

We further stratified patients with GCA according to the state of the disease at the time of sample collection: patients with active disease, patients in remission with treatment and patients in remission without treatment (detailed description in online supplemental material). To better characterise the methylation alterations driving the molecular mechanisms responsible of the active state of GCA, we compared the methylation landscape of patients with GCA with active disease with those showing no sign or symptoms of this vasculitis (healthy controls and patients in remission with and without GC treatment). In addition, we also aimed to evaluate the influence of GC on the DNA methylation patterns of CD14+ monocytes in disease remission by comparing treated and non-treated patients.

We observed a large number of significant DNA methylation alterations between patients with active disease compared with controls and those patients in remission. In contrast, only several CpGs were differentially methylated between patients in remission with and without treatment. A summary of the results obtained in each analysis is shown in figure 2A.

Global DNA methylation alterations reflect the active state of the disease

We first assessed the differences between the methylation status of CD14+ monocytes from patients with active disease and healthy controls, identifying a total of 1444 DMPs (507 hypomethylated and 937 hypermethylated in active patients) (figure 3A and online supplemental tables 7-8). Interestingly, some of these DMPs mapped to genes encoding chemokine receptors such as *CX3CR1*, *CXCR2P1* and *CXCR4*, members of the interferon regulatory transcription factor (IRF) family, including *IRF2* and *IRF8*, as well as genes previously described as susceptibility factors for systemic vasculitides, including *TYK2* and *KDM4C*[15 16]. In addition, hypermethylated DMPs were enriched in multiple pathways and biological processes, including positive regulation of cell activation and cell–cell adhesion and response to tumour necrosis factor (TNF) (figure 3E and online supplemental table 9). Regarding hypomethylated DMPs, a significant enrichment in interesting pathways in the context of GCA pathogenesis was detected, such as regulation of macrophage activation involved in immune response, positive regulation of lymphocyte migration and regulation of inflammatory response, among others (figure 3E and online supplemental table 10).

A

Comparisons	Differentially Methylated Positions (DMPs)		Differentially Expressed Genes (DEGs)	
	Hypermethylated	Hypomethylated	Upregulated	Downregulated
Active-Controls	937	507	209	83
Active-Remission with treatment	105	583	2,701	2,732
Active-Remission without treatment	152	668	327	224
Remission with treatment-Remission without treatment	2	25	1,818	1,732

B

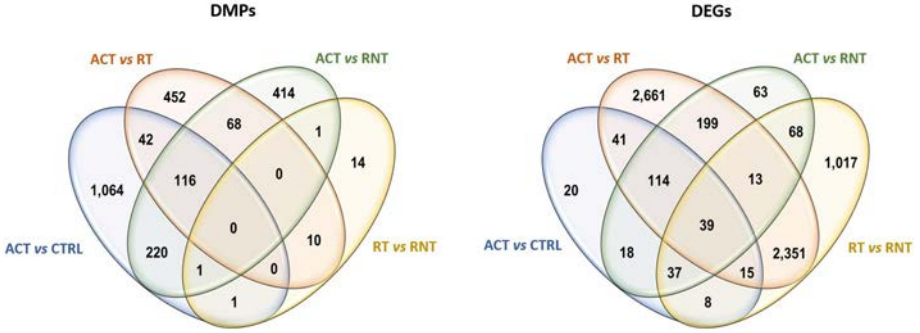

Figure 2 Summary of the results obtained from the stratified analysis of patients according to the state of the disease. (A) Number of significant differentially methylated positions (DMPs) and differentially expressed genes (DEGs) obtained in each comparison. (B) Venn diagrams showing the overlap of significant DMPs (left panel) and DEGs (right panel) among the different comparisons performed. ACT, active disease; CRTL, controls; RNT, remission without treatment; RT, remission with treatment.

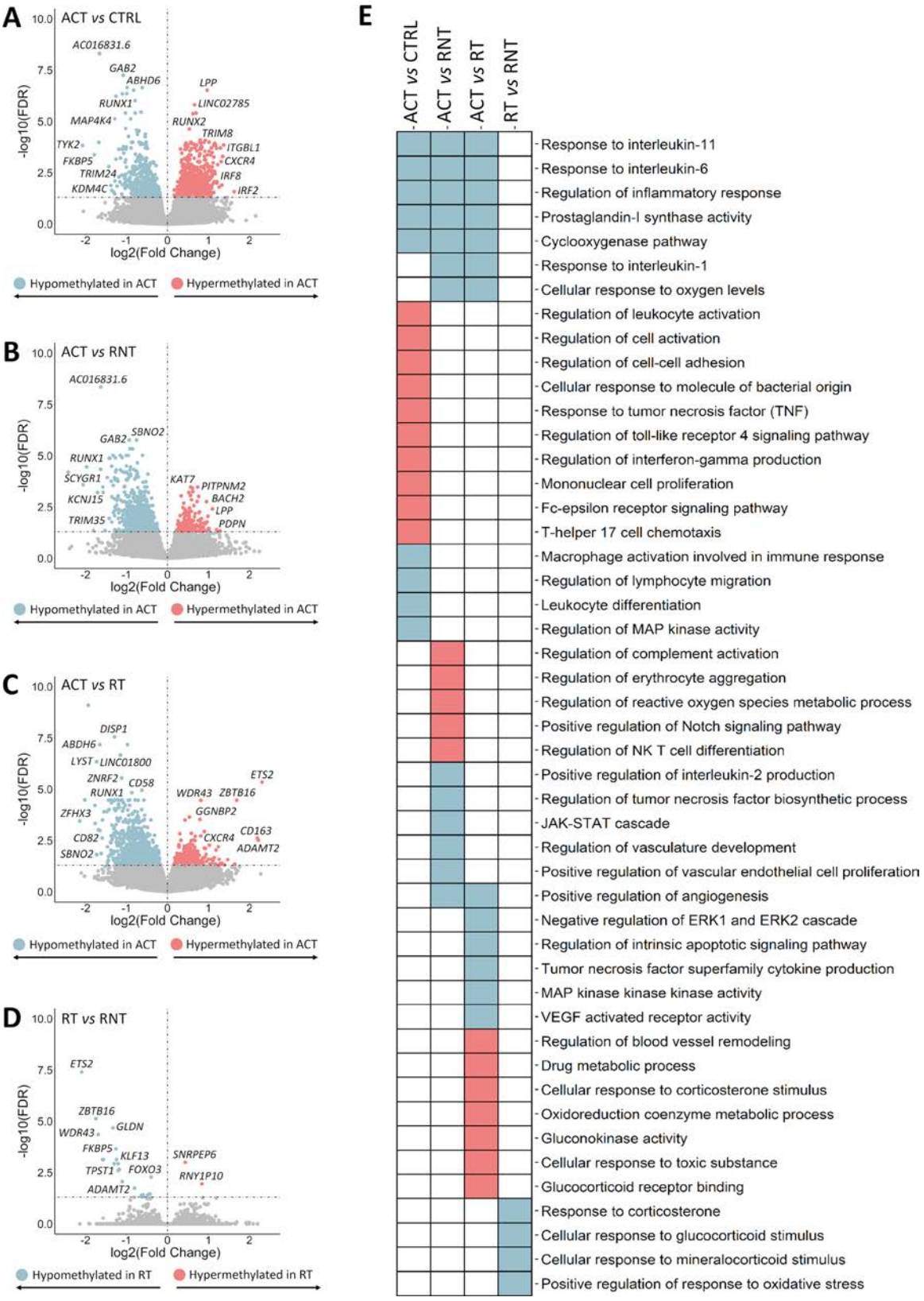

Figure 3 Results of the epigenome-wide association study obtained from the stratified analysis of patients according to the state of the disease. (A–D) Volcano plots showing the results of the epigenome-wide association study for each comparison performed. False discovery rate (FDR) values are represented on the –log10 scale in the y-axis. Significant threshold (FDR<0.05) is marked by a dashed line. The effect size and direction obtained for each CpG site is depicted in the x-axis. Pink and blue dots represent hypermethylated and hypomethylated differentially methylated positions (DMPs), respectively. (E) Scheme summarising the results from the gene ontology enrichment analysis performed using the GREAT online tool. Columns show the different comparisons carried out in the stratified analysis and rows represent selected gene ontology categories. Pink colour denotes statistical significant enrichment of hypermethylated DMPs and blue colour indicates statistical significant enrichment of hypomethylated DMPs. ACT, active disease; CRTL, controls; RT, remission with treatment; RNT, remission without treatment.

Subsequently, we identified substantial alterations in the methylation patterns of monocytes from active disease compared with patients in remission without treatment. Specifically, we identified 820 DMPs (668 hypomethylated and 152 hypermethylated in active patients) (figure 3B and online supplemental tables 11 and 12). Noteworthy, a considerable part of these findings was similar to those obtained in the previous comparison (figure 2B). Aside from the common features, hypomethylated DMPs identified when compared active patients with patients in remission without treatment were enriched in additional biological pathways, such as regulation of reactive oxygen species metabolic processes, positive regulation of IL-2 production, negative regulation of TNF biosynthetic process and regulation of angiogenesis. Moreover, hypermethylated DMPs were enriched in regulatory processes of complement activation, positive regulation of erythrocyte aggregation, regulation of the metabolic process of reactive oxygen species (figure 3E and online supplemental tables 13 and 14).

As expected, CD14+ monocytes from patients with active disease showed large differences in the methylation landscape compared with those from patients in remission with treatment. We identified a total of 688 DMPs, of which 85% presented low levels of methylation in patients with active disease (figure 3C and online supplemental tables 15 and 16). These hypomethylated DMPs were enriched in pathways implicated in the immunopathogenic processes of GCA, including the cellular response to IL-6 as well as response to other members of the IL-6 family, specifically IL-11 (figure 3E and online supplemental table 17).[17 18] In contrast, hypermethylated DMPs were enriched in pathways of drug metabolism, cellular response to GC stimulus and regulation of blood vessel remodelling (figure 3E and online supplemental table 18).

Analysis of the transcription factor (TF) binding motifs showed that the hypomethylated DMPs identified in the active patients compared with controls and patients in remission with and without treatment were enriched in the basic region-leucine zipper (bZIP) family, suggesting that factors in this family might play a key role in the regulation of the molecular mechanisms implicated in the active state of the disease (online supplemental figure 1). Particularly, the bZIP TF family has been reported to regulate the expression of genes involved in angiogenesis, fibrosis and Th17 cells plasticity control.[19–22] Furthermore, IRF family, known to be involved in monocyte/macrophage polarisation,[23] was significantly enriched in the cluster of hypermethylated DMPs in active patients in comparison with controls and patients in remission without treatment.

Remarkably, 65% from the total of DMPs observed in the comparisons between active patients and patients in remission with and without treatment were common (figure 2B). This similarity might be reflecting the success of the GC treatment.

GC treatment greatly affects methylation levels of glucocorticoid receptor target genes

We then compared patients in remission with and without treatment in order to assess the impact of the GC treatment in the DNA methylation landscape of CD14+ monocytes, which revealed 27 CpG sites showing different methylation levels between the two groups (figure 3D and online supplemental table 19). Remarkably, these DMPs, most of which were hypomethylated in patients in remission with treatment, showed high differences in their beta values, for example, *ETS2* (ΔBeta=−0.39) and *ZBTB16* (ΔBeta=−0.38). It should be mentioned that, despite the low number of DMPs, enrichment of biological processes such as response to corticosterone and cellular response to GC stimulus was significantly detected (figure 3E and online supplemental table 20). Furthermore, the analysis of the TF binding motifs revealed that the cluster of hypomethylated DMPs in patients in remission with GC treatment was enriched in GC response elements (online supplemental figure 1), a family of TF reported to repress the activity of the nuclear factor-kappa B (NF-kB) pathway.[24 25]

GCA remission leads to reversal of methylation changes

It should be noted that a significant part of the DMPs identified in the comparison between active patients and controls is common to those detected between active patients and patients in remission without treatment (figure 2B), suggesting that the DNA methylation landscapes of these two groups of individuals are similar. Consistently, no significant DMPs were observed between patients in remission without treatment and healthy controls. This evidence indicates that the DNA methylation alterations occurring in monocytes from individuals with active disease are reverted when the disease subsides.

Identification of aberrant gene expression profiles in CD14+ monocytes through stratified analysis of patients based on clinical status and treatment

Following the same reasoning aforementioned, and considering the few differences observed in the comparison between global GCA patients and healthy controls, we also performed a stratified analysis of the gene expression landscape in patients with GCA according to the state of the disease: patients with active disease, patients in remission with treatment and patients in remission without treatment (detailed description in online supplemental material).

Figure 2A summarises the results obtained through each analysis. Our results revealed that the gene expression profile of CD14+ monocytes largely varies according to the clinical status and treatment. The validity of these results was supported by the high correlation observed for eight deregulated genes (*ITGA7*, *CD63*, *CCRL2*, *CD300E*, *CD163*, *ETS2*, *FKBP5* and *SBNO2*) between ΔCt values obtained by qPCR and their normalised intensities from the RNA-seq (Spearman rank, $|R|=0.79$) (online supplemental figure 2). Furthermore, as expected, we have confirmed the significant deregulation of these eight genes observed among subgroups (online supplemental table 21).

Gene expression deregulation in monocytes affects relevant molecular mechanisms during the active state of the disease

The comparison between the expression patterns of CD14+ monocytes from patients with GCA with active disease and controls revealed 292 DEGs (figure 4A and online supplemental tables 22 and 23). The majority (72%) of DEGs presented higher levels of expression in the subgroup of patients with active disease. Interestingly, overexpressed genes were found to be enriched in related biological processes such as cell adhesion, extracellular matrix disassembly, cell–matrix adhesion, integrin complex, among others. The enrichment in negative regulation of angiogenesis and the positive regulation of TNF production should also be mentioned (figure 4E and online supplemental table 24). Remarkably, the downregulated DEGs were enriched in the major histocompatibility complex (MHC) class II protein complex binding including genes like *HLA-DMB* and *HLA-DOA* (figure 4E and online supplemental table 25).

Subsequently, we compared the gene expression patterns of CD14+ monocytes from active disease and patients in

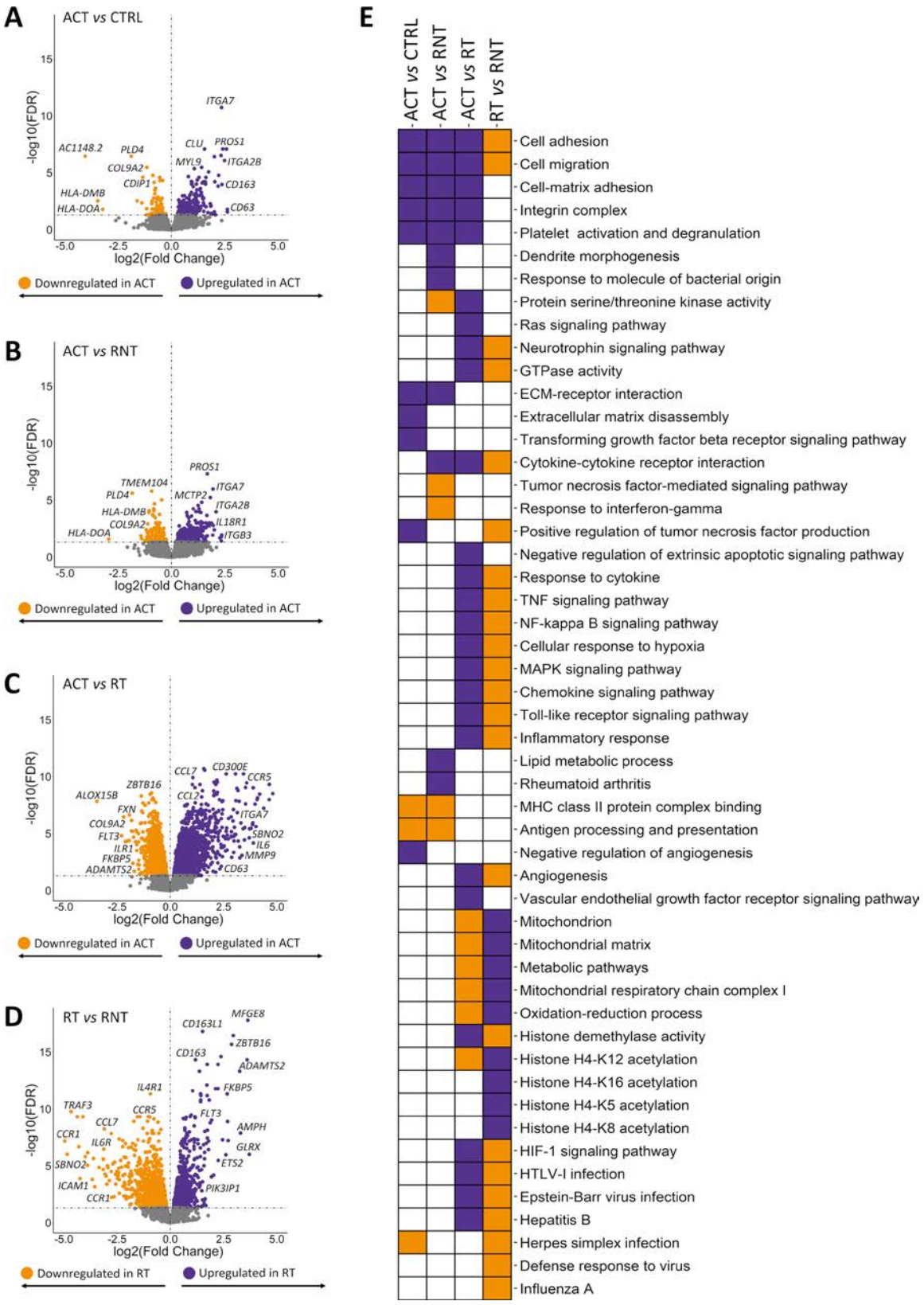

Figure 4 Results of the transcriptome-wide association study obtained from the stratified analysis of patients according to the state of the disease. (A–D) Volcano plots showing the results of the transcriptome-wide association study for each comparison. False discovery rate (FDR) values are represented on the –log10 scale in the y-axis. Significant threshold (FDR<0.05) is marked by a dashed line. The effect size and direction obtained for each gene is depicted in the x-axis. Purple and orange dots represent upregulated and downregulated differentially expressed genes (DEGs), respectively. (E) Scheme summarising the results from the gene ontology enrichment analysis performed using the DAVID online tool. Columns show the different comparisons carried out in the stratified analysis and rows represent selected gene ontology categories. Purple colour denotes statistical significant enrichment of upregulated DEGs and orange colour indicates statistical significant enrichment of downregulated DEGs. ACT, active disease; CRTL, controls; RT, remission with treatment; RNT, remission without treatment.

remission without treatment, identifying 551 DEGs (327 upregulated and 224 downregulated in active patients) (figure 4B and online supplemental tables 26 and 27). The cluster of 327 upregulated DEGs was enriched in pathways including platelet degranulation, cell–matrix adhesion, integrin-mediated signalling pathway, lipid metabolic process, rheumatoid arthritis and cytokine–cytokine receptor interaction (figure 4E and online supplemental table 28). Of special mention, *HLA-DMB*, *HLA-DOA* and *HLA-DRA* were underexpressed in active patients (online supplemental table 27). In this context, these results are in accordance with previous studies reporting a lower expression of *HLA-DRA* in monocytes in inflammatory conditions like sepsis and the immune dysregulation caused by SARS-CoV-2.[26 27] Indeed, among the most significant results of the gene ontology analysis, we observed antigen processing and presentation and MHC class II protein complex binding. In addition, it is also worth highlighting the enrichment of important inflammatory pathways such as TNF-mediated and response to IFN-γ (figure 4E and online supplemental table 29).

Afterwards, we observed greater significant differences between the expression profiles of CD14+ monocytes from patients with GCA with active disease and patients in remission with treatment. 5433 DEGs were identified, of which 2701 and 2732 DEGs were upregulated and downregulated in the active disease group, respectively (figure 4C and online supplemental tables 30 and 31). These results were consistent with the previous knowledge of GCA pathogenesis. In this sense, *IL-6* and *MMP9,* as well as other members of the MMP family (*MMP2*, *MMP24*, *MMP14*, *MMP19* and *MMP25*), were upregulated in active patients. We also detected overexpression of several genes of the integrin family, such as *ITGA2B*, *ITGA5*, *ITGA6*, *ITGA7*, *ITGAX*, *ITGAV*, *ITGB1, ITGB3*, *ITGB5, ITGB7* and *ITGB8*, as well as other remarkable genes that are important in monocyte cell biology like *CCR2*, *CCL2*, *CCL7*, *CXCL5*, *CXCL2* and *CXCL3* (online supplemental table 30).[28] Of note, important biological processes and pathways involved in GCA pathogenesis such as angiogenesis, TNF signalling pathway, vascular endothelial growth factor (VEGF) receptor pathway, chemokine signalling, mitogen-activated protein kinase (MAPK) cascade, Toll-like receptor signalling (TLR) pathway and cellular response to IL-6, were enriched among the set of upregulated genes in patients with active disease (figure 4E and online supplemental table 32). Among the large number of downregulated DEGs, it is notable the presence of genes related with GC such as *FKBP5*, a cochaperone that modulates GC receptor (GR) activity, *ZBTB16*, transcriptional factor contributing to energy balance after GR activation and *HPGD* which encondes a dehydrogenase expressed on dexamethasone (online supplemental table 31).[29–32] Accordingly, mitochondrial metabolic process characteristic of drug metabolism, specifically GC, were significantly enriched (figure 4E and online supplemental table 33). In addition, we also found enrichment in negative regulation of the type I interferon production and the innate response as well as metabolic pathways like oxidative phosphorylation or glucose metabolic process. Of special interest is the enrichment of the apoptotic process that included underexpression of crucial genes in the context of immune-mediated disorders like *TNFAIP3*, *DNASE1*, *AIM2* and *PTK2B,* among others. We also detected downregulation of known autoimmunity-related genes, as examples: *PLD4*, *FLT3*, *ERAP2, BTK, MEFV, DNASE1, PADI14, JAZF1* and *GIMAP* family members (*GIMAP1*, *GIMAP2*, *GIMAP4, GIMAP6, GIMAP7* and *GIMAP8*) (online supplemental table 31).

GC treatment reshapes the gene expression landscape of circulating monocytes

The gene expression landscapes of patients in remission with and without treatment were largely different, with 3550 DEGs when comparing both subgroups (1818 upregulated and 1732 downregulated in treated patients) (figure 4D and online supplemental tables 34 and 35). As reflected in gene ontology analysis, GC treatment might be altering a large number of molecular mechanisms, especially those related with mitochondrial function. Interestingly, as similar to the previous comparison, we also observed enrichment in acetylation of several histones, which could indicate that other epigenetic mechanisms, besides DNA methylation, might be affected (figure 4E and online supplemental table 36). On another side, treated patients showed downregulation in multiple inflammatory-related pathways, apoptotic processes and, notably, defence response to viruses, including pathways such as Herpes simplex infection, Epstein-Barr virus infection, Hepatitis B and Influenza A, among others (figure 4E and online supplemental table 37). Unlike the similarity observed in DNA methylation patterns, the large differences identified in the gene expression patterns between treated and untreated patients are supported by previous evidence describing, in other immune contexts, that the GC treatment has an important effect in reshaping the gene expression landscape but relatively low impact in the DNA methylation profile.[13]

Aberrant gene expression profile of monocytes in the active state of the disease is lost after remission

Consistent to what we observed in the methylation analyses, the gene expression patterns of patients in remission without treatment and healthy controls were similar. Indeed, 208 DEGs identified in the comparisons of active patients with patients in remission without treatment and controls were common (figure 2B), and only one gene showed significantly different expression levels between patients in remission without treatment and healthy controls. Specifically, higher expression levels of *MTCO3P12* were observed in patients in remission without treatment (FDR=0.04, logFC=1.51).

Integrative analysis revealed the existence of relationships between DNA methylation changes and gene expression alterations

Finally, by performing an integrative analysis, we aimed to investigate the potential relationship between DNA methylation alterations and gene expression in GCA. We found 10 191 significant CpG-gene expression interactions (FDR<0.05). To focus on the interactions that might be potentially relevant for GCA pathogenesis, we selected 470 CpG-gene expression interactions showing both methylation and gene expression levels significantly associated in at least one of the comparisons performed. Of these, 34 CpG–gene expression interactions were significant in more than one comparison. Among the 436 unique interactions, we detected 409 DEGs (254 upregulated and 155 downregulated) and 195 DMPs (176 hypomethylated and 19 hypermethylated). In addition, we found that 65.53% and 34.47% of the total interactions associated with disease or clinical status were negative and positive correlations, respectively. Finally, when studying the distribution of CpGs in relation to the genes that they interact with, we identified that 10.39% of the

negative and 1.23% of the positive correlations were located at the gene promoter (figure 5A and online supplemental table 38).

We detected interactions that involved relevant genes in the context of the pathophysiology of GCA. One example was *ITGA7*, upregulated in GCA patients with active disease, in which it showed three interactions with different CpGs located in intergenic regions, involving one positive correlation with cg24773560 (r=0.44, FDR=3.03E-02) and two negative correlations with cg08420353 (r=−0.51, FDR=1.85E-03) and cg17016513 (r=−0.41, FDR=4.31E-02). Interestingly, these last two CpGs also showed negative correlation with the *CD63* gene (r=−0.41, FDR=4.35E-02 and r=−0.41, FDR=4.92E-02, respectively), which was also upregulated in active disease (figure 5B).

Correlation between DNA methylation and gene expression levels was also evident for several known GR target genes, all of which were upregulated in the subgroup of patients in remission with treatment. These genes include *FKBP5*, which correlated negatively with cg03546163 (r=−0.51, FDR=1.24E-03); *ETS2*, which showed a negative interaction with cg06804705 that was located in the promoter region of this gene (r=−0.68, FDR=8.12E-09); *ADAMTS2*, which correlated negatively with cg14727962 (r=0.57, FDR=7.52E-06) and cg09068128 (r=−0.60, FDR=1.54E-10), positively with cg00854503 (r=0.55, FDR=3.07E-04) and cg02052156 (r=0.51, FDR=1.63E-03); and *ZBTB16*, which correlated negatively with cg14388315 (r=−0.60, FDR=8.35E-05) and cg25345365 (r=−0.73, FDR=1.23E-11) (figure 5B).

DISCUSSION

The results of the first methylome and transcriptome profiling of GCA monocytes have yielded evidence supporting that the observed widespread alterations are implicated in the molecular mechanisms underlying this disorder. We also found a significant number of genes whose dysregulation in GCA was mediated by an aberrant DNA methylation. In addition, the stratification of patients according to disease activity allowed us to obtain a clearer picture of the changes in both methylation and expression driving the molecular processes involved in disease activity and molecular response to GC treatment.

Monocytes from active patients seemed to have a more proinflammatory phenotype than controls and patients in remission. Supporting the reliability of our results, we observed a dysregulation of pathways involving cytokines and growth factors already known to have a key role in GCA, such as IL-6, TNF, IL-1, IL-4, IL-2, PDGF and VEGF.[6] Interestingly, the response to IL-11 pathway was enriched among the DMPs hypomethylated in active patients with respect to controls and patients in remission with and without treatment. Although additional evidence is needed to establish the response to IL-11 as a new molecular mechanism involved in GCA, the potential role that might play in the active state of the disease is intriguing. IL11, a member of the IL-6 family, has been implicated in a range of disease pathologies by exerting diverse immunological roles.[33] On the one hand, IL11 inhibits activated macrophages by blocking NF-κB translocation,[34] however, several studies have described a proinflammatory function. For example, it has been reported that IL-11 promotes the differentiation of CD4+ T cells into Th17 cells, an important cell type in GCA pathogenesis,[35] in multiple sclerosis, and it has been also implicated in angiogenesis in rheumatoid arthritis patients.[36] Additionally, it has been reported that IL11 is involved in vascular smooth muscle cell phenotype switching, a mechanism that has been proposed to contribute to vascular remodelling in GCA.[37 38] Therefore, further studies will be needed in order to determine the impact of this cytokine in GCA.

Interestingly, functional categories enriched among the set of differential methylated genes and DEGs in active patients suggest a relevant role of monocytes in GCA by recruiting immune cells and through their interaction with lymphocytes, mainly by promoting their differentiation and activation. In this sense, a significant number of genes encoding chemokines, such as *CCL2* and *CCL7*, involved in the recruitment of monocytes, were overexpressed in active patients.[39] Furthermore, genes encoding several integrins were also overexpressed. Specifically, the expression level of *ITGA7* showed the greatest differences between active patients and healthy controls. Integrins are essential in a wide variety of biological processes, including migration, proliferation, cytokine production and activation, apoptosis and angiogenesis,[40] all of which appear to be dysregulated in active patients. Additionally, hypomethylation of both *ITGA7* and *CCRL2*, a chemokine receptor involved in both innate and adaptive immune responses and known to be upregulated in activated cells,[41] correlated with higher gene expression levels in active patients. Interestingly, two of the CpGs affecting *ITGA7* expression also correlated with overexpression of *CD63*. Notably, *CD63* encodes a tetraspanin family member that interacts with integrins, being crucial for the fusion of monocytes to form multinucleated giant cells, which is the hallmark cell type of GCA.[42–45] Finally, *CD300E*, an immune-activating receptor that promotes the expression of activation markers and the production of proinflammatory cytokines and reactive oxygen species in monocytes as well as the survival of this cell type,[46 47] was also among the most significantly upregulated genes in active patients. Interestingly, expression of this gene was proposed to be restricted to CD115+Ly-6Clow/int peripheral blood monocytes in mice, which corresponds to human non-classical (CD14dimCD16+) and intermediate (CD14brightCD16+) monocytes.[48] Accordingly, increased levels of CD16+ monocytes have been found in temporal artery biopsies of patients with GCA. This subset of monocytes is characterised to be more proinflammatory than the classical one (CD14brightCD16neg) and shows a higher capacity to adhere to endothelial cells via CX3CR1,[7] which, notably, also appeared to be hypermethylated in active patients with respect to controls. Taken together, these results could indicate that, similarly to what has been described in GCA biopsies, increased levels of CD16+ monocytes could be present in peripheral blood of active patients.

It should be noted that similar results were found when active patients were compared with both healthy controls and patients in remission, with and without treatment, which suggests that the proinflammatory methylation and expression profiles observed in the active disease are lost during remission. In fact, no differences were found when DNA methylation and gene expression levels were compared between patients in remission without treatment and healthy controls.

Furthermore, our results suggest that GC therapy remodel the epigenome and, more robustly, the transcriptome, resulting in downregulation of genes involved in pathways with a relevant role in GCA pathogenesis, including cell migration and proliferation, apoptosis, angiogenesis, NF-κB, TNF, IFNγ and TLR signalling pathways and positive regulation of cytokines, such as IL-6 and IL-2. Accordingly, several known target genes for GR that are involved in controlling inflammation, such as *ETS2*, *ZBTB16*, *FKBP5*, and *ADAMTS2*,[49–51] appeared among the most upregulated genes in patients in remission with treatment. Notably, expression levels of these four genes negatively

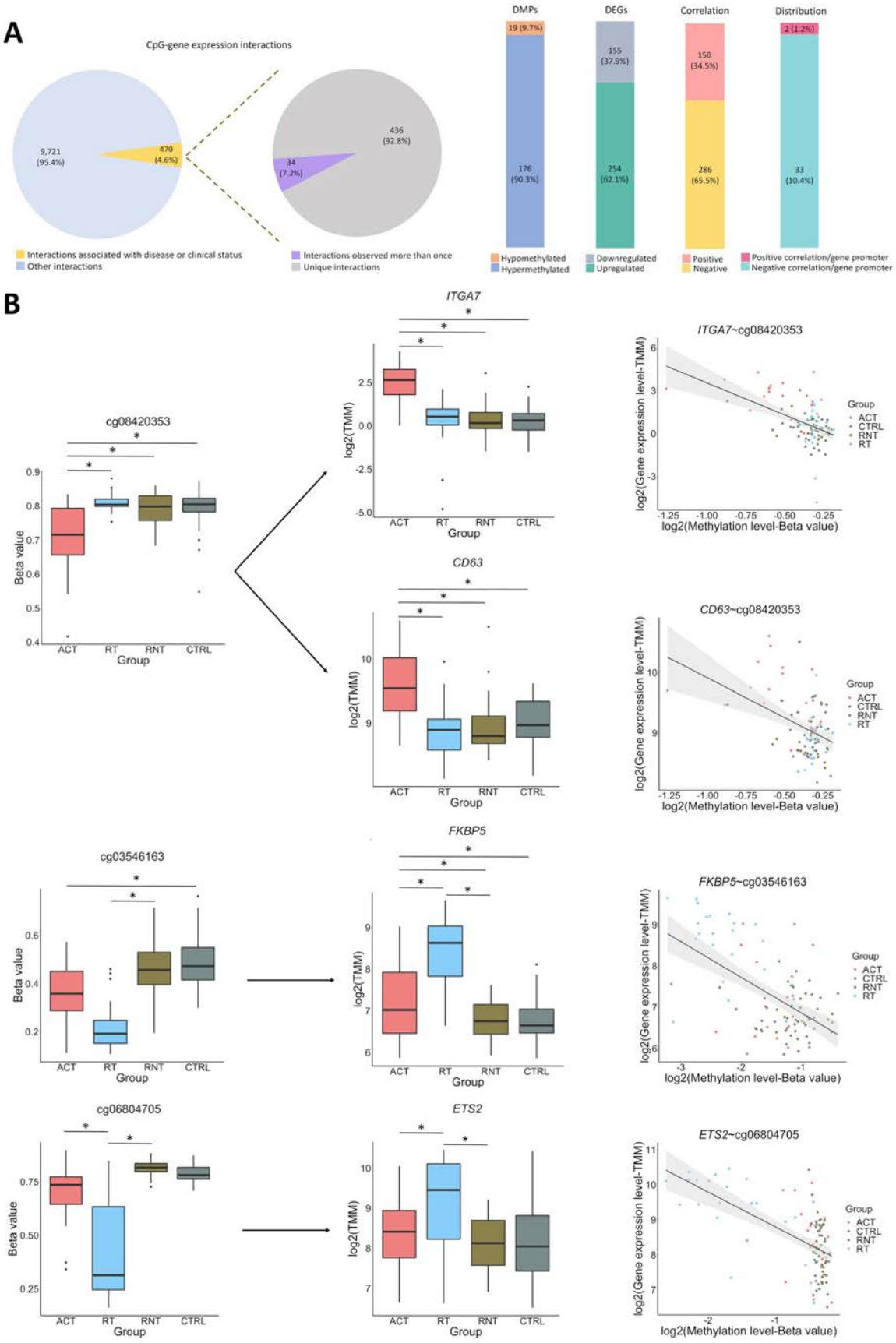

Figure 5 Integrative analysis of DNA methylation and gene expression. (A) General description of the significant CpG-gene expression interactions identified in our analysis. (B) Selected examples of specific CpG-gene expression interactions. Box plots representing both the differentially methylated positions (DMPs) and the differentially expressed genes (DEGs). Significant differences are marked (*FDR<0.05). Graphical representation of the correlation of DNA methylation and gene expression of DMP-DEG pairs is also shown. DNA methylation and gene expression levels are illustrated in each subset of individuals. ACT, active disease; CRTL, controls; RT, remission with treatment; RNT, remission without treatment.

correlated with methylation levels, suggesting that GCs modify gene expression levels through DNA demethylation of target genes.

Interestingly, two scavenger receptors, *CD163* and *CD163L1*, were the most significant overexpressed genes in the subgroup of patients in remission with treatment compared with non-treated patients. CD163 is considered a phenotypic marker of monocytes with anti-inflammatory potential. Specifically, this receptor binds haemoglobin–haptoglobin complexes triggering endocitosis and activating a signalling cascade that results in the production of anti-inflammatory molecules, such as IL-10,[52] another gene upregulated in patients in remission with treatment. Additionally, *CD163L1* has been described to have a role in resolution of inflammation.[53] It should be also noted that, conversely, both *CD163* and *CD163L1* presented higher levels of methylation in active patients compared with patients in remission with treatment that correlated with a decreased gene expression, thus supporting the anti-inflammatory role of these molecules. Remarkably, *CD163* has been found to be significantly increased in temporal artery biopsies from patients with GCA treated with GC.[54]

It has been described that GC can act on naïve monocytes inducing monocytes with an anti-inflammatory profile that may suppress T cell activation, present an increased phagocytic capacity and release anti-inflammatory mediators.[55] Consistently, our results indicate that, in addition to its role promoting the expression of GR-target genes involved in the suppression of inflammation, GC treatment may also promote the expansion of monocytes with an anti-inflammatory phenotype in GCA. In addition, the large differences observed between patients in remission with and without GC treatment suggest that the alterations derived from GC therapy could be reversed in the absence of treatment.

Besides contributing to the elucidation of the pathogenic mechanisms involved in GCA, our study has revealed the existence of specific methylation and transcription profiles in active and GC-treated patients that could potentially improve the clinical care of this vasculitis. In this regard, evaluation of the molecular pattern of GCA monocytes could be especially relevant for early and differential diagnosis as well as for therapy monitoring, thus avoiding a delay in treatment and the use of ineffective drugs. Moreover, we have provided a significant number of molecules that could be targeted in future functional studies and potentially used as biomarkers. For example, *CD163*, which is upregulated after GC treatment in circulating monocytes and temporal artery biopsies, represents an interesting candidate to assess the molecular response to this therapy.

In conclusion, we have performed an exhaustive analysis of the methylome and transcriptome of one of the most relevant cell types in GCA, exploring for the first time the contribution of epigenetic to the disease activity and molecular response to GC in a large cohort of patients. Nevertheless, despite our relevant findings, evaluation of methylation and transcription profiles in additional peripheral cell types and, specially, in temporal artery biopsies will be essential to obtain a clearer picture of the molecular network involved in this type of vasculitis.

Author affiliations
[1]Institute of Parasitology and Biomedicine López-Neyra (IPBLN), Spanish National Research Council (CSIC), Granada, Spain
[2]Epigenetics and Immune Disease Group, Josep Carreras Research Institute (IJC), Badalona, Barcelona, Spain
[3]Department of Autoimmune Diseases, Hospital Clinic, University of Barcelona, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain
[4]Systemic Autoimmune Diseases Unit, Hospital Clinico San Cecilio, Instituto de Investigación Biosanitaria de Granada ibs.GRANADA, Granada, Spain

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Contributors EE-M and LO-F: data analysis, interpretation of the results and manuscript drafting. EA-L and LCT-C: data analysis. TL, LC, GE-F and SP-G.: sample and data collection. JH-R and MCC: study design, sample and data collection. AM, EB and JM: study design, interpretation of the results and manuscript drafting. LO-F and JM: guarantors of the study. All authors read and approved the manuscript.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval The study was approved by the ethical committees of all institutions involved in this study. However, these institutions do not provide a reference number or ID for the ethics approval. Participants gave informed consent to participate in the study before taking part.

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Data availability statement Data are available in a public, open access repository. DNA methylation and expression data for this publication have been deposited in the NCBI Gene Expression Omnibus and are accessible through GEO SuperSeries accession number GSE201754. The code used for the analyses is available from https://github.com/lterroncamero/GCA_CD14_Analysis.git.

ORCID iDs
Elkyn Estupiñán-Moreno http://orcid.org/0000-0002-5818-4389
Lourdes Ortiz-Fernández http://orcid.org/0000-0002-0247-4280
Maria Cinta Cid http://orcid.org/0000-0002-4730-0938
Ana Márquez http://orcid.org/0000-0001-9913-7688
Esteban Ballestar http://orcid.org/0000-0002-1400-2440
Javier Martín http://orcid.org/0000-0002-2202-0622

REFERENCES

1 Jennette JC, Falk RJ, Bacon PA, *et al*. 2012 revised international chapel Hill consensus conference Nomenclature of vasculitides. *Arthritis & Rheumatism* 2013;65:1–11.
2 Gonzalez-Gay MA, Vazquez-Rodriguez TR, Lopez-Diaz MJ, *et al*. Epidemiology of giant cell arteritis and polymyalgia rheumatica. *Arthritis Rheum* 2009;61:1454–61.
3 Koster MJ, Matteson EL, Warrington KJ. Large-Vessel giant cell arteritis: diagnosis, monitoring and management. *Rheumatology* 2018;57:ii32–42.
4 Weyand CM, Goronzy JJ. Immune mechanisms in medium and large-vessel vasculitis. *Nat Rev Rheumatol* 2013;9:731–40.
5 Soriano A, Muratore F, Pipitone N, *et al*. Visual loss and other cranial ischaemic complications in giant cell arteritis. *Nat Rev Rheumatol* 2017;13:476–84.

6 Terrades-Garcia N, Cid MC. Pathogenesis of giant-cell arteritis: how targeted therapies are influencing our understanding of the mechanisms involved. *Rheumatology* 2018;57:ii51–62.
7 van Sleen Y, Wang Q, van der Geest KSM, *et al*. Involvement of monocyte subsets in the immunopathology of giant cell arteritis. *Sci Rep* 2017;7:6553.
8 Foell D, Hernández-Rodríguez J, Sánchez M, *et al*. Early recruitment of phagocytes contributes to the vascular inflammation of giant cell arteritis. *J Pathol* 2004;204:311–6.
9 Cid MC, Cebrián M, Font C, *et al*. Cell adhesion molecules in the development of inflammatory infiltrates in giant cell arteritis: inflammation-induced angiogenesis as the preferential site of leukocyte-endothelial cell interactions. *Arthritis Rheum* 2000;43:184–94.
10 Watanabe R, Maeda T, Zhang H, *et al*. MMP (Matrix Metalloprotease)-9-Producing Monocytes Enable T Cells to Invade the Vessel Wall and Cause Vasculitis. *Circ Res* 2018;123:700–15.
11 Holliday R. Epigenetics: a historical overview. *Epigenetics* 2006;1:76–80.
12 McDermott E, Ryan EJ, Tosetto M, *et al*. Dna methylation profiling in inflammatory bowel disease provides new insights into disease pathogenesis. *J Crohns Colitis* 2016;10:77–86.
13 Morante-Palacios O, Ciudad L, Micheroli R, *et al*. Coordinated glucocorticoid receptor and MafB action induces tolerogenesis and epigenome remodeling in dendritic cells. *Nucleic Acids Res* 2022;50:108–26.
14 Carmona FD, Vaglio A, Mackie SL, *et al*. A genome-wide association study identifies risk alleles in plasminogen and P4HA2 associated with giant cell arteritis. *Am J Hum Genet* 2017;100:64–74.
15 Ortiz-Fernández L, López-Mejias R, Carmona FD, *et al*. The role of a functional variant of Tyk2 in vasculitides and infections. *Clin Exp Rheumatol* 2020;38:949–55.
16 Ortiz-Fernández L, Carmona FD, López-Mejías R, *et al*. Cross-phenotype analysis of Immunochip data identifies *KDM4C* as a relevant *locus* for the development of systemic vasculitis. *Ann Rheum Dis* 2018;77:589–95.
17 Hernández-Rodríguez J, Segarra M, Vilardell C, *et al*. Tissue production of pro-inflammatory cytokines (IL-1beta, TNFalpha and IL-6) correlates with the intensity of the systemic inflammatory response and with corticosteroid requirements in giant-cell arteritis. *Rheumatology* 2004;43:294–301.
18 Cook SA, Schafer S. Hiding in plain sight: interleukin-11 emerges as a master regulator of fibrosis, tissue integrity, and stromal inflammation. *Annu Rev Med* 2020;71:263–76.
19 Hamik A, Wang B, Jain MK. Transcriptional regulators of angiogenesis. *Arterioscler Thromb Vasc Biol* 2006;26:1936–47.
20 Rajasekaran S, Vaz M, Reddy SP. Fra-1/Ap-1 transcription factor negatively regulates pulmonary fibrosis in vivo. *PLoS One* 2012;7:e41611.
21 Chow FY, Nikolic-Paterson DJ, Ozols E, *et al*. Monocyte chemoattractant protein-1 promotes the development of diabetic renal injury in streptozotocin-treated mice. *Kidney Int* 2006;69:73–80.
22 Renoux F, Stellato M, Haftmann C, *et al*. The AP1 transcription factor Fosl2 promotes systemic autoimmunity and inflammation by repressing Treg development. *Cell Rep* 2020;31:107826.
23 Lehtonen A, Veckman V, Nikula T, *et al*. Differential expression of IFN regulatory factor 4 gene in human monocyte-derived dendritic cells and macrophages. *J Immunol* 2005;175:6570–9.
24 Hudson WH, Vera IMSde, Nwachukwu JC, *et al*. Cryptic glucocorticoid receptor-binding sites pervade genomic NF-κB response elements. *Nat Commun* 2018;9:1337.
25 Xavier AM, Anunciato AKO, Rosenstock TR, *et al*. Gene expression control by glucocorticoid receptors during innate immune responses. *Front Endocrinol* 2016;7:31.
26 Giamarellos-Bourboulis EJ, Netea MG, Rovina N, *et al*. Complex immune dysregulation in COVID-19 patients with severe respiratory failure. *Cell Host Microbe* 2020;27:992–1000.
27 Lukaszewicz A-C, Grienay M, Resche-Rigon M, *et al*. Monocytic HLA-DR expression in intensive care patients: interest for prognosis and secondary infection prediction. *Crit Care Med* 2009;37:2746–52.
28 Gren ST, Rasmussen TB, Janciauskiene S, *et al*. A single-cell gene-expression profile reveals Inter-Cellular heterogeneity within human monocyte subsets. *PLoS One* 2015;10:e0144351.
29 Kokkinopoulou I, Moutsatsou P. Mitochondrial glucocorticoid receptors and their actions. *Int J Mol Sci* 2021;22:6054.
30 Zannas AS, Wiechmann T, Gassen NC, *et al*. Gene-Stress-Epigenetic regulation of FKBP5: clinical and translational implications. *Neuropsychopharmacology* 2016;41:261–74.
31 Cheng H, Pablico SJ, Lee J, *et al*. Zinc finger transcription factor Zbtb16 coordinates the response to energy deficit in the mouse hypothalamus. *Front Neurosci* 2020;14:592947.
32 Tong M, Tai H-H. 15-Hydroxyprostaglandin dehydrogenase can be induced by dexamethasone and other glucocorticoids at the therapeutic level in A549 human lung adenocarcinoma cells. *Arch Biochem Biophys* 2005;435:50–5.
33 Fung KY, Louis C, Metcalfe RD, *et al*. Emerging roles for IL-11 in inflammatory diseases. *Cytokine* 2022;149:155750.
34 Schwertschlag US, Trepicchio WL, Dykstra KH, *et al*. Hematopoietic, immunomodulatory and epithelial effects of interleukin-11. *Leukemia* 1999;13:1307–15.
35 Zhang X, Tao Y, Chopra M, *et al*. Il-11 induces Th17 cell responses in patients with early relapsing-remitting multiple sclerosis. *J Immunol* 2015;194:5139–49.
36 Elshabrawy HA, Volin MV, Essani AB, *et al*. Il-11 facilitates a novel connection between RA joint fibroblasts and endothelial cells. *Angiogenesis* 2018;21:215–28.
37 Lim W-W, Corden B, Ng B, *et al*. Interleukin-11 is important for vascular smooth muscle phenotypic switching and aortic inflammation, fibrosis and remodeling in mouse models. *Sci Rep* 2020;10:17853.
38 Planas-Rigol E, Terrades-Garcia N, Corbera-Bellalta M, *et al*. Endothelin-1 promotes vascular smooth muscle cell migration across the artery wall: a mechanism contributing to vascular remodelling and intimal hyperplasia in giant-cell arteritis. *Ann Rheum Dis* 2017;76:1624–34.
39 Tsou C-L, Peters W, Si Y, *et al*. Critical roles for CCR2 and MCP-3 in monocyte mobilization from bone marrow and recruitment to inflammatory sites. *J Clin Invest* 2007;117:902–9.
40 Mezu-Ndubuisi OJ, Maheshwari A. The role of integrins in inflammation and angiogenesis. *Pediatr Res* 2021;89:1619–26.
41 Del Prete A, Bonecchi R, Vecchi A, *et al*. CCRL2, a fringe member of the atypical chemoattractant receptor family. *Eur J Immunol* 2013;43:1418–22.
42 Parthasarathy V, Martin F, Higginbottom A, *et al*. Distinct roles for tetraspanins CD9, CD63 and CD81 in the formation of multinucleated giant cells. *Immunology* 2009;127:237–48.
43 Tugues S, Honjo S, König C, *et al*. Tetraspanin CD63 promotes vascular endothelial growth factor receptor 2-β1 integrin complex formation, thereby regulating activation and downstream signaling in endothelial cells in vitro and in vivo. *J Biol Chem* 2013;288:19060–71.
44 Mantegazza AR, Barrio María Marcela, Moutel S, *et al*. CD63 tetraspanin slows down cell migration and translocates to the endosomal-lysosomal-MIICs route after extracellular stimuli in human immature dendritic cells. *Blood* 2004;104:1183–90.
45 Pfistershammer K, Majdic O, Stöckl J, *et al*. CD63 as an activation-linked T cell costimulatory element. *J Immunol* 2004;173:6000–8.
46 Borrego F. The CD300 molecules: an emerging family of regulators of the immune system. *Blood* 2013;121:1951–60.
47 Brckalo T, Calzetti F, Pérez-Cabezas B, *et al*. Functional analysis of the CD300e receptor in human monocytes and myeloid dendritic cells. *Eur J Immunol* 2010;40:722–32.
48 Isobe M, Izawa K, Sugiuchi M, *et al*. The CD300e molecule in mice is an immune-activating receptor. *J Biol Chem* 2018;293:3793–805.
49 Mullick J, Anandatheerthavarada HK, Amuthan G, *et al*. Physical interaction and functional synergy between glucocorticoid receptor and Ets2 proteins for transcription activation of the rat cytochrome P-450c27 promoter. *J Biol Chem* 2001;276:18007–17.
50 Zannas AS, Jia M, Hafner K, *et al*. Epigenetic upregulation of FKBP5 by aging and stress contributes to NF-κB–driven inflammation and cardiovascular risk. *Proc Natl Acad Sci U S A* 2019;116:11370–9.
51 Hofer TPJ, Frankenberger M, Mages J, *et al*. Tissue-Specific induction of ADAMTS2 in monocytes and macrophages by glucocorticoids. *J Mol Med* 2008;86:323–32.
52 Philippidis P, Mason JC, Evans BJ, *et al*. Hemoglobin scavenger receptor CD163 mediates interleukin-10 release and heme oxygenase-1 synthesis: antiinflammatory monocyte-macrophage responses in vitro, in resolving skin blisters in vivo, and after cardiopulmonary bypass surgery. *Circ Res* 2004;94:119–26.
53 Moeller JB, Nielsen MJ, Reichhardt MP, *et al*. CD163-L1 is an endocytic macrophage protein strongly regulated by mediators in the inflammatory response. *J Immunol* 2012;188:2399–409.
54 Wagner AD, Wittkop U, Thalmann J, *et al*. Glucocorticoid effects on tissue residing immune cells in giant cell arteritis: importance of GM-CSF. *Front Med* 2021;8:709404.
55 Ehrchen JM, Roth J, Barczyk-Kahlert K. More than suppression: glucocorticoid action on monocytes and macrophages. *Front Immunol* 2019;10:2028.

TRANSLATIONAL SCIENCE

Multi-trait and cross-population genome-wide association studies across autoimmune and allergic diseases identify shared and distinct genetic component

Yuya Shirai,[1,2] Yoshimitsu Nakanishi,[2,3,4,5] Akari Suzuki,[6] Hachirou Konaka,[7] Rika Nishikawa,[8] Kyuto Sonehara,[1] Shinichi Namba,[1] Hiroaki Tanaka,[1,9] Tatsuo Masuda,[10,11] Moto Yaga,[2] Shingo Satoh,[2] Mayuko Izumi,[2] Yumiko Mizuno,[2] Tatsunori Jo,[2] Yuichi Maeda ,[2,4,12] Takuro Nii,[2,12,13] Eri Oguro-Igashira ,[2,12] The Biobank Japan Project,[14] Takayuki Morisaki,[15,16] Yoichiro Kamatani,[17] Shingo Nakayamada,[9] Chikako Nishigori,[8] Yoshiya Tanaka ,[9] Yoshito Takeda,[2] Kazuhiko Yamamoto ,[6] Atsushi Kumanogoh,[2,3,4,18] Yukinori Okada [1,18,19,20,21]

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For numbered affiliations see end of article.

Correspondence to
Dr Yukinori Okada, Department of Statistical Genetics, Osaka University Graduate School of Medicine, Suita 565-0871, Japan;
yokada@sg.med.osaka-u.ac.jp

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ABSTRACT

Objectives Autoimmune and allergic diseases are outcomes of the dysregulation of the immune system. Our study aimed to elucidate differences or shared components in genetic backgrounds between autoimmune and allergic diseases.

Methods We estimated genetic correlation and performed multi-trait and cross-population genome-wide association study (GWAS) meta-analysis of six immune-related diseases: rheumatoid arthritis, Graves' disease, type 1 diabetes for autoimmune diseases and asthma, atopic dermatitis and pollinosis for allergic diseases. By integrating large-scale biobank resources (Biobank Japan and UK biobank), our study included 105 721 cases and 433 663 controls. Newly identified variants were evaluated in 21 778 cases and 712 767 controls for two additional autoimmune diseases: psoriasis and systemic lupus erythematosus. We performed enrichment analyses of cell types and biological pathways to highlight shared and distinct perspectives.

Results Autoimmune and allergic diseases were not only mutually classified based on genetic backgrounds but also they had multiple positive genetic correlations beyond the classifications. Multi-trait GWAS meta-analysis newly identified six allergic disease-associated loci. We identified four loci shared between the six autoimmune and allergic diseases (rs10803431 at *PRDM2*, OR=1.07, $p=2.3\times10^{-8}$, rs2053062 at *G3BP1*, OR=0.90, $p=2.9\times10^{-8}$, rs2210366 at *HBS1L*, OR=1.07, $p=2.5\times10^{-8}$ in Japanese and rs4529910 at *POU2AF1*, OR=0.96, $p=1.9\times10^{-10}$ across ancestries). Associations of rs10803431 and rs4529910 were confirmed at the two additional autoimmune diseases. Enrichment analysis demonstrated link to T cells, natural killer cells and various cytokine signals, including innate immune pathways.

Conclusion Our multi-trait and cross-population study should elucidate complex pathogenesis shared components across autoimmune and allergic diseases.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Autoimmune and allergic diseases are distinct outcome of the dysregulation of the immune system, while their differences, or shared components, in genetic backgrounds are elusive.

⇒ The long-term risks of autoimmune diseases are significantly higher in patients with allergic diseases, but the mechanism is unknown.

WHAT THIS STUDY ADDS

⇒ Our study clearly depicted distinct disease classifications between autoimmune and allergic diseases due to different polygenic architecture. On the other hand, our study also showed several multiple positive genetic correlations beyond the classifications.

⇒ Our multi-trait and cross-population analysis identified four loci shared between autoimmune and allergic diseases (*PRDM2*, *HBS1L*, *G3BP1* and *POU2AF1*), which showed population-specific or cross-populational effects. Such shared loci were characterised as associations with genes involved in innate immunity or humoral immunity.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE AND/OR POLICY

⇒ The shared effects identified in this study may be responsible for both autoimmune and allergic diseases. Our multi-trait approach proposes effective strategies to identifying shared genetic components, which contributes to understanding a set of complex human traits such as immune-related diseases.

INTRODUCTION

Genetic background contributes to the development of common and complex diseases, and genome-wide association studies (GWASs) have

identified a number of genetic loci that affect a variety of disease risk.[1] Genetic backgrounds of diseases can be decomposed into disease-specific effects and those shared across diseases. While understanding disease-specific effects helps us comprehend the individual disease pathologies, understanding shared effects is also important to reveal underlying pathologies across diseases and provide opportunities for reciprocal drug repositioning. Previous GWAS integrating allergic diseases have revealed their shared genetic background among allergic diseases (eg, asthma, pollinosis (PO) and eczema[2 3]). Autoimmune diseases are another outcome of dysregulation of the immune system. Several GWASs dealing with multiple autoimmune diseases successfully identified the genetic overlap existing in autoimmunity.[4–6] By integrating similar diseases, these studies have advanced the knowledge of the shared aetiology in each immune dysfunction. While autoimmune and allergic diseases are pathogenetically distinct conditions, several elements such as antibodies, T cells, mast cells and cytokines are involved in both.[7] Furthermore, several allergic diseases are associated with the long-term risks of developing autoimmune diseases.[8] These observations suggest shared genetic components across autoimmune and allergic diseases, but there have been few genetic studies that conducted multi-trait integrative analysis. Furthermore, majority of such approaches focused on a single ancestry, thereby lacking global landscape of human disease genetics.

Biobanks have been accumulating genotypes and medical records on a huge scale,[9 10] including autoimmune and allergic diseases, which encourage us to elucidate the genetic background of immune dysfunction. In this study, we estimated the genetic correlations among three autoimmune (rheumatoid arthritis (RA), Graves' disease (GD) and type 1 diabetes (T1D)) and three allergic diseases (bronchial asthma (BA), PO and atopic dermatitis (AD)) by using the BioBank Japan (BBJ) and UK Biobank (UKB) resources.[11 12] To identify shared genetic components, we conducted multi-trait and cross-population meta-analyses integrating the GWAS datasets. We further performed enrichment analyses of cell types and biological pathways to highlight shared and distinct perspectives in biological functions.

METHODS

Study cohorts and subjects

All the Japanese subjects enrolled in this study were collected through BBJ, which is a hospital-based registry with multiomics data from genotype to multitude phenotype of approximately 200 000 patients with 1 of 47 diseases.[11] We extracted the subjects with autoimmune and allergic diseases registered in BBJ, which composed of AD (2472 cases), BA (7522 cases), GD (2041 cases), PO (5308 cases), anticyclic citrullinated peptide-positive RA (2370 cases) and T1D without a record of type 2 diabetes (638 cases). The controls were the subjects without medical records of any immune-related diseases.

For the European subjects, we obtained the data of UKB, which is a population-based registry on approximately 500 000 individuals aged between 40 and 69 recruited in the UK.[12] Analogous to BBJ, we selected the six autoimmune and allergic diseases as the following definition. AD cases were the subjects registered as AD in hospital records or eczema/dermatitis in self-reported diagnosis (12 285 cases). BA cases were the subjects registered as asthma in either hospital records or self-reported diagnosis (54 872 cases). GD cases were the subjects registered as thyrotoxicosis with diffuse goitre in hospital records or GD in self-reported diagnosis (614 cases). PO cases were the subjects registered as allergic rhinitis due to pollen in hospital records or hayfever/allergic rhinitis in self-reported diagnosis (26 758 cases). RA cases were the subjects registered as seropositive RA in hospital records or RA in self-reported diagnosis (5065 cases). T1D cases were registered as insulin-dependent diabetes mellitus in hospital records or T1D in self-reported diagnosis without the following medical records: insulin-independent diabetes mellitus in hospital records, type 2 diabetes mellitus or gestational diabetes mellitus in self-reported diagnosis (914 cases). The controls were subjects with no records of any immune-related diseases in hospital records or self-reported diagnosis.

The summary of the study cohorts and subjects is described in online supplemental table 1. All the subjects agreed with informed consent based on the approval of the institutional ethical committee. This study was approved by the ethical committee of Osaka University (Approval ID: 734–14).

Genotyping and imputation

The BBJ subjects were genotyped with the Illumina HumanOmniExpressExome BeadChip or a combination of the Illumina HumanOmniExpress and HumanExome BeadChips.[13] Quality control of participants and genotypes was performed as described elsewhere.[14] In this study, we extracted East Asian subjects based on a principal components analysis of the genotypes. We performed haplotype phasing of the genotype data using Eagle (V.2.3) and imputed genotype dosages using Minimac V.3 with the population-specific reference panel of Japanese, which was integrated whole-genome sequence data of 1000 Genomes Project Phase 3 (V.5) and 1037 Japanese.[15]

The UKB subjects were genotyped with the Applied Biosystems UK BiLEVE Axiom Array or the Applied Biosystems UKB Axiom Array. After quality control as described elsewhere,[10] haplotype phasing was performed using SHAPEIT3 and genotype dosages were imputed using IMPUTE4 with the merged UK10K and 1000 Genomes phase 3 reference panels. We extracted Caucasian subjects based on a principal components analysis of the genotypes for subsequent analysis.

Individual-trait GWAS

We performed a GWAS for the individual autoimmune and allergic disease with a generalised linear mixed model implemented in SAIGE.[16] Age, sex and the top five principal components were included as covariates in the regression model. We applied the leave-one-chromosome-out approach to calculate the genetic relation matrix. We excluded the variants with either imputation quality *Rsq* <0.7, minor allele frequency <0.005 or minor allele count<3 from the GWAS. The genome-wide significance threshold was adopted at the level of $p=5.0 \times 10^{-8}$. We considered the human leucocyte antigen (HLA) region (chr6:26Mb-34Mb) as one locus considering its complex and strong linkage disequilibrium (LD) structure within the region.[13]

Heritability and genetic correlation

We estimated heritability and confounding bias for the individual traits using LD score regression (LDSC) analysis[17] with 1000 Genomes phase 3 East Asian (1000G-EAS) reference panel for the BBJ GWAS data sets and 1000 Genomes phase 3 European (1000G-EUR) reference panel for the UKB GWAS data sets. To assess genetic correlations among the six autoimmune or allergic diseases, we used high-definition likelihood (HDL) inference,[18] which is an extension of LDSC in that it thoroughly exploits the information of the variance–covariance matrix of the *Z*-score from GWAS summary statistics. Because HDL needed a larger reference sample for accurate estimation than LDSC, we

prepared a custom reference panel from 1000G-EAS and BBJ genotype data to analyse the BBJ GWAS data sets. We used the prebuilding UKB reference panel to analyse the UKB GWAS data sets. We excluded the variants within the HLA region for the estimation in both LDSC and HDL. Hierarchical clustering for the genetic correlation matrix was performed with Ward's method using 1 - r_g as distance metrics.

Local heritability and genetic correlation

We applied SUPERGNOVA[19] to estimate local heritability and genetic covariance in the prespecified LD-independent segments by ldetect.[20] While SUPERGNOVA can effectively estimate local genetic covariance accounting for sample overlap, local genetic correlation estimates are numerically unstable due to the noise in the estimates of local heritability. We assessed the significance of the local genetic correlations based on the significance of local genetic covariances as was done in the paper of SUPERGNOVA because they are statistically equivalent.

Meta-analysis for autoimmune and allergic diseases

We conducted fixed effect meta-analyses with the Lin-Sullivan method,[21] taking into account sample overlap among GWAS data sets. To account for the effects of heterogeneity, we applied Metasoft to calculate heterogeneity index I^2 and p value based on Cochran's Q test (P_{het}). When heterogeneity was suggested ($I^2 \geqq 50$ or $P_{het}<0.05$), we prioritised the p value in the random effect model calculated with RE2C.[22] First, we performed two types of meta-analyses that integrated three autoimmune diseases or three allergic diseases GWAS data sets. Second, we performed a multi-trait meta-analysis integrating six GWAS data sets. Finally, we performed a cross-population meta-analysis that integrated all of the 12 GWAS data sets. We calculated the genomic control factor λ_{GC} using R statistical software. Genome-wide significance threshold was adopted at the level of $p=5.0\times10^{-8}$. We applied FUMA[23] to define independent associated loci using the default r^2 threshold. As the LD reference panel for FUMA, we referred to 1000G-EAS reference panel for the BBJ meta-analysis and 1000G-EUR reference panel for the UKB meta-analysis. For the cross-population meta-analysis, we referred 1000G-ALL reference panel, which is the only available cross-population LD reference panel in FUMA. We defined a novel locus if all the variants and genes in identified loci were not associated with diseases included in the meta-analysis by querying GWAS catalogue,[24] PheWeb,[25] PheWeb.jp,[3] PhenoScanner (v2)[26] and Open Targets Genetics.[27] We additionally defined an independent locus if a lead variant was located in previously reported genes but not LD (r^2 <0.1) with the reported variants. We created regional plots using LocusZoom for novel and independent loci.

Fine-mapping and functional annotation

We used SuSiE[28] to find 95% credible sets of causal variants accounting for LD in the loci identified in our study. In SuSiE, the LD information was referred to the 1000G-EAS and BBJ reference panel for the BBJ meta-analysis, the 1000G-EUR reference panel for the UKB meta-analysis and the reference panel integrating 1000G-EAS and 1000G-EUR for the cross-population meta-analysis. We obtained functional annotations of the lead variants using ANNOVAR.[29] Annotation of promotor and enhancer marks for the individual lead variants were searched through HaploReg (V.4.1). Quantitative effects on gene expression levels of the variants (ie, eQTL effect) were queried according to GTEx Portal (V.8)[30] and ImmuNexUT,[31] that is the latest eQTL data set of 28 immune cells in Japanese population. Because we could access the summary statistics of ImmuNexUT, we performed colocalisation analysis using eCAVIAR[32] to assess the sharing causal variants between the BBJ GWAS data sets and ImmuNexUT eQTL data sets. We set CLPP ≧0.03 as a threshold for significant colocalisation as was done in the paper of ImmuNexUT.

Cell-type enrichment analysis

To assess the enrichment of the autoimmune and allergic GWAS data sets in immune cell types, we used stratified LDSC[33] for the gene annotations with the highest specific expression in 292 immune cell types from the ImmGen Consortium.[34] We used the 1000G–EAS and 1000G–EUR baseline V.1.2 LD score in BBJ and UKB, respectively, and excluded the variants within the HLA region from the analysis. We calculated the p value of the regression coefficient τ_c of the individual annotation. We set the threshold for significant enrichment as p=0.05/292, adjusted by Bonferroni correction. We performed hierarchical clustering on the matrix of enrichment significance in the 292 cell-type-specific annotations, using Euclidean distance and Ward's method.

Pathway enrichment analysis

We evaluated the association between the GWAS data sets and molecular pathways using PASCAL.[35] PASCAL calculates gene-based scores by integrating p values of variants and estimate pathway enrichment scores by merging gene-based scores belonging to the same pathway. As the reference panel, we used the custom 1000G-EAS genotype data for the BBJ GWAS data sets and the 1000G-EUR genotype data provided by the authors for the UKB GWAS data sets. To assess the enrichment within the immune pathway, we obtained the curated gene sets derived from the Reactome pathway database in MSigDB collections[36] and extracted 150 gene sets in the lower layers of 'immune system'. We set the threshold for significant enrichment as p=0.05/150, adjusted by Bonferroni correction. For the visualisation of the enriched pathways, we used Cytoscape[37] to create a network diagram.

Replication analysis for additional autoimmune diseases

We additionally evaluated the association of the four variants newly identified in the multi-trait analysis of the six autoimmune and allergic diseases with two additional autoimmune diseases: psoriasis (PsO) and systemic lupus erythematosus (SLE). We meta-analysed overall 11 807 cases and 696 291 controls in PsO and 9987 cases and 712 510 controls in SLE. For the EAS cohort, we used the imputed dosage data of the subjects in BBJ, Osaka University Graduate School of Medicine and previous GWAS summary statistics.[38] For the EUR cohort, we used the imputed dosage data of UKB and previous GWAS summary statistics.[39] The summary of the data sets for the replication analysis is described in online supplemental table 2.

As for the dosage data, we performed association analyses for the individual data set using SAIGE in the same condition as our GWAS. Subsequently, we integrated the summary statistics with Metasoft for each disease in the population-specific and cross-population manner. Finally, we conducted multi-trait meta-analyses with RE2C, dealing with sample overlap.

Drug target analysis

We queried the genes associated with autoimmune and allergic diseases to STRING V.11.5,[40] a database that collected protein–protein interaction (PPI) networks. In STRING, each PPI is annotated with a score between 0 and 1 based on physical

and functional information. Biologically related neighbourhood genes were defined as genes with a high confidence score (combined score excluding 'text mining score' >0.7) to the queried target genes. We confirmed whether the target and neighbourhood genes were drug targets by searching in DrugBank[41] and Therapeutic Target Database (TTD).[42]

Patient and public involvement

This research was done without patient and public involvement. Patients and public were not invited to comment on the study design and were not consulted to develop patient relevant outcomes or interpret the results.

RESULTS

Overview of the subjects

Our study focused on six immune-related diseases included in BBJ target diseases. In UKB, we extracted the six autoimmune and allergic diseases corresponding to the BBJ target diseases. The autoimmune diseases consisted of RA (2370 cases in BBJ and 5065 cases in UKB), GD (2041 cases in BBJ and 614 cases in UKB) and T1D (638 cases in BBJ and 914 cases in UKB). The allergic diseases consisted of BA (7522 cases in BBJ and 54 872 cases in UKB), AD (2472 cases in BBJ and 12 285 cases in UKB) and PO (5308 cases in BBJ and 26 758 cases in UKB). We enrolled subjects with no records of any immune-related diseases as control (142 192 controls in BBJ and 291 471 controls in UKB). To enhance power to detect the associated loci, we excluded immune-related diseases from the controls. The summary of the subjects is described in online supplemental table 1.

Individual-trait GWAS analysis in a single ancestry

First, we separately performed a GWAS of individual disease in each ancestry to overview their genetic architecture prior to the meta-analysis. Through the GWASs in BBJ, we observed 4 significant loci in RA, 9 in GD, 1 in T1D, 8 in BA and 9 in AD (online supplemental table 1). Through the GWASs in UKB, we observed 6 significant loci in RA, 2 in GD, 3 in T1D, 88 in BA, 17 in AD and 34 in PO. Although we found no novel loci in the individual-trait GWAS in a single ancestry, all the significant loci were robustly concordant with the previous findings.[3 24–27]

Global genetic relationships across immune-related diseases

We applied LDSC to estimate the heritability of the individual GWAS data sets.[17] The heritability was relatively larger in allergic diseases than in autoimmune diseases (on average, 1.8% in BBJ and 3.8% in UKB for allergic diseases, but 1.4% in BBJ and 0.4% in UKB for autoimmune diseases; figure 1A), although the relatively limited sample sizes and the exclusion of the HLA region in the LDSC framework may have affected the results. Estimates of heritability in the absence of the HLA regions can be underestimated, especially in autoimmune diseases. To finely conduct the subsequent meta-analysis, we applied HDL to more accurately estimate the genetic correlations to find the disease pairs with similar genetic backgrounds.[18] Our genetic correlation analysis showed that the six immune-related diseases could be divided into the two major categories, which corresponded to the original classifications of autoimmune and allergic diseases. Hierarchical clustering based on genetic correlation clearly described these two major categories (figure 1B). Thus, the genetics-based classification of diseases was consistent with the clinical classification. Larger genetic correlation (r_g) estimates were observed among allergic diseases, suggesting close relationship of genetic backgrounds of the allergic diseases assessed in this study. On the other hand, several disease pairs showed a positive genetic correlation across categories, such as RA and BA in BBJ (r_g=0.29, p=2.2×10^{-4}) and UKB (r_g=0.35, p=3.6×10^{-18}). We note that the r_g estimates were generally concordant between BBJ and UKB (r=0.58, p=0.022; figure 1C), indicating the robustness of our assessments.

Local genetic relationships across immune-related diseases

To identify local genetic architecture underlying between two disease categories, we applied SUPERGNOVA to estimate the local heritability and the local genetic correlation per LD-independent segment.[19] In the autoimmune diseases, the local heritability was prominent in the HLA region (online supplemental figures 1 and 2), where strong genetic risk was embedded.[13] In contrast, the local heritability was distributed relatively across genome-wide in the allergic diseases.

In the local genetic correlation analysis, we found multiple regions with positive correlations within allergic diseases in the UKB data sets (online supplemental figure 3). Notably, there were 38 positively correlated regions between BA and PO, suggesting their shared genetic structure in a genome-wide manner. We also observed several genetic regions with positive genetic correlations across the disease categories. Of these, *CLEC16A* at 16p13 was the hub region where nine loci pairs with positive correlations were centralised (online supplemental figure 4). We obtained less evidence for the local genetic correlation in BBJ than UKB, probably reflecting the difference of the sample sizes in the original GWASs.

Multi-trait and cross-population meta-analysis within autoimmune or allergic disease categories

We then performed multi-trait GWAS meta-analyses to evaluate the shared effect among GWAS data sets at the variant level, while local genetic analysis helped us assess prespecified independent regions. Because we expected that statistical power would be enhanced by considering diseases with a shared genetic background together, we first conducted a meta-analysis within each disease category separately (figure 2). In the meta-analysis of autoimmune diseases, we tested 8 371 232 variants in the BBJ data sets, 10 862057 variants in the UKB data sets and 5 965 647 variants in the cross-population data sets. In the meta-analysis of the allergic diseases, we tested 8 368 683 variants in the BBJ data sets, 10 856683 variants in the UKB data sets and 5 965 021 variants in the cross-population data sets. While we observed slight inflation of the genomic control factor (λ_{GC}) in each meta-analysis, LDSC intercept did not obviously deviate from 1.00, suggesting no apparent bias due to confounding population structure (online supplemental figure 5).

In the meta-analysis of the autoimmune diseases, we identified 10, 5 and 11 significant loci in the BBJ, UKB and cross-population data sets, respectively. In the meta-analysis of the allergic diseases, we identified 11, 98 and 99 significant loci in the BBJ, UKB and cross-population data sets, respectively. We found no novel significant loci in the meta-analyses of the autoimmune diseases. On the other hand, we identified three novel loci (rs74052928 G>C at 1p36, *MIIP*, p=3.0×10^{-8}; rs575879774 G>GA at 2q21, *CXCR4*, p=8.4×10^{-9} and rs7773622 C>T at 6q21, *SCML4*, p=2.8×10^{-8}) and two independent novel association signals within the previously reported loci (rs1800440 T>C at 2p22, *CYP1B1*, p=3.6×10^{-9}; rs115257668 A>G at 2q33, *ICOS*, p=1.2×10^{-8}) in the UKB meta-analysis of the allergic diseases (table 1).

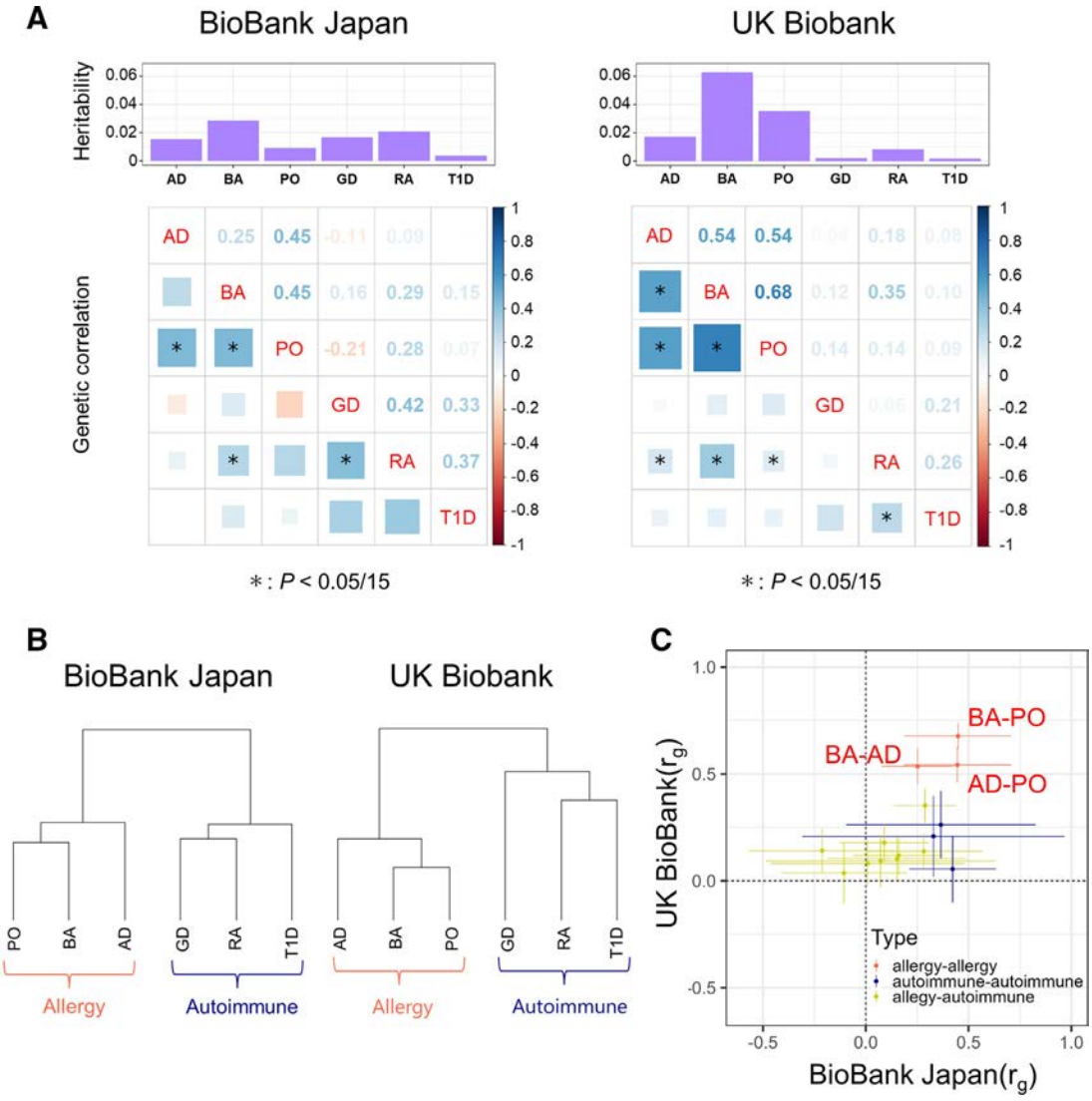

Figure 1 Genetic correlation among the autoimmune and allergic diseases. (A) Histograms of the heritability and genetic correlation matrices of autoimmune and allergic diseases in BBJ (left panel) and UKB (right panel). (B) Dendrograms of the hierarchical clustering based on the genetic correlations in BBJ (left panel) and UKB (right panel). (C) Scatter plots describing the associations between the genetic correlations in BBJ (x-axis) and UKB (y-axis). Dots represent the estimates of the genetic correlation and whiskers represent 95% confidence intervals. The dots are coloured according to disease categories. AD, atopic dermatitis; BA, bronchial asthma; BBJ, BioBank Japan; GD, Grave's diseases; PO, pollinosis; RA, rheumatoid arthritis; T1D, type 1 diabetes; UKB, UK Biobank.

Among the five lead variants, rs1800440 was a missense variant of *CYP1B1*, where the alternative allele was only observed in the UKB data sets (figure 3A). In the statistical fine mapping of putatively causal variants by SuSiE,[28] the 95% credible set included only rs1800440, which supported that rs1800440 was causal in the loci (online supplemental figure 6). The directional effects of the risk allele of rs1800440-C were concordant among the three allergic diseases, demonstrating nominal association significance in BA and PO ($p<0.05$). Pathogenicity scores supported that this missense mutation was constrained (GERP ++score = 5.95) and deleterious to human health (CADD=21.8).

We found an additional novel locus in the cross-population meta-analysis of allergic diseases (rs16902902 G>A at 8q24, *LINC00824*, $p=2.1\times10^{-9}$). The allele of rs16902902-A was suggested to have a protective effect for allergic diseases in the BBJ data set ($p=6.8\times10^{-7}$) and the UKB data set ($p=6.2\times10^{-4}$) and exceeded the genome-wide significance level in the cross-population meta-analysis. None of the identified variants showed apparent heterogeneity (I^2 <30% and $P_{het}>0.2$).

Multi-trait meta-analysis of the autoimmune and allergic diseases

Our genetic correlation analysis showed cross-category correlations like RA and BA. This suggested that common genetic elements cause both autoimmune and allergic diseases. Thus, we conducted a cross-trait meta-analysis integrating the six GWAS datasets of autoimmune and allergic diseases, first in a single ancestry manner.

In the BBJ GWAS meta-analysis, we identified 10 significant loci, including two novel loci (rs10803431 G>C at 1p36, *PRDM2*, $p=2.3\times10^{-8}$; rs2053062 C>T at 5q33, *G3BP1*, $p=2.9\times10^{-8}$) and one independent locus (rs2210366 G>A at 6q23, *HBS1L*, $p=2.5\times10^{-8}$). Although the variants were nominally but not genome-wide significant in the individual analysis, they became significant after integrating the six GWAS data sets (online supplemental figure 7). The minor allele frequencies of the three lead single-nucleotide polymorphisms (SNPs) were higher in non-Europeans. Especially, rs2053062-T was specific

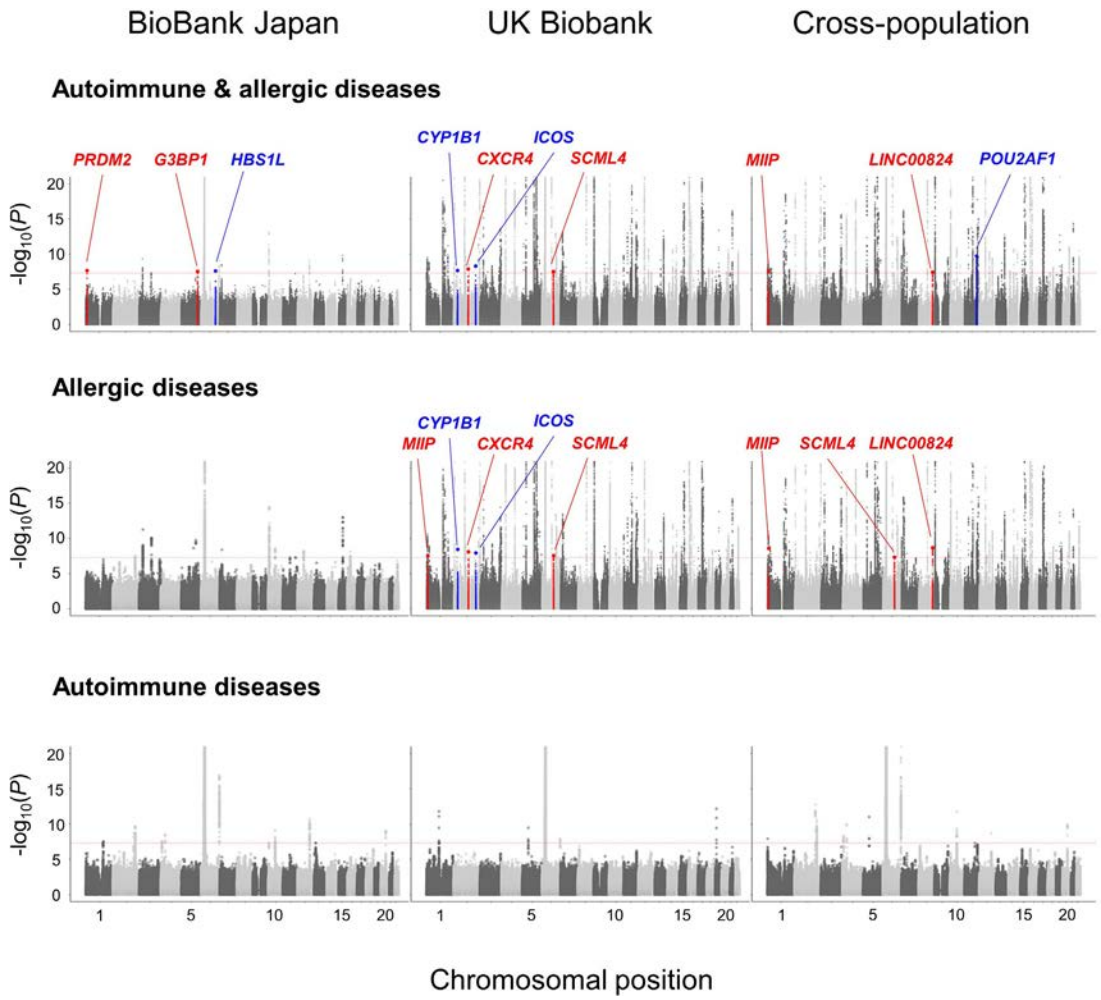

Figure 2 Manhattan plots of the GWAS meta-analysis for the autoimmune and allergic diseases. Manhattan plots of the GWAS meta-analysis of six autoimmune and allergic diseases (upper), three allergic diseases (middle) and three autoimmune diseases (lower). The y-axis indicates $-\log_{10}(P)$ of association of each variant calculated in three cohorts: BBJ (left), UKB (middle), cross-population (right). The upper limit is set to 20 for the sake of clarity. In the loci which we identified, the novel ones are coloured in red and independent ones are coloured in blue. The horizontal dashed red line indicates the genome-wide significance threshold ($p=5.0\times10^{-8}$). BBJ, BioBank Japan; GWAS, genome-wide association study; UKB, UK Biobank.

to East Asians (mainly Japanese) and Americans but not included in the UKB data set (figure 3B), highlighting population-specific disease genetic architecture.

The lead SNP of rs2053062 was the *G3BP1* intron variant. The directional effects of the protective allele of rs2053062-T were concordant among the six immune-related diseases, demonstrating nominal association significance in BA, AD, PO and RA. We evaluated the positional overlap between rs2053062 and cell type-specific chromatin states with Haploreg. The variant was located in a region considered to be an enhancer, which was supported by multiple Chip-seq data for T cells. Furthermore, the protective allele of rs2053062-T has been reported as an eQTL that decreases *G3BP1* expression levels in effector regulatory T cells in ImmunNexUT database[31] (online supplemental figure 8). Our colocalisation analysis supported that rs2053062 affected both the disease risk and the expression levels of *G3BP1* in various lymphocyte cell types (online supplemental figure 9), proposing the expression level as an endophenotype to disease susceptibility.

We identified 98 significant loci in the UKB GWAS meta-analysis, but no novel loci were identified in addition to the meta-analysis of allergic diseases.

Finally, we performed a cross-population meta-analysis integrating the 12 GWAS data sets obtained from the BBJ and UKB. We identified 90 lead variants, one of which was an independent variant that newly satisfied genome-wide significance level (rs4529910 T>G at 11q23, *POU2AF1*, $p=1.9\times10^{-10}$). Because the effect of rs4529910 was suggested to be heterogeneous ($I^2=53.5\%$ and $P_{het}=0.014$), we re-evaluated the association of rs4529910 in the random effect model. Consequently, we observed a more robust association of rs4529910 with the autoimmune and allergic diseases ($p=5.8\times10^{-11}$). The lead SNP of rs4529910 was the *POU2AF1* intron variant. Several variants around *POU2AF1* had been reported to be associated with the allergic diseases, including BA, PO and AD. However, these known variants were not in LD ($r^2<0.1$) with the newly identified risk variant of rs4529910. The statistical fine-mapping analysis by SuSiE described that there were two distinct signals in the loci, which indicated that rs4529910 had a different genetic effect from the reported ones (online supplemental figure 6). The effect allele of rs4529910-G was protectively associated with autoimmune and allergic diseases across ancestries, except for the BBJ PO data set. In Haploreg, the variant was located in a region considered to be an enhancer, which was supported

Table 1 Summary of the multi-trait meta-analyses

Locus discovery	Cohort	Chr:position	SNP	Gene	Annotation	BioBank Japan					UK BioBank					Cross-population				
						EAF (control)	OR	P*	I^2	P_{het}†	EAF (control)	OR	P*	I^2	P_{het}†	EAF (control)	OR	P*	I^2	P_{het}†
Meta-analysis of allergic diseases																				
Novel	UKB	1:12 080122	rs74052928	*MIIP*	Intron	0.094	0.95	0.029	31	0.24	0.14	0.95	3.0×10^{-8}	0	0.76	0.13	0.95	2.6×10^{-9}	0	0.63
Novel	UKB	2:136 809603	rs575879774	*CXCR4*	Intergenic	0.28	1.01	0.46	0	0.78	0.015	1.16	8.4×10^{-9}	0	0.49	0.10	1.05	3.4×10^{-4}	83	1.8×10^{-5}
Novel	UKB	6:108 131 958	rs7773622	*SCML4*	Intron	0.086	1.01	0.81	67	0.047	0.16	0.96	2.8×10^{-8}	0	0.92	0.13	0.96	2.5×10^{-7}	54	0.053
Novel	Cross-population	8:129 428 433	rs16902902	*LINC00824*	Intron	0.33	0.93	6.8×10^{-7}	66	0.051	0.037	0.95	6.2×10^{-4}	0	0.89	0.13	0.94	2.1×10^{-9}	27	0.23
Known ‡	UKB	2:38 298139	rs1800440	*CYP1B1*	Missense	–	–	–	–	–	0.18	1.05	3.6×10^{-9}	0	0.38	–	–	–	–	–
Known ‡	UKB	2:205 032 379	rs115257668	*ICOS*	Intergenic	–	–	–	–	–	0.018	1.14	1.2×10^{-8}	0	0.84	–	–	–	–	–
Meta-analysis of autoimmune and allergic diseases																				
Novel	BBJ	1:14 206917	rs10803431	*PRDM2*	Intergenic	0.49	1.07	2.3×10^{-8}	3	0.39	0.37	1.00	0.42	0	0.96	0.41	1.01	0.058	73	2.7×10^{-5}
Novel	BBJ	5:151 169881	rs2053062	*G3BP1*	Intron	0.11	0.90	2.9×10^{-8}	0	0.54	–	–	–	–	–	–	–	–	–	–
Known ‡	BBJ	6:135 415208	rs2210366	*HBS1L*	Intron	0.36	1.07	2.5×10^{-8}	40	0.14	0.25	1.02	0.03	0	0.62	0.29	1.03	2.9×10^{-6}	62	0.0021
Known ‡	Cross-population	11:111 243102	rs4529910	*POU2AF1*	Intron	0.41	0.96	8.3×10^{-4}	71	0.0044	0.73	0.96	5.7×10^{-8}	25	0.25	0.63	0.96	1.9×10^{-10}	54	0.014

*P-value in the fixed effect model by Lin-Sullivan method.
†P-value based on Cochran's Q test.
‡Newly identified risk variant independent of the previously known risk variant within the locus.
BBJ, BioBank Japan; Chr, chromosome; EAF, effect allele frequency; SNP, single nucleotide polymorphism; UKB, UK Biobank.

by several Chip-seq data for B cells. The protective allele of rs4529910-G has been reported as an eQTL that decreases *POU2AF1* expression levels in B cells in the ImmunNexUT database (online supplemental figure 10).

Cell-type enrichment in the autoimmune and allergic diseases

Our local heritability analysis suggested that the two disease categories were characterised by the different distribution of genetic risk on the genome. To interpret the biological consequences, we performed the enrichment analysis with the 292 immune cell types in ImmGen data set.[34] Many T cell and natural killer cell subsets were associated with BA or PO at the nominal significance level (figure 4A). Among them, regulatory T and natural killer T cells were significantly enriched in both BA and PO in UKB even after multiple testing correction (table 2). We observed no significant enrichment in the autoimmune diseases potentially due to biased polygenicity resulting from the centralisation of heritability on the HLA region.

Pathway enrichment in the autoimmune and allergic diseases

To elucidate pathogenicity, we conducted pathway enrichment analysis of the autoimmune and allergic disease GWASs with 150 gene sets in the lower layers of 'immune system' in Reactome. In the BBJ and UKB data sets, allergic diseases were significantly enriched in multiple gene sets in the lower layers of 'cytokine signalling', including IL−4, 5 and 13 involved in type 2 inflammation and IL-1,6, and TNF involved in non-type 2 inflammation (figure 4B). In the lower layers of 'innate immune system', BA is significantly associated with C-type lectin receptors and Dectin1 signalling, which is involved in house dust mite-induced allergic airway inflammation. As observed in the cell-type enrichment analysis, we observed less significant enrichment of the pathways in the autoimmune diseases. Only RA in BBJ was significantly associated with NOD1/2 signalling.

Pervasive effect of the multitrait-associated variants on additional autoimmune diseases

We evaluated the effects of the four variants associated with autoimmune and allergic diseases on PsO and SLE by collecting additional individual data. Our replication meta-analysis included overall 21 778 cases and 712 767 controls in PsO and SLE (online supplemental table 2). We found nominally significant results consistent with our original multitrait GWAS meta-analysis for the two variants (rs10803431, OR=1.06, p=0.024 in EAS and rs4529910, OR=0.95, $p=2.1\times10^{-4}$ in EUR and OR=0.96, $p=1.9\times10^{-4}$ in cross-population; figure 5 and online supplemental table 3). The effect size of the EAS specific variant rs2053062 for PsO was similar to our multitrait analysis, while not significantly due to the limited sample size (OR=0.90, p=0.29 in EAS). From these results, our approach revealed the novel associations between genetic variants and additional autoimmune diseases.

Drug targets for immune-related diseases at the identified multi-trait-associated loci

We found the biologically related genes in the allergic associated loci (68 in *CXCR4*, 19 in *CYP1B1*, and 8 in *ICOS*) and autoimmune and allergic associated loci (1 in *PRDM2*, 13 in *G3BP1*, 88 in *HBS1L*, 1 in *POU2AF1*) by using STRING V.11.5[40] (online supplemental figure 11A). By querying them through DrugBank[41] and TTD,[42] we found that *CXCR4* and its functionally related genes have been therapeutic targets of various autoimmune and allergic diseases (online supplemental figure 11B). This result would be plausible given that chemokines involved

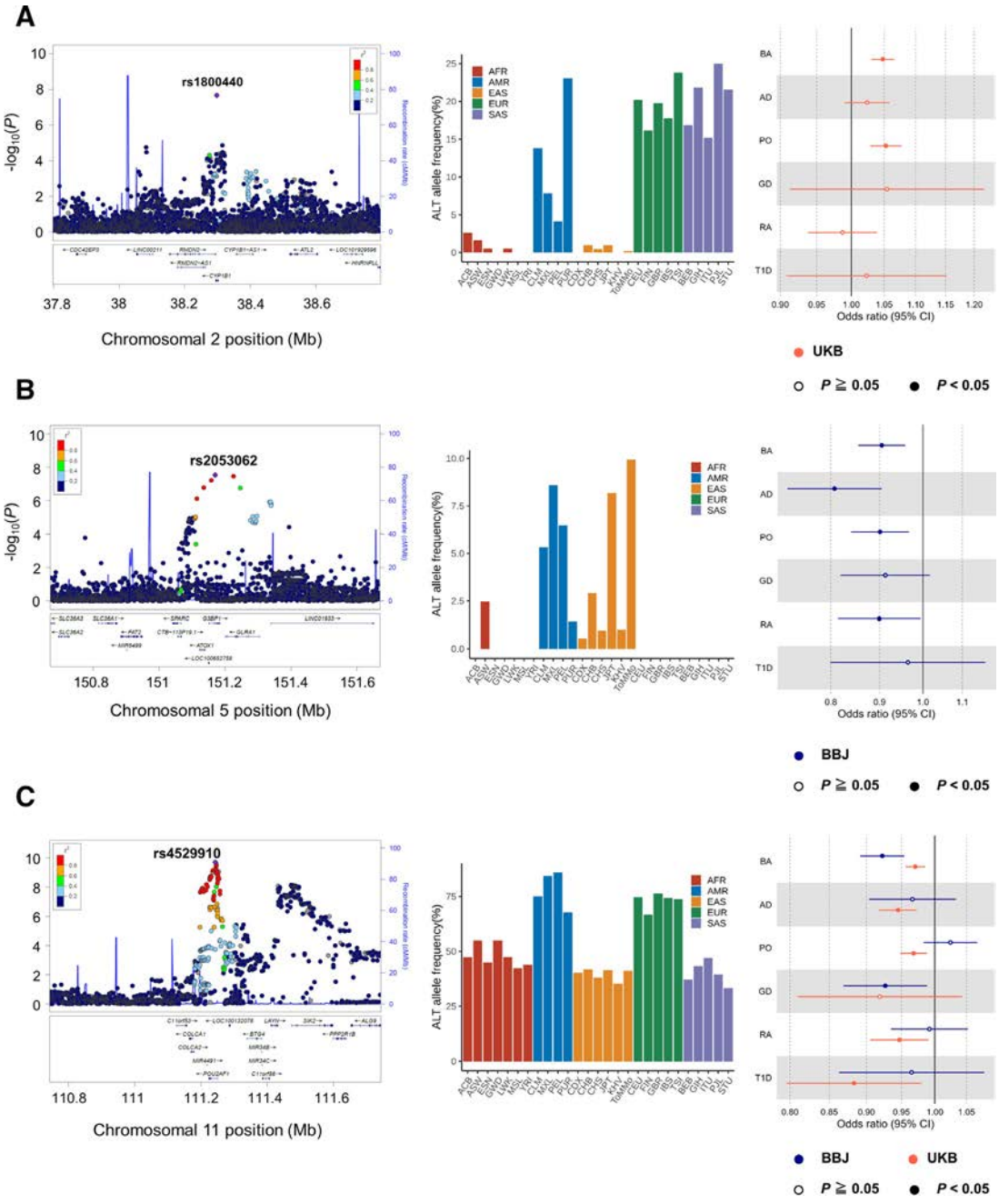

Figure 3 Population-specific and cross-populational disease-associated loci. (A) *CYP1B1* locus, observed in only the UKB datasets, (B) *G3BP1* locus, observed in only the BBJ datasets, and (C) *POU2AF1* locus, observed consistent effect in both ancestries are described as follows. (Left) Regional plot of the individual locus. The lead variants are coloured in purple and all the other variants are coloured based on LD with the lead variant as in the legend. (Middle) Histograms of the alternative allele frequency of the lead variants, which are coloured according to continental populations. (right) Forest plot of the individual lead variants. The dots indicate the OR in each dataset and the whiskers represent 95% confidence intervals. AD, atopic dermatitis; BA, bronchial asthma; BBJ, BioBank Japan; GD, Grave's diseases; PO, pollinosis; RA, rheumatoid arthritis; T1D, type 1 diabetes; UKB, UK Biobank.

in *CXCR4* broadly control the immune system.[43] We also found that *ICOS* and its functionally related genes have been expected to be therapeutic targets of several autoimmune diseases. Given its ability to enhance T cell responses against foreign antigens,[44] *ICOS* has the potential to be a common therapeutic target for autoimmune and allergic diseases.

DISCUSSION

In this study, the multitrait and cross-population GWAS meta-analysis depicted shared and distinct genetic components across the six immune-related diseases, which enabled de novo categorical classification of the autoimmune and allergic diseases solely based human genetics. Our study newly identified six loci associated with allergic diseases (*MIIP*, *CXCR4*, *SCML4*, *CYP1B1*, *ICOS* and *LINC00824*) and four pleiotropic loci associated with both autoimmune and allergic diseases (*PRDM2*, *G3BP1*, *HBS1L* and *POU2AF1*). While the variants identified in the meta-analysis in BBJ or UKB were ancestry specific (ie, almost monomorphic in the other ancestry), cross-population meta-analysis successfully enhanced the power to identify the variants with

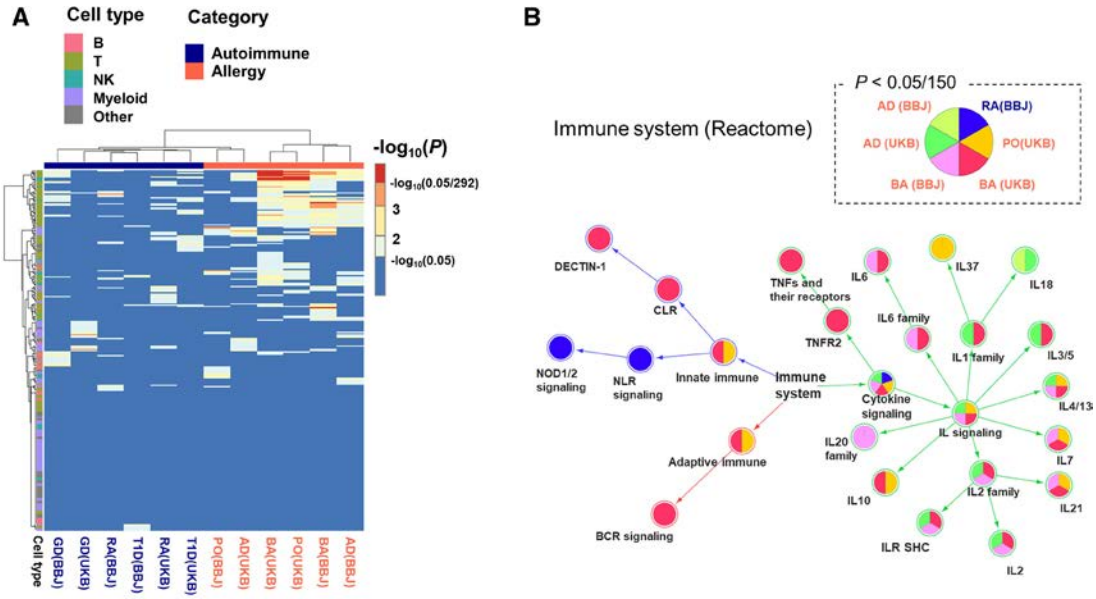

Figure 4 Enrichment analysis for six autoimmune and allergic diseases. (A) A heatmap describing the 292 immune cell-type enrichment using LDSC referring ImmGen gene expression data. The row and column are hierarchically clustered. The row annotations are coloured based on five cell types (B cell, T cell, NK cell, myeloid cell, and others), and the column annotations are coloured according to whether it is an autoimmune or an allergic disease. (B) The pathway network of immune system in Reactome database. The nodes are coloured according to whether the individual GWAS data are significantly enriched at a significance level of 0.05/150. For the sake of clarity, only nodes that have at least one enriched disease are shown. AD, atopic dermatitis; BA, bronchial asthma; BBJ, BioBank Japan; GD, Grave's diseases; GWAS, genome-wide association study; LDSC, linkage disequilibrium score regression; PO, pollinosis; RA, rheumatoid arthritis; T1D, type 1 diabetes; UKB, UK Biobank.

common effects between ancestry, thereby showing a value of both population-specific and cross-population approaches.

The European-specific *CYP1B1* missense variant of rs1800440 (N453S) was associated with allergic diseases susceptibility. Of note, another *CYP1B1* missense variant of rs1056836-G (V432L) was previously associated with BA susceptibility through a candidate gene approach ($p=0.045$),[45] of which independent protective effect was also confirmed in our study ($p=2.7\times10^{-5}$). *CYP1B1* is a member of the cytochrome P450 superfamily of enzymes and performs ligand degradation in aryl hydrocarbon receptor (AHR)-dependent signalling pathway.[46] AHR-dependent signalling pathway plays important roles in the immune response to molecular changes provided by the environment, diet, commensal flora and host metabolism.[47] The missense variants of *CYP1B1* are involved in developing allergic diseases through the dysregulation of immune responses to external molecules.

The East Asian-specific putative causal variants in *G3BP1* were associated with autoimmune and allergic disease susceptibility. The lead variant of rs2053062 has been reported as an eQTL that affects *G3BP1* expression levels in multiple immune cells. Colocalisations between the eQTL and the GWAS data sets in a set of lymphocyte cell types suggested *G3BP1* as a potential risk gene in the loci. *G3BP1* plays a positive role in activating the STING pathway, resulting in type 1 interferon response.[48] *G3BP1* expression levels have been reported to be high in autoimmune diseases involved in type 1 interferon, such as RA, myositis and SLE.[31] Because rs2053062-T has been reported to decrease *G3BP1* expression levels, this variant may have a protective effect on disease susceptibility by suppressing type 1 interferon activation. Notably, the protective effect of rs2053062-T was also observed in the allergic diseases in our analysis, implying the involvement of type I interferon in allergy.

Table 2 Significantly enriched cell type in autoimmune and allergic diseases

		P value		
Category	**Cell type**	**BA (UKB)**	**PO (UKB)**	**BA (BBJ)**
T cell	T.4Mem.Sp	**3.8×10^{-6}**	2.5×10^{-4}	0.0015
T cell	T.4Mem44h62l.Sp	**1.3×10^{-5}**	1.9×10^{-4}	5.3×10^{-4}
Natural killer cell	NKT.4-.Sp	**3.1×10^{-5}**	**4.6×10^{-5}**	0.0048
Natural killer cell	NKT.4+.Lv	**3.5×10^{-5}**	**1.1×10^{-4}**	0.019
T cell	LN.TR.14w.B6	**4.4×10^{-5}**	**3.6×10^{-5}**	7.4×10^{-4}
T cell	ABD.TR.14w.B6	**5.4×10^{-5}**	**6.5×10^{-5}**	2.2×10^{-4}
T cell	T.4Mem44h62l.LN	**1.4×10^{-4}**	5.6×10^{-4}	0.0078
T cell	CD4Control	3.3×10^{-4}	**9.1×10^{-5}**	0.0013
T cell	T.8Mem.Sp	0.010	0.0064	**1.5×10^{-4}**
T cell	T.8Eff.Tbet+.Sp.OT1.d6LisOVA	0.053	0.051	**1.5×10^{-4}**

P values satisfying the threshold of 0.05/292 for Bonferroni multiple testing are shown in bold.
BA, bronchial asthma; BBJ, BioBank Japan; PO, pollinosis; UKB, UK Biobank.

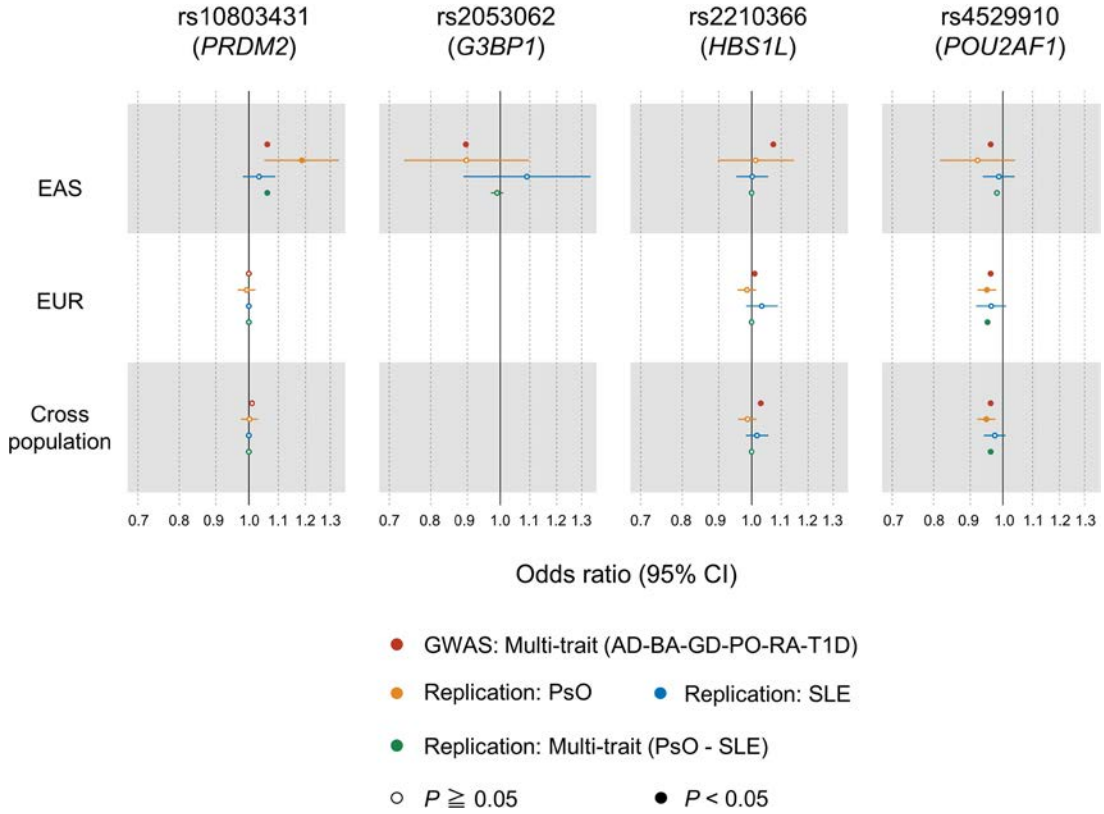

Figure 5 Forest plots of the replication meta-analysis for psoriasis and SLE. Odds ratio of the autoimmune and allergic associated variants are indicated by the individual population. The results of the original GWAS multi-trait analysis that integrates six autoimmune and allergic diseases are shown in red. The whiskers represent 95% CIs. AD, atopic dermatitis; BA, bronchial asthma; GD, Grave's diseases; PO, pollinosis; PsO, psoriasis; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; T1D, type 1 diabetes.

The cross-population meta-analysis identified the ancestry common variant in *POU2AF1*, which was associated with autoimmune and allergic disease susceptibility. The lead variant of *POU2AF1* also showed consistent effects on PsO and SLE. The *SIK2* locus, located downstream of *POU2AF1*, was previously reported to associate with allergic diseases. Several studies have annotated *POU2AF1* and *SIK2* together as the single risk locus. However, we found that these signals were independent. *POU2AF1* is essential for the response of B cells to antigens and required for the formation of germinal centres. *POU2AF1* is expressed in a highly cell-specific manner, being most abundant in B cells.[49] The protective allele rs4529910-G has been reported to decrease *POU2AF1* expression levels in B cells.[31] Therefore, we think that rs4529910-G has a protective effect for autoimmune and allergic diseases by attenuating humoral immunity.

Local heritability of allergic diseases was distributed across genome-wide, while it was relatively centralised in the HLA region in the autoimmune diseases. This difference might have resulted in heterogeneity in the enrichment analysis of cell type and biological pathway. In the cell-type enrichment analysis, regulatory T and natural killer cells were significantly enriched in allergic diseases, indicating the involvement of both adaptive and innate immune systems. The pathway enrichment analysis also showed that the allergic diseases were involved in various cytokine signals, including type 1 interferon. Non-type 2 inflammatory asthma is mainly caused by neutrophil inflammation involving IL6 and TNF-α, which is important pathogenesis as a cause of steroid refractory.[50] Thus, our study captured the diverse aetiologies that compose the immune-related diseases.

We also acknowledge potential discussions. First, BBJ is a hospital-based cohort, while UKB is a population-based cohort. The difference in cohort characteristics, including prevalence and diagnosis criteria, may have affected the results. Second, the inclusion of the HLA region in estimating genome-wide heritability is challenging due to its complex genetic architecture. The general framework used in our analysis, LDSC, estimates polygenic effects without the HLA region. The relatively small polygenic effects in autoimmune diseases make several complex analyses more challenging (eg, cross-population genetic correlation[51]). Third, we reported the genetic loci satisfying the genome-wide significance threshold at the level of $p=5.0\times10^{-8}$ without multiple testing correction of the number of the GWAS. Recent multi-trait GWASs adopt the nominal genome-wide significance threshold of $p=5.0\times10^{-8}$.[3] We note that the number of the significant loci was two (rs16902902 on *LINC00824* and rs4529910 on *POU2AF1*) when we strictly controlled multiple testing by Bonferroni correction ($p<5.0\times10^{-8}$/(12 independent GWASs and 9 meta-analyses)$=P<2.4\times10^{-9}$).

In summary, our multi-trait and cross-population approaches utilising the large-scale biobank resources demonstrated evidence of both distinct and shared genetic components across the autoimmune and allergic diseases. We also provided identification of novel loci linked to the immune-related diseases as well as elucidation of disease pathogenicity. Our approach proposes novel strategies to understand genetic backgrounds, biology, therapeutic targets of a set of complex human traits such as immune-related diseases.

Author affiliations
[1]Department of Statistical Genetics, Osaka University Graduate School of Medicine, Suita, Japan
[2]Department of Respiratory Medicine and Clinical Immunology, Osaka University Graduate School of Medicine, Suita, Japan
[3]Department of Immunopathology, Immunology Frontier Research Center (WPI-IFReC), Osaka University, Suita,Japan, Japan
[4]Integrated Frontier Research for Medical Science Division, Institute for Open and Transdisciplinary Research Initiatives (OTRI), Osaka University, Suita, Japan
[5]Department of Advanced Clinical and Translational Immunology, Osaka University Graduate School of Medicine, Suita, Japan
[6]Laboratory for Autoimmune Diseases, RIKEN Center for Integrative Medical Sciences, Yokohama, Japan
[7]Department of Respiratory Medicine and Clinical Immunology, Public Interest Incorporated Foundation, Nippon Life Saiseikai, Nippon Life Hospital, Osaka, Japan
[8]Division of Dermatology, Department of Internal Related, Kobe University Graduate School of Medicine, Kobe, Japan
[9]The First Department of Internal Medicine, University of Occupational and Environmental Health, School of Medicine, Kitakyushu, Fukuoka, Japan
[10]Department of Obstetrics and Gynecology, Osaka University Graduate School of Medicine, Suita, Japan
[11]StemRIM Institute of Regeneration-Inducing Medicine, Osaka University, Suita, Japan
[12]Laboratory of Immune Regulation, Department of Microbiology and Immunology, Osaka University Graduate School of Medicine, Suita, Japan
[13]Department of Respiratory Medicine, National Hospital Organization Osaka Toneyama Medical Center, Toyonaka, Japan
[14]Institue of Medical Science, The University of Tokyo, Tokyo, Japan
[15]Division of Molecular Pathology, Institute of Medical Science, The University of Tokyo, Tokyo, Japan
[16]Department of Internal Medicine, Institute of Medical Science, The University of Tokyo Hospital, Tokyo, Japan
[17]Laboratory of Complex Trait Genomics, Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, The University of Tokyo, Tokyo, Japan
[18]Center for Infectious Diseases for Education and Research (CiDER), Osaka University, Suita, Japan
[19]Laboratory for Systems Genetics, RIKEN Center for Integrative Medical Sciences, Yokohama, Japan
[20]Laboratory of Statistical Immunology, Immunology Frontier Research Center (WPI-IFReC), Suita, Japan
[21]Department of Genome Informatics, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

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Contributors YS and YO designed the study and wrote the manuscripts. YS, AS, KS, SN, TMa and YK performed the analysis. YN, HK, MY, SS, MI, YMi, and TJ conducted the experiments. RN, HT, YMa, TN, EO-I, TMo, SNak, CN, and YTan constructed the clinical and genotype data. YTak, KY, AK and YO supervised the study. YO is responsible for the overall content as guarantor. All authors contributed to the article and approved the submitted version.

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Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval All the subjects agreed with informed consent based on the approval of the institutional ethical committee. This study was approved by the ethical committee of Osaka University (Approval ID: 734-14). Participants gave informed consent to participate in the study before taking part.

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Data availability statement Data are available upon reasonable request. The summary statistics of the GWAS results has been deposited in the National Bioscience Database Center (NBDC) Human Database (https://humandbs.biosciencedbc.jp/en/) under the accession number of hum0197 [https://humandbs.biosciencedbc.jp/en/hum0197-latest]. Data can also be browsed at our pheweb.jp website [https://pheweb.jp/].

ORCID iDs
Yuichi Maeda http://orcid.org/0000-0002-6831-8205
Eri Oguro-Igashira http://orcid.org/0000-0001-5863-0775
Yoshiya Tanaka http://orcid.org/0000-0002-0807-7139
Kazuhiko Yamamoto http://orcid.org/0000-0001-9037-3625
Yukinori Okada http://orcid.org/0000-0002-0311-8472

REFERENCES

1 Okada Y, Wu D, Trynka G, *et al*. Genetics of rheumatoid arthritis contributes to biology and drug discovery. *Nature* 2014;506:376–81.
2 Ferreira MA, Vonk JM, Baurecht H, *et al*. Shared genetic origin of asthma, hay fever and eczema elucidates allergic disease biology. *Nat Genet* 2017;49:1752–7.
3 Sakaue S, Kanai M, Tanigawa Y, *et al*. A cross-population atlas of genetic associations for 220 human phenotypes. *Nat Genet* 2021;53:1415–24.
4 Li YR, Li J, Zhao SD, Bradfield JP, *et al*. Meta-Analysis of shared genetic architecture across ten pediatric autoimmune diseases. *Nat Med* 2015;21:1018–27.
5 Márquez A, Kerick M, Zhernakova A, *et al*. Meta-Analysis of immunochip data of four autoimmune diseases reveals novel Single-Disease and cross-phenotype associations. *Genome Med* 2018;10:1–13.
6 Acosta-Herrera M, Kerick M, González-Serna D, *et al*. Genome-wide meta-analysis reveals shared new *loci* in systemic seropositive rheumatic diseases. *Ann Rheum Dis* 2019;78:311–9.
7 Rottem M, Gershwin ME, Shoenfeld Y. Allergic disease and autoimmune effectors pathways. *Dev Immunol* 2002;9:161–7.
8 Krishna MT, Subramanian A, Adderley NJ, *et al*. Allergic diseases and long-term risk of autoimmune disorders: longitudinal cohort study and cluster analysis. *Eur Respir J* 2019;54. doi:10.1183/13993003.00476-2019. [Epub ahead of print: 14 11 2019].
9 Nagai A, Hirata M, Kamatani Y, *et al*. Overview of the Biobank Japan project: study design and profile. *J Epidemiol* 2017;27:S2–8.
10 Bycroft C, Freeman C, Petkova D, *et al*. The UK Biobank resource with deep phenotyping and genomic data. *Nature* 2018;562:203–9.
11 Hirata M, Kamatani Y, Nagai A, *et al*. Cross-Sectional analysis of Biobank Japan clinical data: a large cohort of 200,000 patients with 47 common diseases. *J Epidemiol* 2017;27:S9–21.
12 Sudlow C, Gallacher J, Allen N, *et al*. Uk Biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLoS Med* 2015;12:e1001779–10.
13 Hirata J, Hosomichi K, Sakaue S, *et al*. Genetic and phenotypic landscape of the major histocompatibilty complex region in the Japanese population. *Nat Genet* 2019;51:470–80.
14 Akiyama M, Ishigaki K, Sakaue S, *et al*. Characterizing rare and low-frequency height-associated variants in the Japanese population. *Nat Commun* 2019;10:4393.
15 Okada Y, Momozawa Y, Sakaue S, *et al*. Deep whole-genome sequencing reveals recent selection signatures linked to evolution and disease risk of Japanese. *Nat Commun* 2018;9:1631.
16 Zhou W, Nielsen JB, Fritsche LG, *et al*. Efficiently controlling for case-control imbalance and sample relatedness in large-scale genetic association studies. *Nat Genet* 2018;50:1335–41.
17 Bulik-Sullivan BK, Loh P-R, Finucane HK, *et al*. LD score regression distinguishes confounding from polygenicity in genome-wide association studies. *Nat Genet* 2015;47:291–5.
18 Ning Z, Pawitan Y, Shen X. High-Definition likelihood inference of genetic correlations across human complex traits. *Nat Genet* 2020:1–6.
19 Zhang Y, Lu Q, Ye Y, *et al*. SUPERGNOVA: local genetic correlation analysis reveals heterogeneous etiologic sharing of complex traits. *Genome Biol* 2021;22:1–30.
20 Berisa T, Pickrell JK. Approximately independent linkage disequilibrium blocks in human populations. *Bioinformatics* 2016;32:btv546.
21 Lin D-Y, Sullivan PF. Meta-Analysis of genome-wide association studies with overlapping subjects. *Am J Hum Genet* 2009;85:862–72.
22 Lee CH, Eskin E, Han B. Increasing the power of meta-analysis of genome-wide association studies to detect heterogeneous effects. *Bioinformatics* 2017;33:i379–88.

23 Watanabe K, Taskesen E, van Bochoven A, *et al*. Functional mapping and annotation of genetic associations with FUMA. *Nat Commun* 2017;8:1–10.
24 Buniello A, MacArthur JAL, Cerezo M, *et al*. The NHGRI-EBI GWAS catalog of published genome-wide association studies, targeted arrays and summary statistics 2019. *Nucleic Acids Res* 2019;47:D1005–12.
25 Gagliano Taliun SA, VandeHaar P, Boughton AP, *et al*. Exploring and visualizing large-scale genetic associations by using PheWeb. *Nat Genet* 2020;52:550–2.
26 Kamat MA, Blackshaw JA, Young R, *et al*. PhenoScanner V2: an expanded tool for searching human genotype-phenotype associations. *Bioinformatics* 2019;35:4851–3.
27 Ghoussaini M, Mountjoy E, Carmona M, *et al*. Open targets genetics: systematic identification of trait-associated genes using large-scale genetics and functional genomics. *Nucleic Acids Res* 2021;49:D1311–20.
28 Wang G, Sarkar A, Carbonetto P, *et al*. A simple new approach to variable selection in regression, with application to genetic fine mapping. *J R Stat Soc B* 2020;82:1273–300.
29 Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res* 2010;38:e164–7.
30 GTEx Consortium. The GTEx Consortium atlas of genetic regulatory effects across human tissues. *Science* 2020;369:1318–30.
31 Ota M, Nagafuchi Y, Hatano H, *et al*. Dynamic landscape of immune cell-specific gene regulation in immune-mediated diseases. *Cell* 2021;184:3006–21.
32 Hormozdiari F, van de Bunt M, Segrè AV, *et al*. Colocalization of GWAS and eQTL signals detects target genes. *Am J Hum Genet* 2016;99:1245–60.
33 Finucane HK, Reshef YA, Anttila V, *et al*. Heritability enrichment of specifically expressed genes identifies disease-relevant tissues and cell types. *Nat Genet* 2018;50:621–9.
34 Heng TSP, Painter MW, Elpek K, Immunological Genome Project Consortium. The immunological genome Project: networks of gene expression in immune cells. *Nat Immunol* 2008;9:1091–4.
35 Lamparter D, Marbach D, Rueedi R, *et al*. Fast and rigorous computation of gene and pathway scores from SNP-based summary statistics. *PLoS Comput Biol* 2016;12:e1004714–20.
36 Subramanian A, Tamayo P, Mootha VK, *et al*. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A* 2005;102:15545–50.
37 Shannon P, Markiel A, Ozier O, *et al*. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res* 2003;13:2498–504.
38 Wang Y-F, Zhang Y, Lin Z, *et al*. Identification of 38 novel loci for systemic lupus erythematosus and genetic heterogeneity between ancestral groups. *Nat Commun* 2021;12:1–13.
39 Bentham J, Morris DL, Graham DSC, *et al*. Genetic association analyses implicate aberrant regulation of innate and adaptive immunity genes in the pathogenesis of systemic lupus erythematosus. *Nat Genet* 2015;47:1457–64.
40 Szklarczyk D, Gable AL, Lyon D, *et al*. String v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res* 2019;47:D607–13.
41 Wishart DS, Feunang YD, Guo AC, *et al*. DrugBank 5.0: a major update to the DrugBank database for 2018. *Nucleic Acids Res* 2018;46:D1074–82.
42 Zhou Y, Zhang Y, Lian X, *et al*. Therapeutic target database update 2022: facilitating drug discovery with enriched comparative data of targeted agents. *Nucleic Acids Res* 2022;50:D1398–407.
43 García-Cuesta EM, Santiago CA, Vallejo-Díaz J, *et al*. The role of the CXCL12/CXCR4/ACKR3 axis in autoimmune diseases. *Front Endocrinol* 2019;10:1–16.
44 Hutloff A, Dittrich AM, Beier KC, *et al*. ICOS is an inducible T-cell co-stimulator structurally and functionally related to CD28. *Nature* 1999;397:263–6.
45 Polonikov AV, Ivanov VP, Solodilova MA. Genetic variation of genes for xenobiotic-metabolizing enzymes and risk of bronchial asthma: the importance of gene-gene and gene-environment interactions for disease susceptibility. *J Hum Genet* 2009;54:440–9.
46 Quintana FJ. *Review regulation of the immune response by the aryl hydrocarbon receptor*, 2018: 19–33.
47 Rothhammer V, Quintana FJ. The aryl hydrocarbon receptor: an environmental sensor integrating immune responses in health and disease. *Nat Rev Immunol* 2019;19:184–97.
48 Wiser C, Kim B, Ascano M. G3Bp1 enhances cytoplasmic DNA pattern recognition. *Nat Immunol* 2019;20:5–7.
49 Strubin M, Newell JW, Matthias P. Obf-1, a novel B cell-specific coactivator that stimulates immunoglobulin promoter activity through association with octamer-binding proteins. *Cell* 1995;80:497–506.
50 Israel E, Reddel HK, Severe RHK. Severe and difficult-to-treat asthma in adults. *N Engl J Med* 2017;377:965–76.
51 Galinsky KJ, Reshef YA, Finucane HK, *et al*. Estimating cross-population genetic correlations of causal effect sizes. *Genet Epidemiol* 2019;43:180–8.

EPIDEMIOLOGICAL SCIENCE

Optimising both disease control and glucocorticoid dosing is essential for bone protection in patients with rheumatic disease

Edgar Wiebe,[1] Dörte Huscher,[2] Désireé Schaumburg,[1] Andriko Palmowski,[1] Sandra Hermann,[1] Thomas Buttgereit,[3,4] Robert Biesen ,[1] Gerd-Rüdiger Burmester ,[1] Yannick Palmowski,[5] Maarten Boers ,[6] John H Stone ,[7] Christian Dejaco ,[8,9] Frank Buttgereit [1]

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For numbered affiliations see end of article.

Correspondence to
Professor Frank Buttgereit, Department of Rheumatology and Clinical Immunology, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany; frank.buttgereit@charite.de

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ABSTRACT

Objectives Inflammatory rheumatic and musculoskeletal diseases (iRMDs) are associated with increased systemic bone loss that is mediated by chronic inflammation, treatment with glucocorticoids (GCs) and other factors. Our objective was to analyse the impact of variables that influence osteoporosis (OP) in patients with iRMD treated with GC.

Methods Rh-GIOP (acronyme) is a prospective observational cohort study investigating bone health in consecutive patients with iRMD and current or prior GC treatment. We present an analysis of the patients' baseline data here. Bone mineral density (BMD) measured by dual X-ray absorptiometry was the primary outcome. Multivariable linear regression models were performed to identify variables associated with BMD.

Results Data from 1066 patients with iRMD were analysed. GC doses of <5 mg prednisone equivalent per day, cumulative dose and duration of GC therapy were not associated with negative effects on BMD. Dosages of ≥5 mg/day lost their negative association with BMD after adjustment for confounders. When subanalysing patients with exactly 5 mg/day, no negative effect was seen. For patients with rheumatoid arthritis (RA), GC doses of >7.5 mg/day showed a negative association with BMD overall, but this effect seemed to be specific only to patients with moderate or high disease activity (Disease Activity Score 28–C reactive protein >3.2).

Conclusions GCs of ≤5 mg/day did not seem to be associated with a reduction of BMD in patients with iRMD and current or prior exposure to GC. This is most likely due to the dampening of inflammation by GC, which exerts a mitigating effect on the risk of OP. In RA, current GC doses of >7.5 mg/day were negatively associated with BMD, but only in patients with moderate to high disease activity.

Trial registration number NCT02719314.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Patients with inflammatory rheumatic and musculoskeletal diseases (iRMD) have an increased risk of osteoporosis and fragility fractures. The influence of glucocorticoid (GC) therapy on this risk has been controversial for years.

WHAT THIS STUDY ADDS

⇒ In this large cross-sectional study of patients previously or currently exposed to GCs, doses of ≤5 mg/day prednisone equivalent did not seem to be associated with negative effects on bone mineral density (BMD).

⇒ Higher daily GC dosages lost their negative association with BMD after adjustment for confounding factors.

⇒ In patients with rheumatoid arthritis, GC doses of >7.5 mg/day seemed to be negatively associated with BMD only in combination with moderate or high disease activity.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE AND/OR POLICY

⇒ GCs should be used in an optimum dose, titrated with both benefit and harm in mind, in order to achieve remission and to support bone health in patients with iRMD.

INTRODUCTION

Glucocorticoids (GCs) exert powerful anti-inflammatory and immunosuppressive effects[1 2] and are widely used to treat inflammatory rheumatic diseases (eg, rheumatoid arthritis (RA), vasculitis, lupus and inflammatory myopathies). In addition to their beneficial effects on reducing inflammation, GCs are also associated with many well-known adverse effects. Their use often elicits fierce debates on the benefit–risk profile.[3] Among the most worrisome and unwanted effects of GC therapy is osteoporosis (OP).[4] Despite the common use of these drugs, now employed for the treatment of inflammatory diseases for more than 70 years, there remain many unanswered questions about their use, such as the following: is there a safe (long-term) dose for bone? what is the dose-dependent effect size of GC therapy on bone health compared with other influencing factors?

This study focuses on 'glucocorticoid-induced' osteoporosis (GIOP), the most common form of secondary OP.[5] This condition affects up to one-third of GC-treated patients suffering from inflammatory rheumatic and musculoskeletal diseases (iRMDs).[6] The deleterious bone effects of GC at dosages above 10 mg/day of prednisone equivalent

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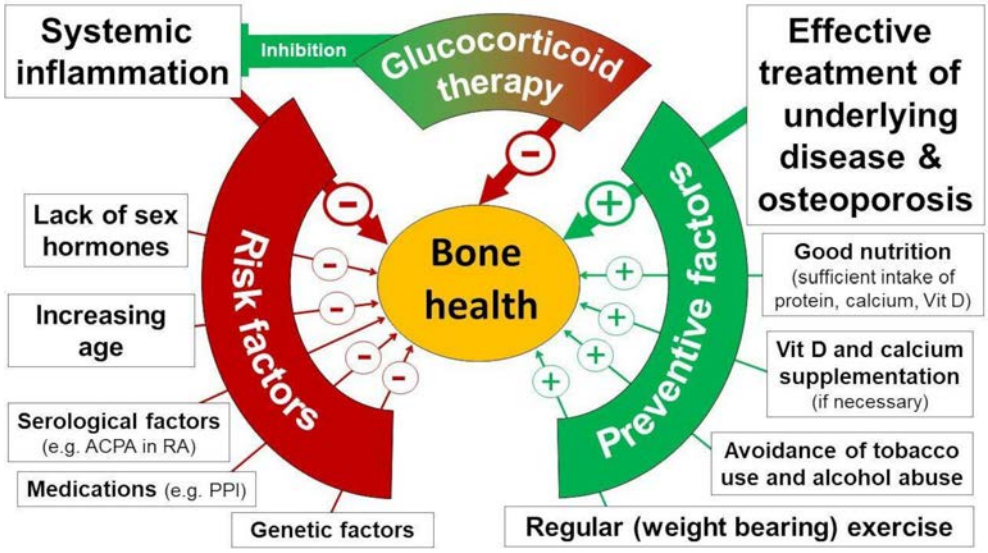

Figure 1 Protective and risk factors for osteoporosis-related bone health. This figure illustrates selected factors influencing bone health according to current evidence but is not meant to be exhaustive. + indicates that the factors exert a protective effect on bone; – indicates a negative impact on bone health. Font size reflects presumed importance. ACPA, anticitrullinated protein antibody; PPI, proton pump inhibitor; RA, rheumatoid arthritis.

for prolonged periods are undisputed.[7] In recent years, however, it has been recognised that overall bone health on GC treatment is actually a result not only of the dose and duration of GC treatment, but also of several highly interactive factors that impact the potential for OP both positively and negatively (see also online supplemental box S1).[8 9] For instance, inflammation also has deleterious effects and, in turn, is dampened by GC. In patients with iRMD, the net effect of GC treatment is modified by other factors such as inflammatory activity, age, regular exercise (which, in turn, is also determined by disease activity and pain), menopausal status, vitamin D levels and current therapy of both the underlying disease and OP (figure 1).

This background was the driving force to initiate the Inflammatory Rheumatic and Musculoskeletal Diseases and Glucocorticoid-Induced Osteoorosis (Rh-GIOP) open cohort study in 2015. Its aim is to examine the effects of protective and risk factors contributing to bone health in iRMD in a comprehensive manner, including 'old' as well as 'new' antirheumatic and antiosteoporotic therapies. We present the results of the first analysis of baseline data including more than 1000 patients.

METHODS

Study design and patient involvement

Rh-GIOP is an ongoing single-centre open cohort study designed to collect and analyse disease-related and bone-related data from patients with iRMD with prior or current exposure to GC. We partnered with a patient representative from the Deutsche Rheuma-Liga to centre our research outcomes and questionnaire on patients' preferences. Patients receiving longitudinal care at the inpatient or outpatient clinic of the Department of Rheumatology and Clinical Immunology of Charité University Medicine are eligible. Data collection started in July 2015, and data are entered into an access database (programmed by Medikadat, Leverkusen, Germany). Five years of data extending through July 2020 are included.

Eligibility criteria

Inclusion criteria include (1) age at least 18 years, (2) clinical diagnosis of an iRMD, (3) current or previous treatment with GC, and (4) eligibility for OP diagnostics as recommended by the Dachverband Osteologie (http://dv-osteologie.org; see online supplemental box S2).

Key exclusion criteria: (1) pregnancy or lactation and (2) inability to provide informed consent for any reason (for full description, see online supplemental table S1).

Data collection

Data collected on each patient are summarised in table 1.

Bone densitometry

Bone mineral density (BMD) was measured at the lumbar spine and bilateral proximal femur by dual X-ray absorptiometry (DXA). All participants were scanned on Lunar Prodigy bone densitometers (GE Medical Systems Lunar Corporation, Madison, Wisconsin, USA) per manufacturer recommendations and analysed with enCORE Software. The results are presented as T-scores. Scores <-1.0 to >-2.5 were classified as osteopenic ('low bone mass') and ≤ -2.5 osteoporotic.

Statistical analysis

The primary outcome was BMD expressed by the T-score, more specifically, the lowest (minimum) T-score measured at either the lumbar spine (L1–L4), the left and right femoral neck or total hip. Secondary outcomes were the lowest T-score of the individual lumbar vertebrae (L1–L4) and the lower T-score obtained at the left and right femoral neck. To identify variables associated with the T-score, multivariable linear regression models were formed that included a full set of factors preselected according to published evidence, medical and clinical expertise and subcategorised into factors known to have an impact on bone health (online supplemental table S2). Our aim was data mining but not a specific regression model, to identify variables strongly associated with the respective T-scores out of a large pool of potential factors competing in one model. The full model, including non-significant variables, is reported. In addition, we performed a sensitivity analysis that excluded patients treated with anti-OP drugs (bisphosphonates, denosumab and teriparatide). Collinearity between explanatory variables was ignored. Multiple imputation with 10 replications were used to address missing

Table 1 Data collected in each patient (by questionnaire and measurements)

Demographics and general information	Age, sex, *BMI*, smoking status, alcohol consumption, type and frequency of physical exercise, exposure to direct sunlight, daily calcium intake, use of care services and socioeconomic status
Description of GC therapy	Current GC dose, mean daily GC dose, cumulative (lifetime) GC dose* and duration of GC therapy
Description of underlying disease	Onset of disease, current disease activity (DAS28–ESR, DAS28–CRP, CDAI, SDAI, SLEDAI, BASDAI, BASMI, BVAS, concomitant diseases and organ manifestation of iRMD (such as diabetes, hypertension, stroke, cancer, pericarditis in SLE, lung fibrosis in systemic sclerosis, etc), selected patient-reported outcomes (pain according to numerical rating scale, health assessment questionnaire, bath ankylosing spondylitis functional index), and past and current antirheumatic drugs
General bone-relevant parameters	Vitamin D and calcium supplementation, treatment with antiosteoporotic drugs, treatment with drugs having a known or potential impact on bone (eg, proton pump inhibitors)
Clinical bone-relevant parameters	Family history of osteoporosis/osteoporotic fractures, *frailty assessment (timed-up-and-go test, chair-rising test and tandem stand)*, back pain, prior low-trauma vertebral and non-vertebral fractures,† date of fracture, management of fractures, fracture sequalae, weight loss, loss of height, past falls, risk assessment of falls, back pain, menarche/menopause/pregnancies/lactation/past use of hormone-based contraceptives
Technical bone-relevant parameters	*Routine laboratory parameters such as calcium, phosphate, vitamin D levels (1, 25 and 25), iPTH, bone alkaline phosphatase, crosslinks and other, BMD/T-score measured by DXA and TBS*

Parameters in *italic* were retrieved through measurements. All other parameters were assessed through a questionnaire. When patients were not able to provide full or detailed information, patient charts were used to complement the investigated parameters.
*Cumulative GC dose was calculated meticulously from patients' self-reported dose and duration of GC therapy with the help of supplemental data retrieved from patient charts.
†History of fractures was self-reported and verified from patient charts, if available. Fractures were adjudicated under osteoporotic fractures when having occurred due to inadequate trauma or fall from standing height.
BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASMI, Bath Ankylosing Spondylitis Metrology Index; BMD, bone mineral density; BMI, body mass index; BVAS, Birmingham Vasculitis Activity Score; CDAI, Clinical Disease Activity Index; CRP, C reactive protein; DAS28, Disease Activity Score 28; DXA, dual-energy X-ray absorptiometry; ESR, erythrocyte sedimentation rate; GC, glucocorticoid; HAQ, Health Assessment Questionnaire; iPTH, intact parathyroid hormone; iRMD, inflammatory rheumatic and musculoskeletal disease; SDAI, Simplified Disease Activity Index; SLEDAI, Systemic Lupus Erythematodes Disease Activity Index; TBS, trabecular bone score.

data. Variables categorised a priori as having weak effects on BMD were excluded if values were missing in more than 30% of the patients. Exceptions were made for alanine transaminase (31% missing), alkaline phosphatase (32%) and urinary deoxypyridinoline (34%) due to their known relevance as laboratory markers for the assessment of bone health.

To explore the impact of current GC dose on the T-score, three commonly discussed GC threshold doses were tested as binary variables in separate multivariable models: <5, ≤5 and ≤7.5 mg/day prednisone equivalent. Subdivisions at doses below 5 mg/day were not feasible due to low case numbers. The models with the lowest cut-off with significant impact for any of the considered T-scores are reported. The following categorical variables were analysed in three models: (1) in crude models without any adjustment; (2) in models adjusted for age, sex, menopause, body mass index (BMI), disease duration, alkaline phosphatase, and the use of denosumab and bisphosphonates; and (3) in models specifically adjusted for those variables retained from the data mining processes after backward selection. The results are displayed in forest plots for comparison. Because the majority of patients received 5 mg/day, another categorisation of current GC dose was analysed in the process described previously with 'no GC', '>0 mg/day to <5 mg/day', '5 mg/day' and '>5 mg/day', with a specific focus on 5 mg/day.

Currently, no generic clinical composite score of iRMDs is available to assess the influence of disease activity on BMD. For patients with RA—the largest patient subgroup—the DAS28–CRP as a specific composite score of disease activity was available in 93% of individuals. The interplay of GC dose and disease activity on the lowest T-score was explored with a combination variable of dose and activity in a separate model including all variables that were significant in the prior model selection for patients with RA. Apart from RA, the number of scored patients in individual disease groups was too small to perform a subgroup analysis for associations between composite score and BMD.

In order to investigate the impact of anticitrullinated protein antibodies (ACPAs)/rheumatoid factor (RF), four variants of possible singlets/combinations were considered in separate multivariable linear regression models as described previously (online supplemental table S3): (1) positive ACPA status, (2) positive RF status, defined as either IgA or IgM positivity; (3) double positive, defined as both positive ACPA and RF status; and (4) double negative.

Values are reported as mean/SD for normally distributed data and median/inner quartiles for non-normally distributed continuous variables. Subgroup comparisons were performed by non-parametric tests for continuous variables and χ^2 tests for categorical variables. P values lower than 0.05 were considered significant. Given the explorative nature of this study, no adjustment for multiple testing was done. IBM SPSS Statistics V.27.0 was used for analysis.

RESULTS

A total of 1246 patients were enrolled, comprising >95% of eligible patients (ie, those who met inclusion/exclusion criteria after screening). Approximately 60% of the patients enrolled were from the outpatient clinic, and the remainder were from day or in-hospital care. Patients with the following iRMD were included in the current analysis: RA (n=434), connective tissue diseases (CTDs) (n=281), vasculitides (n=173) and spondyloarthritides, including psoriatic arthritis (n=178). Patients in other disease groups, totaling 174, were excluded because of low numbers in any individual disease group. Six patients were excluded because of clinically manifest hyperparathyroidism, leaving 1066 patients for the final analysis. The patients' age was 62 (±13) years, and 76% were women, of whom 89% were postmenopausal (table 2). Further baseline characteristics are summarised in tables 2–4, and details on disease, treatment and bone health are listed.

Two-thirds were taking GC at baseline; the median daily dose was 5 mg. OP, as indicated by the lowest measured T-score, was present in 22% of patients and osteopenia in 49%; 31% had fragility fractures. Of note, 24% of the latter group had normal T-scores; 44% were osteopenic; and 32% osteoporotic. Overall, 43% had OP (12% OP by DXA, 21% by fragility fracture and 10% by both).

Prevalence of OP risk modifiers

Most patients had low CRP levels (median 2.3 mg/L, normal <5), suggesting low systemic inflammatory activity. In patients with RA, mean DAS28–CRP score was 2.7 (±1.3). Disease duration in the entire cohort was 12 (±10) years, with mild to moderate

Table 2 Demographics, GC therapy and bone status*†

	All	RA‡	CTD§	Vasculitides¶	Spondyloarthritides**
	N=1066	N=434	N=281	N=173	N=178
Demographics					
Age (years)	62.2 (±13)	64.2 (±12)	57.5 (±15)	67.6 (±12)	59.4 (±12)
Female patients	806 (76)	348 (80)	240 (85)	115 (67)	103 (58)
Menopause	706 (89)	314 (91)	193 (81)	111 (97)	88 (87)
BMI (kg/m²)	27.1 (±5.4)	27.7 (±5.6)	25.3 (±5.2)	26.6 (±4.4)	28.7 (±5.7)
GC therapy					
Patients with current GC†† therapy	705 (66)	311 (72)	201 (72)	150 (87)	43 (24)
Current GC dose (mg/day), median (IQ)	5.0 (5–10)	5.0 (4–8)	5.0 (5–10)	8.2 (5–30)	10.0 (5–40)
≤2.5 (% of total current GC)	85 (12)	48 (15)	17 (9)	16 (11)	4 (9)
>2.5–4.9	75 (11)	40 (13)	20 (10)	11 (7)	4 (9)
5.0–7.4	285 (40)	143 (46)	92 (46)	41 (27)	9 (21)
7.5–10.0	108 (15)	41 (13)	38 (19)	20 (13)	9 (21)
>10.0	152 (22)	39 (13)	34 (17)	62 (41)	17 (40)
Cumulative GC dose (g)‡‡	18.2 (±24.7)	18.0 (±23.8)	23.4 (±26.3)	13.9 (±22.6)	12.9 (±26.0)
Duration of GC therapy (years)	8.2 (±8.8)	8.7 (±9.1)	10.5 (±9.1)	5.0 (±6.2)	6.1 (±8.2)
Bone status (T-score§§)					
Spine	−0.7 (±1.5)	−0.8 (±1.5)	−1.0 (±1.3)	−0.6 (±1.5)	−0.6 (±1.5)
Normal	512 (51)	205 (51)	116 (44)	91 (56)	100 (61)
Osteopenia	374 (38)	157 (39)	109 (41)	60 (37)	48 (29)
OP	107 (11)	40 (10)	39 (15)	11 (7)	17 (10)
Left femoral neck	−1.1 (±1.1)	−1.1 (±1.0)	−1.2 (±1.2)	−1.2 (±1.0)	−0.9 (±1.1)
Normal	395 (41)	155 (39)	97 (38)	53 (34)	90 (54)
Osteopenia	486 (50)	196 (50)	135 (52)	89 (57)	66 (39)
OP	94 (9)	42 (11)	26 (10)	14 (9)	12 (7)
Right femoral neck	−1.1 (±1.1)	−1.1 (±1.1)	−1.2 (±1.1)	−1.2 (±1.0)	−0.9 (±1.2)
Normal	395 (41)	156 (40)	96 (37)	58 (37)	85 (51)
Osteopenia	475 (49)	193 (40)	133 (51)	85 (54)	64 (38)
OP	101 (11)	38 (10)	31 (12)	14 (9)	18 (11)
Osteoporotic fractures¶¶					
Vertebral	67 (6)	34 (8)	12 (4)	14 (8)	7 (4)
Non-vertebral	290 (27)	124 (29)	70 (25)	41 (24)	55 (31)

*Categorical variables are presented as number and per cent of valid observations (%) unless otherwise noted.
†Continuous variables are presented as mean values with SD unless otherwise noted.
‡RA comprises patients with seropositive and seronegative RA as well as late-onset RA.
§CTDs include patients with systemic lupus erythematosus, progressive systemic sclerosis, limited cutaneous systemic sclerosis, mixed CTD, polymyositis, undifferentiated CTD, antisynthetase syndrome, eosinophilic fasciitis, inclusion body myositis, dermatomyositis, scleroderma with overlap RA and Sjogren's syndrome.
¶Vasculitides include polymyalgia rheumatica, giant cell arteritis, panarteritis nodosa, microscopic polyangiitis, granulomatosis with polyangiitis, eosinophilic granulomatosis with polyangiitis, Cogan's syndrome, anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis and undifferentiated vasculitis.
**Spondyloarthritides include psoriatic arthritis and ankylosing spondylitis.
††GCs include both oral and intravenous application forms of prednis(ol)one, methylprednisolone and modified-release prednisone. All doses are given in prednisone equivalent.
‡‡Cumulative GC dose is an estimate calculated from information provided by the patient with the help of patient charts for the entire duration of GC therapy.
§§BMD and T-score are measured with GE Healthcare Lunar Prodigy DF+15629 dual X-ray absorptiometry scanner. Normal, ≥−1.0; osteopenia, <−1.0; and >−2.5; OP, ≤−2.5.
¶¶History of fractures was self-reported and/or verified from patient charts, if available. In case of clinical suspicion of a vertebral fracture, a conventional X-ray examination was performed. Fractures were adjudicated under osteoporotic fractures when having occurred due to inadequate trauma or fall from standing height.
BMD, bone mineral density; BMI, body mass index; CTD, connective tissue disease; GC, glucocorticoid; IQ, inner quartile; OP, osteoporosis; RA, rheumatoid arthritis.

disability (mean HAQ score 0.8, table 3). Fifty-one per cent required (mostly low-level) support from care services. Some patients were on antirheumatic or antiosteoporotic drugs at baseline; others were not (table 4).

Factors associated with BMD

Age, male sex, menopause and HAQ were negatively associated with T-scores. For laboratory parameters, only alkaline phosphatase (negative) and gamma-GT levels (positive) were associated with T-scores (all patients, online supplemental table S4A; patients with RA, online supplemental table S4B; sensitivity analysis excluding patients on anti-OP drugs, online supplemental table S4C).

Treatment with antiosteoporotic medication was strongly associated with low T-scores at any site, with regression coefficients of −0.42 for denosumab and −0.45 for bisphosphonates. Prior vertebral (−0.39) and non-vertebral fractures were associated with low BMD, with the latter, however, only at the femoral neck (−0.53). Non-steroidal anti-inflammatory drugs were positively associated with T-scores (+0.10) at the femoral neck, while proton pump inhibitors (PPIs) were negatively associated with the lowest T-score (−0.19) at all sites measured (online supplemental table S4A).

All variables emerging in the three models were confirmed in regression analyses with backward selection except for diabetes and calcium supplementation for lumbar T-score (online supplemental table S5).

Of note, disease-modifying antirheumatic drug (DMARD) use, including biological, conventional synthetic and targeted synthetic agents, was not associated with an impact on BMD.

Table 3 Risk factors for OP*†

	All	RA	CTD	Vasculitides	Spondyloarthritides
	N=1066	N=434	N=281	N=173	N=178
Disease activity					
HAQ score	0.8 (±0.8)	0.9 (±0.8)	0.9 (±0.9)	0.6 (±0.8)	0.8 (±0.7)
S-CRP mg/L (<5), median (IQR)	2.3 (0.8–6.6)	2.4 (0.8–6.7)	1.6 (0.7–4.9)	4.8 (1.3–10.9)	2.0 (0.8–4.9)
RA–DAS28–CRP score			2.7 (±1.3)		
Disease duration (years)	11.9 (±10)	11.9 (±10)	12.8 (±9)	5.4 (±6)	17.2 (±13)
Use of care services‡	473 (51)	189 (49)	147 (59)	53 (36)	84 (57)
Age (years)					
Age group					
<50	164 (15)	43 (10)	80 (9)	12 (7)	29 (16)
50–64	427 (40)	180 (42)	103 (27)	50 (29)	94 (53)
65–84	458 (43)	203 (47)	97 (25)	107 (62)	51 (29)
≥85	17 (2)	8 (2)	1 (<1)	4 (2)	4 (2)
Underweight (BMI <18.5 kg/m²)	28 (3)	8 (2)	18 (6)	2 (1)	0
Family history					
OP	212 (27)	100 (30)	54 (25)	22 (19)	36 (33)
Osteoporotic fractures	101 (13)	44 (14)	28 (14)	13 (11)	16 (14)
Comedication					
Proton pump inhibitors	468 (44)	175 (40)	138 (49)	98 (57)	57 (32)
NSAIDs	249 (23)	117 (27)	45 (16)	14 (8)	73 (41)
Antidepressants	75 (7)	18 (4)	38 (14)	6 (4)	13 (7)
Oral antidiabetics	61 (6)	26 (6)	4 (1)	11 (6)	20 (11)
Insulin	49 (4)	19 (4)	9 (3)	9 (5)	12 (7)
Antihyperuricaemic drugs	42 (4)	18 (4)	9 (3)	8 (5)	7 (4)
Oestrogens (female patients only)	17 (2)	6 (1)	7 (3)	0	5 (5)
Concomitant diseases§					
Osteoarthritis	153 (14)	79 (18)	38 (14)	10 (6)	26 (15)
Diabetes	130 (12)	56 (13)	16 (6)	26 (15)	32 (18)
Dyslipidaemia	119 (11)	43 (10)	32 (11)	25 (15)	19 (11)
Depression	94 (9)	39 (9)	27 (10)	11 (6)	17 (10)
Renal insufficiency	76 (7)	21 (5)	22 (8)	25 (15)	8 (5)
Hyperuricaemia/gout	53 (5)	23 (5)	12 (4)	8 (5)	10 (6)

*Categorical variables are presented as number and per cent of valid observations (%) unless otherwise noted.
†Continuous variables are presented as mean values with SD unless otherwise noted.
‡Use of care services comprises any level of care received, including low-level support. The latter applied for most patients.
§Concomitant diseases: shown are diseases or medications that are either particularly common and/or variables considered to have a 'weakly expected' impact on the T-score. To avoid overfitting, diseases or medications were not considered in our model when case numbers were low (such as history of transplantation, chronic obstructive pulmonary disease, antiepileptic therapy, heart failure, aromatase inhibitors and hypogonadism).
BMI, body mass index; CTD, connective tissue diseases; HAQ, Health Assessment Questionnaire; NSAID, non-steroidal anti-inflammatory drug; OP, osteoporosis; RA, rheumatoid arthritis; S-CRP, serum C reactive protein.

Impact of GCs on BMD

In the crude analysis of the effect of the current GC dose categorised as no GC, >0 mg/day to <5 mg/day, '5.0–7.5 mg/day' and '>7.5 mg/day', we found no differential effects on T-scores between patients on current GC at doses below five and patients not on GC. There were negative effects, however, in patients on dosages >7.5 mg/day (figure 2). At the femoral neck, this effect was already noticeable at dosages of 5.0–7.5 mg/day. However, after adjustment for age, sex, menopause, BMI, disease duration, alkaline phosphatase, and the use of denosumab and bisphosphonates, this effect was seen only for dosages of 5.0–7.5 mg/day (for min- and min-/T-score femoral neck). When further optimising adjustment to include only significant variables for the respective score (online supplemental table S5), the effect estimates shifted even closer to 0, suggesting no meaningful impact of any GC dose (figure 2).

Similar results were obtained with the GC dose categorisation no GC, >0 mg/day to <5 mg/day, 5 mg/day and >5 mg/day. In the crude model the difference in T-score between 5 mg/day compared with no GC use was significant only at the femoral neck, persisting after predefined adjustment but disappearing after specified adjustment.

As described in table 1, we also quantified the GC therapy by estimating the cumulative dose and duration of GC therapy. These did not show strong associations with T-scores.

Since approximately 15% of patients in our cohort received anti-OP drugs at baseline (mostly bisphosphonates), we performed a sensitivity analysis that excluded patients with anti-OP drugs. This did not change our findings and conclusions (online supplemental table S4C).

Impact of GCs, ACPA or RF, and the use of DMARDs on BMD in patients with RA

In patients with RA, current GC doses of >5 mg per day had a significant negative association with the lowest overall (−0.49) and lumbar spine T-score (−0.77), together with age, menopause, BMI, alkaline phosphatase, bisphosphonates, disease duration, denosumab and male sex (compare online supplemental table S4B). These results were confirmed at the higher

Table 4 Factors with a confirmed or potential anti-OP effect and bone turnover markers*†

	All	RA	CTD	Vasculitides	Spondyloarthritides
	N=1066	N=434	N=281	N=173	N=178
Treatment of underlying disease					
csDMARDs‡	637 (60)	288 (66)	210 (75)	81 (47)	58 (33)
Biologics	313 (29)	154 (36)	38 (14)	24 (14)	97 (55)
TNF-alpha antagonists§ (n, % of total biologics)	134 (43)	76 (49)	1 (3)	0	57 (59)
IL-6R antagonists¶	47 (15)	33 (21)	4 (11)	10 (42)	0
Rituximab	57 (18)	25 (16)	18 (47)	14 (58)	0
Abatacept	23 (7)	20 (13)	1 (3)	0	2 (2)
IL-17 and IL-12/23 antagonists**	38 (12)	0	0	0	38 (39)
Belimumab	15 (5)	0	15 (40)	0	0
tsDMARDs††	26 (2)	18 (4)	1 (<1)	0	7 (4)
Antiosteoporotic therapy					
Vitamin D supplementation	865 (81)	365 (84)	250 (89)	144 (83)	87 (49)
Calcium supplementation	51 (5)	24 (6)	18 (6)	6 (4)	3 (2)
Bisphosphonates‡‡	124 (12)	60 (14)	31 (11)	29 (17)	4 (2)
Denosumab	32 (3)	13 (3)	10 (4)	6 (4)	3 (2)
Teriparatide	2 (<1)	2 (1)	0	0	0
Strontium ranelate	1 (<1)	0	1 (<1)	0	0
Behavioural					
Sun exposure (>30 min/day)	490 (47)	218 (51)	111 (40)	82 (49)	79 (44)
Non-smoker (never)	540 (51)	214 (50)	171 (61)	85 (50)	70 (39)
Former smoker	347 (33)	138 (32)	71 (25)	72 (42)	66 (37)
Active smoker§§	171 (16)	78 (18)	38 (14)	13 (7)	42 (24)
No Alcohol consumption	487 (46)	216 (51)	141 (50)	72 (43)	58 (33)
Regular physical exercise	658 (63)	257 (61)	173 (63)	113 (67)	115 (67)
Laboratory tests					
S-25-hydroxy vitamin D (nmol/L) (50–150), median (IQR)	80.0 (61–97)	78.2 (62–96)	85.8 (67.7–103)	86.4 (71.0–97.6)	67.7 (49.8–6.8)
Vitamin D deficiency¶¶	123 (14)	50 (11)	29 (13)	11 (8)	43 (25)
S-osteocalcin (ng/mL) (11.0–46.0)	12.3 (8–18)	12.6 (9–17)	11.8 (8–17)	9.9 (7–16)	14.6 (11–21)
S-BAP (µg/L) (5.5–38.0)	16.9 (13–21)	17.2 (14–22)	15.3 (12–20)	15.0 (11–19)	19.3 (16–25)
S-AP (U/L) (35–130)	66 (66–81)	67 (56–82)	61 (50–75)	64 (54–84)	70 (60–86)
Gamma-GT (U/L) (5–61)	24 (17–39)	23 (16–36)	23 (15–35)	29 (19–48)	24 (17–44)
Urinary deoxypyridinoline (nmol/L) (<64)	43 (23–76)	48 (25–81)	35 (17–76)	39 (18–59)	47 (27–82)

* Continuous variables are presented as mean values with SD unless otherwise noted.
† Categorical variables are presented as number and per cent of valid observations (%) unless otherwise noted.
‡csDMARDs include azathioprine, chloroquine, ciclosporin, cyclophosphamide, hydroxychloroquine, leflunomide, methotrexate, mycophenolate mofetil and sulfasalazine.
§TNF-alpha antagonists include adalimumab, certolizumab, etanercept, infliximab and golimumab, both originator products as well as biosimilars.
¶IL-6R antagonists include tocilizumab and sarilumab.
**IL-17 and IL-12/23 antagonists include secukinumab, ixekizumab, guselkumab, brodalumab and ustekinumab.
††tsDMARDs include tofacitinib, baricitinib and apremilast.
‡‡Bisphosphonates include alendronate, ibandronate, risedronate, pamidronic acid and zoledronate.
§§Active smoking is a known risk factor for OP and is only listed in this table for completeness of information.
¶¶Vitamin D deficiency is defined as serum 25-hydroxy vitamin D level below the lower range of normal <50 nmol/L.
csDMARD, conventional synthetic disease-modifying antirheumatic drug; CTD, connective tissue disease; Gamma-GT, gamma-glutamyltransferase; IL, interleukin; OP, osteoporosis; RA, rheumatoid arthritis; S-AP, serum alkaline phosphatase; S-BAP, serum bone alkaline phosphatase; S-CRP, serum C reactive protein; TNF, tumour necrosis factor; tsDMARD, targeted synthetic disease-modifying antirheumatic drug.

cut-off of >7.5 mg/day (−0.60 for the lowest T-score and −0.90 at the lumbar spine). However, in the interaction analysis of GC with disease activity, T-scores seemed to be only negatively affected in patients with moderate to high disease activity and current GC doses of >7.5 mg/day (figure 3). In other words, doses of 5 mg/day or above did not seem to be associated with lower T-scores in patients either in remission or with low disease activity. In a specific examination of the 5 mg/day group—the largest subgroup—no negative effects were seen at any disease activity level (remission/low −0.12, p=0.38, n=93; moderate/high −0.35, p=0.051, n=45).

No impact of seropositivity for ACPA or RF on BMD was found for any of the combinations explored in patients with RA (online supplemental table S2).

DISCUSSION

In this study of patients with rheumatic disease with prior GC exposure, current GC doses of ≤5 mg/day prednisone equivalent did not seem to be associated with deleterious effects on BMD. For higher GC doses, crude models showed negative associations with lower BMD measured as lowest (minimum) T-score either at the lumbar spine and/or femoral neck and/or total hip. However, after adjusting for age, sex, menopause, BMI, disease duration, alkaline phosphatase, and the use of denosumab and bisphosphonates, these associations disappeared.

GC usage is seen as the main culprit for OP in iRMD.[10] Indeed, a multitude of observational studies have found correlations between current GC use and both low BMD and fracture incidence.[11–13] One report, however, suggested that prednisone

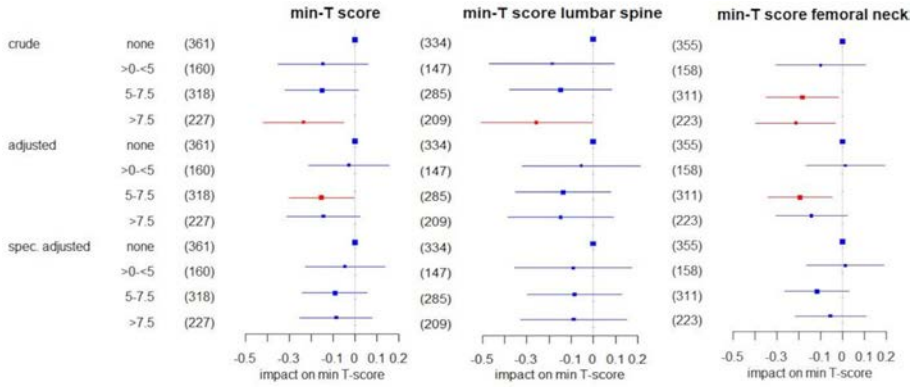

Figure 2 Impact of the current GC dose on the lowest (min) T-score in all patients in linear regression using (1) a crude model only including GC categories; (2) a multivariable model adjusted for age, sex, menopause, body mass index, alkaline phosphatase, disease duration, bisphosphonates and denosumab; and (3) a multivariable model specifically adjusted for the variables that emerged in the data mining process and were confirmed with backward selection for the respective T score (compare online supplemental table S5). The regression coefficient β and respective 95% CIs are shown. Significant coefficients are highlighted in red. The size of the boxes indicates the case numbers, also shown in brackets, of the respective groups; these are the rounded pooled case numbers of the 10 imputed data sets. For 'no GC' as the reference group, no coefficient was estimated. GC, glucocorticoid.

was beneficial for femoral neck BMD in patients with RA with concomitant adalimumab therapy.[14] However, observational research in GC, including our research here, is highly susceptible to confounding/bias by indication.[15 16] In other words, we know that active inflammation itself deteriorates bone health, and patients with the most active disease are precisely the patients most likely to be treated with high doses of GCs.[9 17–19] Thus, a patient's inherent inflammatory state may confound an accurate determination of the specific effects of GC. Even if disease activity is recorded and adjusted for, confounding may persist because one patient may need a higher dose of GC to remain at a certain disease activity level than another, and the motivation for a certain dose is usually not recorded. In our study, we attempted to address this problem by broad and accurate data collection and by adjusting for as many relevant influencing factors as possible.

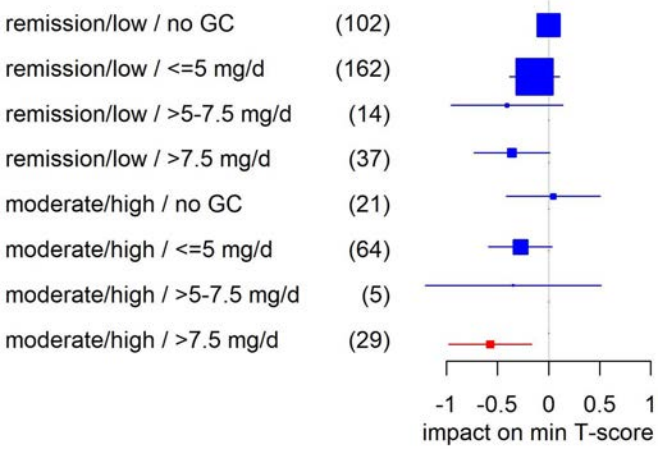

Figure 3 Impact of the interaction of disease activity and current GC dose on the lowest (min) T-Score in patients with rheumatoid arthritis in multivariable linear regression. Adjusted for age, menopause, body mass index, alkaline phosphatase, bisphosphonates, disease duratio, denosumab and male sex (compare online supplemental table S4B). Shown are regression coefficients β and respective 95% CIs. Significant coefficients are highlighted in red. Yhe size of the boxes indicates the case numbers, also shown in brackets, of the respective groups; these are the rounded pooled case numbers of the 10 imputed data sets. For 'remission/low/no GC' as the reference group, no coefficient was estimated. GC, glucocorticoid; min, minimum.

In RA, bone loss occurs even before clinical onset,[20] and the risk of hip and vertebral fracture is doubled merely by the presence of RA (without GC).[21] Previous work demonstrated that small elevations of C reactive protein significantly increased the risk of non-traumatic fractures.[22] Therefore, in observational research, disentanglement of the effects of disease activity and GC (dose) is challenging if not impossible, despite adjustment for these variables in statistical models. Pragmatic trials can solve the confounding problem but cannot be continued long enough to provide full information on long-term effects. Observational studies complement clinical trials and may approach the truth if they feature prospective, high-quality data collection and analyses. Ideally, such observational studies include detailed information on dosing over time and documentation of the motivation for a certain dose and dose changes. The hurdles to achieving such standards in observational studies are substantial.

In our patients with RA, daily intake of higher GC doses seemed to be associated with lower T-scores only in the presence of moderate or high disease activity. Furthermore, our data suggest that in the presence of remission or low disease activity, there is no association between GC dose and low T-scores. These results are consistent with findings from previous studies that also failed to identify links between current or cumulative GC dose and bone loss, vertebral fractures or trabecular bone scores in chronic inflammatory disease.[23–26] In contrast, Tong *et al* recently reported the cumulative GC dose to be associated with vertebral osteoporotic fractures in patients with RA[27] but did not properly adjust for factors with (potential) influence on bone health. Other studies confirmed the association between BMD loss and disease activity.[28 29] Trials offer unconfounded observations. In these, low-dose prednisone has clearly been shown to provide a safe and more sustainable disease control in conjunction with biological DMARDs compared with biological DMARD regimens that do not include simultaneous, continuous GC treatment.[30] Low-dose prednisone also prevents or slows radiographic progression in patients with RA.[31–33] In the recent Glucocorticoid Low-dose Outcome in Rheumatoid Arthritis Study (GLORIA), in which patients with RA aged 65+ were treated with prednisolone 5 mg/day or placebo for 2 years, these beneficial effects were confirmed without relevant

effects on bone health (Boers *et al*, Ann Rheum Dis, in press). Current recommendations in rheumatology agree that the treatment goal in iRMD should be remission. GCs continue to play an important role, but their dosing must be optimised, that is, titrated with a view toward both benefit and harm. In other words, the dose of GCs should be as high as necessary but as low as possible, and the dose must be re-evaluated frequently with an eye toward optimisation. This approach achieves the dual goals of tempering inflammation towards remission while supporting bone health. Thus, at these low dosages, the anti-inflammatory effects of GC can potentially counter their negative effects on BMD.[29]

There is no optimal parameter for homogeneous measurement of systemic inflammatory activity across all iRMDs, but measurement of CRP allows a generic and feasible estimate. It is, however, not perfect because (1) disease activity is not always reflected as CRP elevation; and (2) CRP elevation may have other causes. Nevertheless, associations between elevated CRP levels with inflammation-related complications such as cardiovascular disease and OP are well documented. Gulyás *et al* recently reported in RA and ankylosing spondylitis an inverse correlation of baseline CRP levels with BMD values both at baseline measurement and after 12 months of treatment, suggesting that baseline high-grade inflammation was associated with lower BMD.[34]

Although RA is included as an independent risk factor in fracture risk calculators such as the FRAX, the complexity of interrelating factors is often not adequately appreciated or addressed analytically. For instance, bone loss is also a feature of many other iRMDs.[35] Moreover, analyses that focus on binary representations of exposure to GC (eg, ≥5 mg/day, yes or no?) oversimplify the question because the dosage categorisation is too rough, and the impact of disease activity is excluded.[36] Recent efforts, however, have addressed this situation more definitively. For example, the updated Japanese Society for Bone and Mineral Research guidelines on GC-induced OP evaluate and indeed weight fracture risk and treatment indication according to GC dose categories of <5 mg/day, ≥5 mg/day to <7.5 mg/day and ≥7.5 mg/day.[37] The current German Osteoporosis Guidelines also specifically adjust fracture risk assessment in GC users when RA is present (http://dv-osteologie.org).

As another result, we observed in our patients with RA with long-standing disease that seropositivity for ACPA or RF was not associated with negative effects on BMD, confirming recent evidence[38 39] and suggesting that ACPA positivity is associated with low BMD in early RA only.[39]

Our study offers a more thorough understanding of non-GC factors determining bone fragility in patients with iRMD. We found a relatively high prevalence of reduced bone mass and fragility fractures in our cohort, confirming previous epidemiological studies.[6] Our study also confirms that older age, menopause, prior vertebral and non-vertebral fractures, high AP levels, and intake of PPIs are important risk factors for OP. Moreover, our targeted data mining approach revealed some new findings. First, in contrast to the well-known risks in postmenopausal women,[40 41] we found that men with iRMD in particular had low BMD. As male OP in general remains underdiagnosed and undertreated, our findings suggest we should pay more attention to the bone health of men with iRMD.[42] Second, we found that in patients with iRMD, a higher BMI is associated with higher T-scores.

In our cohort, 81% received vitamin D supplementation. Only 14% had vitamin D deficiency, which is lower than would be expected in a random adult German population (about 30%).[43] This might have been a relevant factor pertaining to the results of our analysis about risk factors for OP in our cohort.

We did not find an association of DMARD use with an impact on BMD. It should be noted, however, that we included in our multivariable model several protective and potential harmful factors whose strength of influence may be greater than that of DMARD therapy. Second, our data are still limited with regard to subanalyses of patients treated with, for example, anti-TNF or IL-6R blocking agents.

Strengths of this study include a large sample size of patients with a variety of iRMD and prospective state-of-the art collection of a very broad spectrum of data, increasing the level of detail in the analysis. Our study also has some limitations. First, we cannot derive causal relationships from our cross-sectional study. The level of evidence will be improved by longitudinal observations. Second, BMD alone may not fully account for the elevated fracture risk in patients treated with GC as suggested by Van Staa *et al*.[44] BMD is at best a surrogate for fragility fractures. In our study, history of fractures was either self-reported and/or verified from patient charts, if available. In case of clinical suspicion of a vertebral fracture, a conventional X-ray examination was performed. This approach holds the possibility that radiographic morphometric vertebral fractures were missed, which is why we did not consider fractures as a primary outcome parameter in this cross-sectional analysis. Concerning Trabecular Bone Score (TBS), a meta-analysis demonstrated the combination of BMD and TBS to provide a better estimation of fracture risk than BMD (or BMD+FRAX) alone.[45] We started TBS measurement in our cohort in July 2019. Consequently, the amount of available data is still too small to allow for meaningful analysis. Third, we conducted a pooled analysis of a variety of iRMDs. While this increased statistical power, the actual benefits and risks of certain factors may vary between diseases. A subgroup analysis of patients with RA was performed and yielded similar results compared with the overall cohort. Subgroup analyses of other diseases were not yet performed due to the rather low numbers of patients.

We conclude that in patients with iRMD, (1) both optimal disease control, optimum GC doses and sufficient OP treatment measures (such as normal vitamin D levels and appropriate use of anti-OP drugs) are essential for bone protection, and (2) low GC dosages (≤5 mg/day), aimed at achieving sustained remission or low disease activity, are likely to be safe in terms of bone health. A final conclusion is that a better term for GIOP might be 'GC-associated' OP.

Author affiliations
[1]Department of Rheumatology and Clinical Immunology, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany
[2]Institute of Biometry and Clinical Epidemiology, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany
[3]Department of Dermatology, Venerology and Allergology, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Berlin, Germany
[4]Institute of Allergology, Charité, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Berlin, Germany
[5]Center for Musculoskeletal Surgery, Charité–Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany
[6]Epidemiology and Data Science, Amsterdam UMC, Vrije Universiteit, Amsterdam, Netherlands
[7]Vasculitis and Glomerulonephritis Center, Rheumatology, Immunology and Allergy Division, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts, USA

[8]Rheumatology, Medical University of Graz, Graz, Austria
[9]Rheumatology, Brunico Hospital (SABES-ASDAA), Brunico, Italy

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Competing interests EW reported consultancy fees, honoraria and travel expenses from Medac and Novartis. DH reported receiving travel expenses from Shire. TB received consultancy fees and honoraria from Roche, Novartis, Sanofi and GSK. RB reported receiving consultancy fees, honoraria and travel expenses from Novartis. GRB reported receiving consultancy fees, honoraria and travel expenses from Roche and Sanofi and grant support from Medac. MB received consulting fees from Novartis. JS reported receiving consulting fees from AbbVie, ChemoCentryx, Sanofi, Spruce, Zenas, Bristol-Myers Squib, Sana, Q32Bio, Novartis, Kyverna, Horizon, Steritas and Argenx. CD reported receiving consultancy fees and honoraria from MSD, Pfizer, UCB, AbbVie, Roche, Novartis, Lilly, Sanofi and Galapagos. FB reported receiving consultancy fees, honoraria and travel expenses from Abbvie, Horizon Therapeutics, Pfizer and Roche, and grant support from Horizon Therapeutics, Roche and Abbvie.

Patient and public involvement Patients and/or the public were involved in the design, conduct, reporting or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by the ethical committee of the Charité Universitätsmedizin Berlin (EA1/367/14). The participants gave informed consent to participate in the study before taking part.

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Data availability statement Data are available upon reasonable request.

ORCID iDs
Robert Biesen http://orcid.org/0000-0002-0434-7832
Gerd-Rüdiger Burmester http://orcid.org/0000-0001-7518-1131
Maarten Boers http://orcid.org/0000-0002-6969-283X
John H Stone http://orcid.org/0000-0001-6588-9435
Christian Dejaco http://orcid.org/0000-0002-0173-0668
Frank Buttgereit http://orcid.org/0000-0003-2534-550X

REFERENCES

1 Palmowski Y, Buttgereit T, Buttgereit F. The 70th anniversary of glucocorticoids in rheumatic diseases: the second youth of an old Friend. *Rheumatology* 2019;58:580–7.
2 Stahn C, Buttgereit F. Genomic and nongenomic effects of glucocorticoids. *Nat Clin Pract Rheumatol* 2008;4:525–33.
3 Buttgereit F, Bijlsma JW. Glucocorticoids in rheumatoid arthritis: the picture is shaping up. *Ann Rheum Dis* 2017;76:1785–7.
4 van der Goes MC, Jacobs JWG, Boers M, *et al*. Patient and rheumatologist perspectives on glucocorticoids: an exercise to improve the implementation of the European League against rheumatism (EULAR) recommendations on the management of systemic glucocorticoid therapy in rheumatic diseases. *Ann Rheum Dis* 2010;69:1015–21.
5 Mirza F, Canalis E. Management of endocrine disease: secondary osteoporosis: pathophysiology and management. *Eur J Endocrinol* 2015;173:R131–51.
6 Rossini M, Viapiana O, Vitiello M, *et al*. Prevalence and incidence of osteoporotic fractures in patients on long-term glucocorticoid treatment for rheumatic diseases: the glucocorticoid induced osteoporosis tool (GIOTTO) study. *Reumatismo* 2017;69:30–9.
7 Strehl C, Bijlsma JWJ, de Wit M, *et al*. Defining conditions where long-term glucocorticoid treatment has an acceptably low level of harm to facilitate implementation of existing recommendations: viewpoints from an EULAR Task force. *Ann Rheum Dis* 2016;75:952–7.
8 Briot K, Geusens P, Em Bultink I, *et al*. Inflammatory diseases and bone fragility. *Osteoporos Int* 2017;28:3301–14.
9 Güler-Yüksel M, Hoes JN, Bultink IEM, *et al*. Glucocorticoids, inflammation and bone. *Calcif Tissue Int* 2018;102:592–606.
10 Leipe J, Holle JU, Weseloh C, *et al*. [German Society of Rheumatology Recommendations for the management of glucocorticoid-induced Osteoporosis. German version]. *Z Rheumatol* 2021;80:670–87.
11 Van Staa TP, Leufkens HG, Abenhaim L, *et al*. Use of oral corticosteroids and risk of fractures. *J Bone Miner Res* 2000;15:993–1000.
12 Steinbuch M, Youket TE, Cohen S. Oral glucocorticoid use is associated with an increased risk of fracture. *Osteoporos Int* 2004;15:323–8.
13 van Staa TP, Leufkens HGM, Cooper C. The epidemiology of corticosteroid-induced osteoporosis: a meta-analysis. *Osteoporos Int* 2002;13:777–87.
14 Wijbrandts CA, Klaasen R, Dijkgraaf MGW, *et al*. Bone mineral density in rheumatoid arthritis patients 1 year after adalimumab therapy: arrest of bone loss. *Ann Rheum Dis* 2009;68:373–6.
15 Boers M. Observational studies on glucocorticoids are harmful! *Lupus Sci Med* 2017;4:e000219.
16 Buttgereit F. Views on glucocorticoid therapy in rheumatology: the age of convergence. *Nat Rev Rheumatol* 2020;16:239–46.
17 Chiodini I, Falchetti A, Merlotti D, *et al*. Updates in epidemiology, pathophysiology and management strategies of glucocorticoid-induced osteoporosis. *Expert Rev Endocrinol Metab* 2020;15:283–98.
18 Dougados M. Comorbidities in rheumatoid arthritis. *Curr Opin Rheumatol* 2016;28:282–8.
19 Briot K, Geusens P, Em Bultink I, *et al*. Inflammatory diseases and bone fragility. *Osteoporos Int* 2017;28:3301–14.
20 Kleyer A, Finzel S, Rech J, *et al*. Bone loss before the clinical onset of rheumatoid arthritis in subjects with anticitrullinated protein antibodies. *Ann Rheum Dis* 2014;73:854–60.
21 Cooper C, Coupland C, Mitchell M. Rheumatoid arthritis, corticosteroid therapy and hip fracture. *Ann Rheum Dis* 1995;54:49–52.
22 Schett G, Kiechl S, Weger S, *et al*. High-Sensitivity C-reactive protein and risk of nontraumatic fractures in the Bruneck study. *Arch Intern Med* 2006;166:2495–501.
23 Lems WF, Baak MME, van Tuyl LHD, *et al*. One-Year effects of glucocorticoids on bone density: a meta-analysis in cohorts on high and low-dose therapy. *RMD Open* 2016;2:e000313.
24 Okano T, Inui K, Tada M, *et al*. High frequency of vertebral fracture and low bone quality in patients with rheumatoid arthritis-Results from tomorrow study. *Mod Rheumatol* 2017;27:398–404.
25 Florez H, Hernández-Rodríguez J, Muxi A, *et al*. Trabecular bone score improves fracture risk assessment in glucocorticoid-induced osteoporosis. *Rheumatology* 2020;59:1574–80.
26 Nowakowska-Płaza A, Wroński J, Sudoł-Szopińska I, *et al*. Clinical utility of trabecular bone score (tbs) in fracture risk assessment of patients with rheumatic diseases treated with glucocorticoids. *Horm Metab Res* 2021;53:499–503.
27 Tong J-J, Xu S-Q, Zong H-X, *et al*. Prevalence and risk factors associated with vertebral osteoporotic fractures in patients with rheumatoid arthritis. *Clin Rheumatol* 2020;39:357–64.
28 Book C, Karlsson M, Akesson K, *et al*. Disease activity and disability but probably not glucocorticoid treatment predicts loss in bone mineral density in women with early rheumatoid arthritis. *Scand J Rheumatol* 2008;37:248–54.
29 Kim J-W, Jung J-Y, Kim H-A, *et al*. Anti-Inflammatory effects of low-dose glucocorticoids compensate for their detrimental effects on bone mineral density in patients with rheumatoid arthritis. *J Clin Med* 2021;10:2944.
30 Burmester GR, Buttgereit F, Bernasconi C, *et al*. Continuing versus tapering glucocorticoids after achievement of low disease activity or remission in rheumatoid arthritis (SEMIRA): a double-blind, multicentre, randomised controlled trial. *Lancet* 2020;396:267–76.
31 Jacobs JWG, van Everdingen AA, Verstappen SMM, *et al*. Followup radiographic data on patients with rheumatoid arthritis who participated in a two-year trial of prednisone therapy or placebo. *Arthritis Rheum* 2006;54:1422–8.
32 Wassenberg S, Rau R, Steinfeld P, *et al*. Very low-dose prednisolone in early rheumatoid arthritis retards radiographic progression over two years: a multicenter, double-blind, placebo-controlled trial. *Arthritis Rheum* 2005;52:3371–80.
33 Svensson B, Boonen A, Albertsson K, *et al*. Low-Dose prednisolone in addition to the initial disease-modifying antirheumatic drug in patients with early active rheumatoid arthritis reduces joint destruction and increases the remission rate: a two-year randomized trial. *Arthritis Rheum* 2005;52:3360–70.
34 Gulyás K, Horváth Ágnes, Végh E, *et al*. Effects of 1-year anti-TNF-α therapies on bone mineral density and bone biomarkers in rheumatoid arthritis and ankylosing spondylitis. *Clin Rheumatol* 2020;39:167–75.
35 Weinstein RS. Clinical practice. Glucocorticoid-induced bone disease. *N Engl J Med* 2011;365:62–70.

36 Kanis JA, Johansson H, Oden A, *et al*. Guidance for the adjustment of FRAX according to the dose of glucocorticoids. *Osteoporos Int* 2011;22:809–16.
37 Suzuki Y, Nawata H, Soen S, *et al*. Guidelines on the management and treatment of glucocorticoid-induced osteoporosis of the Japanese Society for bone and mineral research: 2014 update. *J Bone Miner Metab* 2014;32:337–50.
38 Amkreutz JAMP, de Moel EC, Theander L, *et al*. Association between bone mineral density and autoantibodies in patients with rheumatoid arthritis. *Arthritis Rheumatol* 2021;73:921–30.
39 Wysham KD, Baker JF, Shoback DM. Osteoporosis and fractures in rheumatoid arthritis. *Curr Opin Rheumatol* 2021;33:270–6.
40 Jones G, Nguyen T, Sambrook PN, *et al*. Symptomatic fracture incidence in elderly men and women: the Dubbo osteoporosis epidemiology study (does). *Osteoporos Int* 1994;4:277–82.
41 Kanis JA, Johnell O, Oden A, *et al*. Long-Term risk of osteoporotic fracture in Malmö. *Osteoporos Int* 2000;11:669–74.
42 Kaufman J-M. Management of osteoporosis in older men. *Aging Clin Exp Res* 2021;33:1439–52.
43 Rabenberg M, Scheidt-Nave C, Busch MA, *et al*. Vitamin D status among adults in Germany--results from the German Health Interview and Examination Survey for Adults (DEGS1). *BMC Public Health* 2015;15:641.
44 Van Staa TP, Laan RF, Barton IP, *et al*. Bone density threshold and other predictors of vertebral fracture in patients receiving oral glucocorticoid therapy. *Arthritis Rheum* 2003;48:3224–9.
45 McCloskey EV, Odén A, Harvey NC, *et al*. A meta-analysis of trabecular bone score in fracture risk prediction and its relationship to FRAX. *J Bone Miner Res* 2016;31:940–8.

EPIDEMIOLOGICAL SCIENCE

Risk of cardiovascular events in patients having had acute calcium pyrophosphate crystal arthritis

Sara K Tedeschi ,[1,2] Weixing Huang,[1] Kazuki Yoshida,[1] Daniel H Solomon[1,2]

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[1]Division of Rheumatology, Inflammation, and Immunity, Brigham and Women's Hospital, Boston, Massachusetts, USA
[2]Department of Medicine, Harvard Medical School, Boston, Massachusetts, USA

Correspondence to
Dr Sara K Tedeschi, Division of Rheumatology, Inflammation, and Immunity, Brigham and Women's Hospital, Boston, Massachusetts, USA; stedeschi1@bwh.harvard.edu

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ABSTRACT

Objectives Calcium pyrophosphate deposition (CPPD) disease, broadly defined, has been associated with increased risk of cardiovascular (CV) events. We investigated risk of CV events in patients with acute CPP crystal arthritis, the acute manifestation of CPPD.

Methods Cohort study using Mass General Brigham electronic health record (EHR) data, 1991–2017. Patients with acute CPP crystal arthritis were identified using a published machine learning algorithm with positive predictive value 81%. Comparators were matched on year of EHR entry and index date of patients with acute CPP crystal arthritis (first positive synovial fluid CPP result or mention of 'pseudogout', or matched encounter). Major adverse cardiovascular event (MACE) was a composite of non-fatal CV event (myocardial infarction, acute coronary syndrome, coronary revascularisation, stroke) and death. We estimated incidence rates (IRs) and adjusted hazard ratios for MACE, non-fatal CV event and death, allowing for differential estimates during years 0–2 and 2–10. Sensitivity analyses included: (1) patients with acute CPP crystal arthritis diagnosed during outpatient visits, (2) patients with linked Medicare data, 2007–2016 and (3)patients matched on number of CV risk factors.

Results We matched 1200 acute CPP crystal arthritis patients to 3810 comparators. IR for MACE in years 0–2 was 91/1000 person-years (p-y) in acute CPP crystal arthritis and 59/1000 p-y in comparators. In years 2–10, IR for MACE was 58/1000 p-y in acute CPP crystal arthritis and 53/1000 p-y in comparators. Acute CPP crystal arthritis was significantly associated with increased risk for MACE in years 0–2 (HR 1.32, 95% CI 1.01 to 1.73) and non-fatal CV event in years 0–2 (HR 1.92, 95% CI 1.12 to 3.28) and years 2–10 (HR 2.18, 95% CI 1.27 to 3.75), but not death. Results of sensitivity analyses were similar to the primary analysis; in the outpatient-only analysis, risk of non-fatal CVE was significantly elevated in years 2–10 but not in years 0–2.

Conclusions Acute CPP crystal arthritis was significantly associated with elevated short and long-term risk for non-fatal CV event.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Calcium pyrophosphate deposition (CPPD) disease, broadly defined to include all its manifestations, has been associated with about a 25% greater risk of non-fatal cardiovascular (CV) event in a nationwide cohort study of predominantly male US Veterans.

WHAT THIS STUDY ADDS

⇒ In this cohort study at a large US medical centre, acute CPP crystal arthritis—the acute inflammatory manifestation of CPPD—was associated with twice the risk of non-fatal CV event. We observed similar results when the analysis was restricted to patients diagnosed with acute CPP crystal arthritis in the outpatient setting, and when restricted to patients with linked Medicare claims data allowing ascertainment of CV outcomes at other medical centres.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The association between acute CPP crystal arthritis and elevated risk for CV events provides additional evidence for interleukin-1β as a pathophysiological link between crystalline arthritis and CV events.

INTRODUCTION

Calcium pyrophosphate deposition (CPPD) disease is a common crystalline arthritis estimated to affect 8–10 million US adults.[1] Individuals with CPPD disease may experience one or more symptomatic manifestation, namely, acute CPP crystal arthritis, osteoarthritis with CPPD and chronic CPP crystal inflammatory arthritis.[2] Acute CPP crystal arthritis, historically known as 'pseudogout', is the most widely recognised manifestation of CPPD. This acute inflammatory arthritis is characterised by acute monoarticular or oligoarticular joint pain and swelling, often with warmth and limited range of motion, and recurs in approximately 25% of patients.[3] Episodes of acute CPP crystal inflammatory arthritis are triggered by articular cartilage releasing CPP crystals into the joint, leading to NLRP3 inflammasome activation and interleukin-1 (IL-1) β release.[4 5]

Interest in a possible pathogenic role for IL-1β in cardiovascular (CV) disease provided the basis for the Canakinumab Antiinflammatory Thrombosis Outcome Study (CANTOS) trial, which identified a lower rate of recurrent CV events in subjects randomised to IL-1β blockade versus placebo.[6] Patients with CPPD may also have a greater burden of vascular calcifications than those without CPPD, which could increase risk for CV events.[7 8] Among all patients with CPPD, those with acute CPP crystal arthritis might be at particularly increased risk for CV events due to acute episodes in which IL-1β is released.

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Many types of inflammatory arthritis including gout, rheumatoid arthritis, systemic lupus erythematosus and psoriatic arthritis are associated with increased risk for CV disease.[9–13] CPPD was recently shown to be correlated with CV events after adjustment for traditional CV risk factors in a nationwide cohort of predominantly male Veterans.[14] CPPD was broadly defined in that study to include any manifestation of CPPD identified by one or more billing code for chondrocalcinosis or calcium metabolism disorder. This study investigated the relationship between acute CPP crystal arthritis—the acute inflammatory manifestation of CPPD—and CV events in a well-characterised cohort using electronic health record (EHR) data.

METHODS

Study population and data source

We performed a matched cohort study using EHR data from the Mass General Brigham Research Patient Data Repository (RPDR), 1991–2017. This repository includes data for >5 million patients at Brigham and Women's Hospital, Massachusetts General Hospital and affiliated sites.[15] RPDR data include demographics, body mass index, diagnosis codes, procedure codes and prescription medications from inpatient and outpatient encounters at Mass General Brigham facilities. Vital status is obtained via linkage with the US Social Security Administration Death Master File, which provides date of death and is updated monthly; cause of death is not available.

The acute CPP crystal arthritis cohort was identified by applying a previously published EHR-based algorithm with positive predictive value (PPV) 81% for this acute inflammatory manifestation of CPPD.[16] The algorithm uses machine learning techniques to classify patients as acute CPP crystal arthritis or not, incorporating a range of EHR information including natural language processing of narrative notes and radiology reports, laboratory data including synovial fluid crystal analysis, and structured EHR data such as billing codes. Acute CPP crystal arthritis index date was the earlier of the first positive synovial fluid CPP crystal analysis in laboratory data or the first natural language processing concept of 'pseudogout' in narrative notes.

The comparator cohort included Mass General Brigham patients that the algorithm did not classify as acute CPP crystal arthritis at any time, 1991–2017. Comparators were matched up to 4:1 to acute CPP crystal arthritis patients on year of RPDR entry and index date to provide similar time for covariate assessment. The comparator index date was the date of an encounter within 30 days of the matched patient's index date. We randomly selected 50 comparators for detailed review of clinical notes, radiology reports and synovial fluid data for manifestations of CPPD.

We required age ≥50 at index date for all subjects, as acute CPP crystal arthritis is rare before age 50. All subjects were required to have ≥180 days and ≥2 encounters from RPDR entry through index date. We excluded subjects with non-fatal CV event occurring from RPDR entry through index date. In the primary analysis, follow-up began the day after index date and censoring occurred at the earliest of major adverse cardiovascular event (MACE), 10 years after index date or last encounter before/on 31 December 2017.

Outcomes

The prespecified primary outcome was MACE, a composite of non-fatal CV event (myocardial infarction (MI), acute coronary syndrome (ACS), coronary revascularisation, stroke) and all-cause death. Non-fatal CV event and death were prespecified secondary outcomes. We employed validated algorithms to identify non-fatal CV event using International Classification of Diseases ninth edition (ICD-9) diagnosis codes and procedural codes, updated to include corresponding ICD-10 codes.[17–20] These algorithms defined MI, ACS and stroke using inpatient diagnosis codes. We defined coronary revascularisation using inpatient procedure codes as in validated algorithms, and also included outpatient procedure codes due to secular trends in performing coronary revascularisation in ambulatory settings.[21] For the Medicare subgroup analysis, outcomes were assessed in both Medicare and RPDR; the first occurrence of each individual MACE endpoint in Medicare or RPDR was selected.

Covariate assessment

Comorbidities previously associated with risk of CV disease or death, including diabetes, hypertension, hyperlipidaemia, atrial fibrillation and cancer, were defined by ≥2 ICD-9/10 codes from EHR entry through index date (see table 1 for a complete list of CV risk factors).[20] Requiring at least two ICD codes increases PPV for each comorbidity compared with one code; assessing comorbidities in all available data before index date is not expected to create bias.[22 23] Osteoarthritis, which is common in CPPD and has inconsistently been associated CV risk, was included as a potential confounder; osteoarthritis was considered present if two or more ICD codes were recorded, regardless of anatomic site.[24 25] Age, sex and race were extracted from RPDR. Body mass index was assessed using height and weight measurements nearest to and before index date. Multimorbidity score was calculated using a validated method based on billing codes for 40 chronic conditions in RDPR data.[26] Smoking was defined by ≥1 ICD-9/10 code for tobacco use disorder or ever being prescribed smoking-cessation medication. Healthcare utilisation was assessed by summing the number of inpatient and outpatient encounters at Mass General Brigham (visits, diagnosis codes, laboratory tests and prescriptions) before and through index date. Medications were defined as ever/never prescribed in the 90 days before and through index date and included lipid-lowering agents, anti-hypertensives and aspirin (see table 1 for a complete list). Medications that may be used to treat acute CPP crystal arthritis and affect risk of CV event (colchicine, glucocorticoids, non-steroidal anti-inflammatory drugs (NSAIDs)) were also assessed, as these may have been prescribed for symptoms shortly before index date.

Sensitivity analyses

Outpatient-only analysis

To explore the potential impact of hospitalisation on effect estimates, we performed a post hoc sensitivity analysis restricted to patients without hospitalisation in the 14 days around index date ('outpatients only'). Subjects with no matched pair fulfilling this requirement were excluded.

Medicare subgroup analysis

A prespecified Medicare subgroup analysis aimed to capture outcomes occurring outside of Mass General Brigham, reducing the potential for ascertainment bias. Medicare is a federal health insurance programme for persons ages ≥65 or with certain medical conditions. We included patients with linked Medicare claims data (parts A, B & D), 2007–2016, available through a data use agreement with the Centers for Medicare and Medicaid (#RSCH-2019-53914). Linkage is achieved using the HIC, a unique identifier linking the Mass General Brigham EHR with Medicare data. Subjects were excluded if non-fatal CV event was

Table 1 Baseline characteristics of acute CPP crystal arthritis cohort and comparator cohort

	Acute CPP crystal arthritis cohort n=1200	Comparator cohort n=3810
Age, mean (SD)	72.9 (10.7)	72.7 (12.0)
Age ≥65	74.8	71.5
Male	44.8	40.6
Race		
White	83.2	80.2
Black	7.4	11.1
Other/unknown	9.4	8.8
Body mass index (BMI), kg/m^2*	28.5 (5.7)	27.7 (5.8)
Multimorbidity index score†	16.6 (16.4)	9.7 (12.0)
Comorbidities‡		
Diabetes mellitus, type 1 or 2	20.8	13.3
Hypertension	65.3	45.0
Hyperlipidaemia	49.8	32.4
Atrial fibrillation	16.5	8.6
Cancer	31.9	24.8
Chronic kidney disease	10.0	3.7
Smoking	12.8	8.2
Peripheral artery disease	9.7	4.2
Rheumatoid arthritis	12.8	3.2
Osteoarthritis	59.4	25.5
Gout	16.4	3.1
Prescription medications§		
Aspirin	22.8	9.2
Platelet aggregation inhibitor	1.8	0.8
Antianginal	1.4	0.5
Antiarrhythmic	4.3	1.7
Beta blocker	26.0	12.3
Calcium channel blocker	21.8	10.6
ACE inhibitor or ARB	23.2	12.4
Diuretic	21.0	10.1
Antilipaemic	27.6	14.3
Insulin	5.8	1.1
Non-insulin diabetes medication	7.9	3.7
Oral anticoagulant	9.0	4.0
Heparin or LMWH	16.7	3.5
Glucocorticoid	30.6	8.5
NSAID	21.3	5.9
Colchicine	11.4	0.5
Urate-lowering therapy	6.4	1.1
No of inpatient and outpatient encounters from EHR entry through index date, mean (SD)	130.9 (132.0)	58.0 (68.0)
Hospitalisation in the 14 days around index date	32.1	4.8

Values presented as % or mean (SD).
*BMI available for n=2914 (58.2% of subjects).
†Weighted multimorbidity score based on ICD-9 codes for 40 chronic conditions (Radner *et al*. Seminars Arthritis Rheum 2015;45:167–73), range 0–156.
‡Defined by ≥2 ICD-9/10 codes from EHR entry through index date.
§Prescribed (yes/no) in the 90 days before index date through index date (oral or intravenous).
ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; CPP, calcium pyrophosphate; EHR, electronic health record; ICD-9, International Classification of Diseases 9th edition; LMWH, low molecular weight heparin; NSAID, non-steroidal anti-inflammatory drug.

identified in Medicare and/or RPDR data any time before index date. We required age ≥50 with index date occurring from 2007 to 2016 and before Medicare disenrolment, ≥180 days Medicare enrolment and ≥180 days in RPDR before index date, and ≥1 match for each patient. In the Medicare subgroup analysis, follow-up began the day after index date and censoring occurred at the earliest of MACE, Medicare disenrolment or 31 December 2016.

For the Medicare subgroup analysis, comorbidities and medications were assessed using Mass General Brigham RPDR data and Medicare data prior to and through index date using the definitions above. If a comorbidity or medication met the definition in either dataset, it was considered present.

Matching on CV risk factors

The following traditional CV risk factors were summed for each patient: diabetes, hypertension, hyperlipidaemia, atrial fibrillation, peripheral artery disease, smoking (range: 0–6). Acute CPP crystal arthritis patients were matched to comparators with the same number of CV risk factors; the original matching criteria (index date and year of EHR entry) were also retained.

Statistical analysis

We estimated incidence rates (IRs) and incidence rate ratios (IRRs) with 95% CIs for MACE, non-fatal CV event and death. A Kaplan-Meier curve and log-rank test compared MACE-free survival in the two cohorts. Cox proportional hazards models estimated adjusted HRs for each outcome. The proportional hazard assumption was tested for MACE, the prespecified primary outcome, by plotting Schoenfeld residuals; the plot for cohort status had a non-zero slope over time, indicating violation of the assumption. Histograms of MACE over time revealed approximately equal numbers of events in years 0–2 and years 2–10, thus, we chose to dichotomise the analysis at year 2. On visual inspection of the slope of Schoenfeld residuals in years 0–2 and years 2–10, the slope was closer to zero. Multivariable models were adjusted for age, sex, race, body mass index, comorbidities, medications, multimorbidity index score and healthcare utilisation. Body mass index was missing for 41.8% of subjects and was imputed using multiple imputation; a sensitivity analysis omitting body mass index from models provided similar effect estimates to the primary analysis (data not shown).

In the Medicare subgroup analysis, the proportional hazards assumption was met and Cox proportional hazards models were performed without stratifying for follow-up time.

Analyses were performed using SAS V.9.4 (SAS Institute). A two-sided alpha of 0.05 was considered statistically significant.

RESULTS

The acute CPP crystal arthritis cohort included 1200 patients (mean age 72.9, 44.8% male) that were matched up to 4:1 on year of RPDR entry and index date to 3810 comparators (mean age 72.7, 40.6% male). Traditional CV risk factors such as diabetes and hypertension were more common in the acute CPP crystal arthritis cohort (table 1). Nearly one-third of patients in the acute CPP crystal arthritis cohort were hospitalised in the 14 days around index date, compared with 5% of comparators. Prescriptions for medications with potential CV implications were more frequent in the acute CPP crystal arthritis cohort, in whom use of glucocorticoids (30.6%), NSAIDs (21.3%) and colchicine (11.4%) was common. Among the 50 randomly selected comparators, two had asymptomatic chondrocalcinosis at the pubic symphysis; one had symptomatic CPPD in the wrist

Table 2 Incidence rates (IR), incidence rate ratios (IRR) and HRs for MACE, non-fatal CV event and death

	Acute CPP crystal arthritis cohort		Comparator cohort		Incidence rate ratio	HR (95% CI)
	Events	IR/1000 person-years (95% CI)	Events	IR/1000 person-years (95% CI)	IRR (95% CI)	Multivariable adjusted*
Years 0–2						
MACE	178	90.57 (78.20 to 104.90)	362	59.06 (53.28 to 65.47)	1.53 (1.28 to 1.83)	1.32 (1.01 to 1.73)
Non-fatal CV event	63	32.06 (25.04 to 41.03)	74	12.07 (9.61 to 15.16)	2.65 (1.90 to 3.72)	1.92 (1.12 to 3.28)
Death	131	65.07 (54.83 to 77.22)	312	50.46 (45.16 to 56.38)	1.29 (1.05 to 1.58)	1.19 (0.87 to 1.62)
Years 2–10						
MACE	196	58.30 (50.69 to 67.07)	445	53.15 (48.43 to 58.32)	1.10 (0.93 to 1.30)	1.26 (0.97 to 1.64)
Non-fatal CV event	69	20.53 (16.21 to 25.99)	100	11.94 (9.82 to 14.53)	1.72 (1.26 to 2.34)	2.18 (1.27 to 3.75)
Death	159	43.68 (37.39 to 51.03)	415	45.93 (41.72 to 50.57)	0.95 (0.79 to 1.14)	1.04 (0.78 to 1.39)

*Adjusted for age, sex, race, body mass index, comorbidities (diabetes, hypertension, hyperlipidaemia, atrial fibrillation, cancer, chronic kidney disease, smoking, peripheral artery disease, rheumatoid arthritis, gout, osteoarthritis), medications prescribed in the 90 days prior to index date (aspirin, platelet aggregation inhibitor, antianginal, antiarrhythmic, beta blocker, calcium channel blocker, ACE inhibitor or ARB, diuretic, antilipaemic, insulin, non-insulin diabetes medication, oral anticoagulant, heparin/low-molecular weight heparin, glucocorticoid, NSAID, colchicine, urate-lowering therapy), multimorbidity index score and healthcare utilisation.
ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; CV, cardiovascular; MACE, major adverse cardiovascular event; NSAID, non-steroidal anti-inflammatory drug.

and asymptomatic chondrocalcinosis in the knee; none had a diagnosis of acute CPP crystal arthritis; 17 did not have joint imaging, limiting the ability to assess for chondrocalcinosis.

The IR for MACE was highest in the acute CPP crystal arthritis cohort in the first 2 years of follow-up (90.57 per 1000 person-years, 95% CI 78.20 to 104.90), with an IRR 1.53 (95% CI 1.28 to 1.83) compared with the comparator cohort (table 2). IRs for MACE were similar across cohorts during years 2–10: 58.30 per 1000 person-years in acute CPP crystal arthritis and 53.15 per 1000 person-years in comparator cohort.

Risks of MACE, non-fatal CV event and death

Acute CPP crystal arthritis was significantly associated with MACE (HR 1.32, 95% CI 1.01 to 1.73) and non-fatal CV event (HR 1.92, 95% CI 1.12 to 3.28) in years 0–2 in a multivariable adjusted model.

During years 2–10 of follow-up, risk of non-fatal CV event was elevated in the acute CPP crystal arthritis cohort in a multivariable adjusted model with HR 2.18 (95% CI 1.27 to 3.75); the effect estimate was similar in magnitude in years 0–2. Acute CPP crystal arthritis was not significantly associated with risk of all-cause mortality in multivariable adjusted models in either follow-up period.

Effect estimates for all covariates are presented in online supplemental table 1.

Sensitivity analyses

The outpatient only analysis included 811 patients with acute CPP crystal arthritis (mean age 72.0 years, 42.5% male) and 3245 comparators (mean age 72.6 years, 40.4% male). Risk of non-fatal CV event among outpatients with acute CPP crystal arthritis was of similar magnitude to the primary analysis in years 0–2 (HR 2.24, 95% CI 0.86 to 5.83) but did not reach statistical significance (table 3). Acute CPP crystal arthritis was associated with a significantly increased risk of non-fatal CV event in years 2–10 (HR 2.52, 95% CI 1.23 to 5.16) in a multivariable adjusted model.

The Medicare sensitivity analysis included 438 patients with acute CPP crystal arthritis with linked Medicare data that were matched to 767 comparators with linked Medicare data. Mean age was 76.2 years in the acute CPP crystal arthritis cohort and 77.9 years in comparators (online supplemental table 2). The prevalence of traditional CV risk factors was higher in each cohort in this subgroup analysis compared with their counterparts in the primary analysis. MACE-free survival was less likely in the acute CPP crystal arthritis cohort in this sensitivity analysis (log-rank p=0.006) (see online supplemental figure 1). Acute CPP crystal arthritis was associated with a significantly elevated risk for MACE with HR 1.69 (95% CI 1.10 to 2.62) and non-fatal CV event with HR 2.19 (95% CI 1.02 to 4.70) in multivariable adjusted models (table 4).

The sensitivity analysis matched on number of CV risk factors included 654 patients with acute CPP crystal arthritis and 971 comparators. Acute CPP crystal arthritis was associated with significantly elevated risk for MACE in years 2–10 (HR 2.03, 95% CI 1.23, 3.35) but not in years 0–2 (see online supplemental table 3). Effect estimates for non-fatal CV event were unstable due to smaller number of events and person-years, and large number of covariates in adjusted models (data not shown).

Table 3 HRs and 95% CI for MACE, non-fatal CV event, and death among outpatients (811 acute CPP crystal arthritis patients matched to 3245 comparators (reference))

	Multivariable adjusted*
Years 0–2	
MACE	1.28 (0.86 to 1.90)
Non-fatal CV event	2.24 (0.86 to 5.83)
Death	1.05 (0.67 to 1.67)
Years 2–10	
MACE	1.08 (0.79 to 1.48)
Non-fatal CV event	2.52 (1.23 to 5.16)
Death	0.77 (0.54 to 1.10)

*Adjusted for age, sex, race, body mass index, comorbidities (diabetes, hypertension, hyperlipidaemia, atrial fibrillation, cancer, chronic kidney disease, smoking, peripheral artery disease, rheumatoid arthritis, gout, osteoarthritis), medications prescribed in the 90 days prior to index date (aspirin, platelet aggregation inhibitor, antianginal, antiarrhythmic, beta blocker, calcium channel blocker, ACE inhibitor or ARB, diuretic, antilipaemic, insulin, non-insulin diabetes medication, oral anticoagulant, heparin/low-molecular weight heparin, glucocorticoid, NSAID, colchicine, urate-lowering therapy), multimorbidity index score and healthcare utilisation.
ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; CPP, calcium pyrophosphate; CV, cardiovascular; MACE, major adverse cardiovascular event; NSAID, non-steroidal anti-inflammatory drug.

Table 4 HRs and 95% CI for MACE, non-fatal CV event and death among patients with linked Medicare claims data, 2007–2016 (438 acute CPP crystal arthritis matched to 767 comparators (reference))

	Multivariable adjusted*
MACE	1.69 (1.10 to 2.62)
Non-fatal CV event	2.19 (1.02 to 4.70)
Death	1.56 (0.84 to 2.88)

*Adjusted for age, sex, race, body mass index, comorbidities (diabetes, hypertension, hyperlipidaemia, atrial fibrillation, cancer, chronic kidney disease, smoking, peripheral artery disease, rheumatoid arthritis, gout, osteoarthritis), medications prescribed in the 90 days prior to index date (aspirin, platelet aggregation inhibitor, antianginal, antiarrhythmic, beta blocker, calcium channel blocker, ACE inhibitor or ARB, diuretic, antilipaemic, insulin, non-insulin diabetes medication, oral anticoagulant, heparin/low-molecular weight heparin, glucocorticoid, NSAID, colchicine, urate-lowering therapy), multimorbidity index score and healthcare utilisation.
ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; CPP, calcium pyrophosphate; CV, cardiovascular; MACE, major adverse cardiovascular event; NSAID, non-steroidal anti-inflammatory drug.

DISCUSSION

Patients with at least one episode of acute CPP crystal arthritis had twice the risk of non-fatal CV events compared with those without evidence of this acute crystalline arthritis after adjusting for traditional CV risk factors, medications, and healthcare utilisation in a large academic medical centre. Greater risk for non-fatal CV events was observed both in the initial years after an episode of acute CPP crystal arthritis and persisted up to 10 years later. We observed a similar two-fold risk for non-fatal CV events in years 2–10 among outpatients with acute CPP crystal arthritis, suggesting that hospitalisation (eg, for severe illness that might trigger a flare of acute CPP crystal arthritis) does not fully explain elevated CV risks. Similar results were also observed in the Medicare subgroup analysis that facilitated more complete ascertainment of comorbidities, prescriptions, and MACE outcomes at other medical facilities, and in a sensitivity analysis matched on number of CV risk factors. Acute CPP crystal arthritis was not associated with risk of death.

These results extend the recent finding that CPPD—broadly defined to include acute CPP crystal arthritis as well as other manifestations of CPPD—was associated with a 25% greater risk of non-fatal CV event in a nationwide cohort of US Veterans.[14] Risk of non-fatal CV event was greater in patients with acute CPP crystal arthritis—the acute inflammatory manifestation of CPPD—in this study than among patients with any manifestation of CPPD in the Veterans Administration study, suggesting that inflammation may be a driving force behind elevated CV risk in this disease. Medical record review of 50 comparators confirmed that the comparator cohort includes patients with other forms of CPPD (not acute CPP crystal arthritis), a finding that is not surprising given the prevalence of CPPD in older adults. If CPPD itself contributes toward elevated CV risk, as suggested in the nationwide cohort of Veterans, then the observed effect estimates for acute CPP crystal arthritis would be attenuated toward the null. The observed significantly increased risk of CV events in patients with acute CPP crystal arthritis in this study points to a risk related to the acute inflammatory arthritis itself and not only due to CPPD. Risk of MACE differed over time in this study as indicated by the violation of the proportional hazard assumption; this is likely due to the much higher crude rate of death in the early years after acute CPP crystal arthritis diagnosis (65 per 1000 person-years) compared with later years (44 per 1000 person-years), as death was the most common component in our MACE outcome definition.

This study further addresses questions related to the relationship between hospitalisation, CPPD, and non-fatal CV events. Acute CPP crystal arthritis is commonly diagnosed in the context of acute illness, and approximately one-third of patients in the acute CPP crystal arthritis cohort had been hospitalised within 14 days of index date.[4] Our sensitivity analysis restricted to outpatients at index date reduces the potential for reverse causation, as might be the case if a patient had an MI while hospitalised and subsequently developed acute CPP crystal arthritis in the hospital. In this situation, the billing code for MI would be recorded on the hospital discharge date and would appear to occur after the first mention of “pseudogout” in clinical notes. It is also possible that the reason for hospitalisation, such as heart failure or atrial fibrillation, might itself increase the risk of MACE. The outpatient analysis suggests that acute CPP crystal arthritis is associated with higher risk for CV events many years after an episode of acute crystal arthritis. Risk for CV events was not significantly elevated in the first 2 years after index date among outpatients, suggesting that acute illness may partly underlie CV risk for events occurring soon after diagnosis. Possible mechanisms that might explain the observed increased risk for CV events include systemic inflammation due to IL-1β, greater burden of vascular calcifications, and dysregulated lipid profiles.[4 8 27 28]

Gout and hyperuricaemia have been strongly and consistently associated with elevated risk for CV events in large cohort studies.[10 29 30] Whether gout or hyperuricaemia cause CV events, or whether IL-1β mediates the association between gout and CV events, remains a subject of debate. CPP crystals and monosodium urate crystals both activate the NLRP3 inflammasome, leading to IL-1β release.[4] NLRP3 inflammasome activation, triggered by a range of stimuli including CPP or MSU crystals, damage-associated molecular patterns (DAMPs), and pathogen-associated molecular patterns (PAMPs), leads to altered lipid metabolism, inflammation, and oxidative stress contributing to the pathogenesis of atherosclerosis.[28] The association between acute CPP crystal arthritis and elevated risk for CV events provides additional evidence for IL-1β as a pathophysiologic link between crystalline arthritis and CV events. Presence of crystals alone may not fully explain the increased risk for CV events, however; in vitro studies suggest that CPP and monosodium urate crystals only induced IL-1β expression in the presence of PAMPs or DAMPs such as serum amyloid A.[31] While is beyond the scope of this cohort study to identify mechanisms explaining the long-lasting association between acute CPP crystal arthritis and CV events years later, it is possible that acute CPP crystal arthritis is a proxy for an ongoing process such as subclinical inflammation, vascular calcification, or prolonged exposure to PAMPs/DAMPs with long-term CV consequences.[32] Episodic inflammation due to flares, subclinical inflammation in patients without flares or between flares, and the extent of CPP deposits may all contribute to CV risk, though it may be difficult to assess the relative contributions of these possible mechanisms.

The study has a number of limitations including using EHR data from a single healthcare system to ascertain covariates and MACE outcomes. We addressed this limitation in a subgroup analysis including Medicare claims data from outside healthcare institutions and observed similar effect estimates. The sample size in the outpatient-only sensitivity analysis may have limited power to detect a significant association in years 0–2, as reflected by the wide confidence intervals. Analyses were adjusted for comorbidities previously associated with

CV disease and death, though the acute CPP crystal arthritis cohort had greater healthcare utilisation in the baseline period which raises the possibility of differential ascertainment of comorbidities. Unmeasured confounding is also possible in any observational cohort study. Approximately 25% of patients with one episode of acute CPP crystal arthritis have a subsequent episode, and this study design was unable to assess whether recurrent episodes (or future treatments such as NSAIDs and glucocorticoids) occurred and/or mediate CV risk.[3] The index date of acute CPP crystal arthritis was based on a documented episode and likely does not represent the true onset of CPPD disease; indeed, much remains unknown regarding the natural history of CPPD including disease evolution over time. Patients with acute CPP crystal arthritis frequently have other manifestations of CPPD as well, most often osteoarthritis with chondrocalcinosis.[33] This study was not able to assess the potential contribution of having more than one manifestation of CPPD, nor could it determine the total burden of CPP deposits nor whether patients had experienced recurrent episodes of acute CPP crystal arthritis before or after the index date—any of which could potentially influence CV risk. Comparators were drawn from the Mass General Brigham EHR for feasibility, though this limits generalisability of results. Reverse causation during hospitalisations remains a possibility, though the sensitivity analysis restricted to outpatients reduces the chance of this. While we were not able to identify cause of death in this dataset to assess for CV death specifically, all-cause death has been included in MACE definitions.[34] The study included relatively equal proportions of males and females at an academic medical centre, though our results may not generalise to the general population or to international populations with different CV risk profiles.

Our results raise the possibility that well-characterised acute CPP crystal arthritis is an independent risk factor for non-fatal CV event and replicate the prior report of increased CV risk in a nationwide study of Veterans.[14] CPPD prevalence will increase as the population ages, so understanding the mechanisms linking this common crystalline arthritis to CV risk is a critical next step. Studies of colchicine, a common treatment for CPP crystal arthritis, and CV endpoints will be of interest given that colchicine was protective against CV events in several recent large randomised controlled trials of patients with CV disease.[35 36] Recognising CPPD as a risk factor for CV events highlights the importance of identifying and preventing long-term adverse outcomes of this common crystalline arthritis.

Twitter Kazuki Yoshida @kaz_yos

Contributors SKT conceived the study, oversaw study design, results interpretation and drafted the first version of the manuscript. WH performed data analysis and provided comments on the manuscript. KY aided with biostatistical analysis, results interpretation and provided comments on the manuscript. DHS contributed to study design, results interpretation and provided comments on the manuscript. SKT takes full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish.

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ORCID iD

Sara K Tedeschi http://orcid.org/0000-0001-9475-1363

REFERENCES

1 Abhishek A, Neogi T, Choi H, *et al*. Review: unmet needs and the path forward in joint disease associated with calcium pyrophosphate crystal deposition. *Arthritis Rheumatol* 2018;70:1182–91.
2 Cai K, Fuller A, Hensey O, *et al*. Outcome domains reported in calcium pyrophosphate deposition studies: a scoping review by the OMERACT CPPD Working group. *Semin Arthritis Rheum* 2020;50:719–27.
3 Yates KA, Yoshida K, Xu C, *et al*. Acute calcium pyrophosphate crystal arthritis flare rate and risk factors for recurrence. *J Rheumatol* 2020;47:1261–6.
4 Rosenthal AK, Ryan LM. Calcium pyrophosphate deposition disease. *New England Journal of Medicine* 2016;374:2575–84.
5 Renaudin F, Orliaguet L, Castelli F, *et al*. Gout and pseudo-gout-related crystals promote GLUT1-mediated glycolysis that governs NLRP3 and interleukin-1β activation on macrophages. *Ann Rheum Dis* 2020;79:1506–14.
6 Ridker PM, Everett BM, Thuren T, *et al*. Antiinflammatory therapy with canakinumab for atherosclerotic disease. *N Engl J Med* 2017;377:1119–31.
7 Chen J, Budoff MJ, Reilly MP, *et al*. Coronary artery calcification and risk of cardiovascular disease and death among patients with chronic kidney disease. *JAMA Cardiol* 2017;2:635–43.
8 Abhishek A, Doherty S, Maciewicz R, *et al*. Association between low cortical bone mineral density, soft-tissue calcification, vascular calcification and chondrocalcinosis: a case-control study. *Ann Rheum Dis* 2014;73:1997–2002.
9 Liao KP, Solomon DH. Traditional cardiovascular risk factors, inflammation and cardiovascular risk in rheumatoid arthritis. *Rheumatology* 2013;52:45–52.
10 Choi HK, Curhan G. Independent impact of gout on mortality and risk for coronary heart disease. *Circulation* 2007;116:894–900.
11 Ogdie A, Yu Y, Haynes K, *et al*. Risk of major cardiovascular events in patients with psoriatic arthritis, psoriasis and rheumatoid arthritis: a population-based cohort study. *Ann Rheum Dis* 2015;74:326–32.
12 Hak AE, Karlson EW, Feskanich D, *et al*. Systemic lupus erythematosus and the risk of cardiovascular disease: results from the nurses' health study. *Arthritis Rheum* 2009;61:1396–402.
13 Clarson LE, Hider SL, Belcher J, *et al*. Increased risk of vascular disease associated with gout: a retrospective, matched cohort study in the UK clinical practice research Datalink. *Ann Rheum Dis* 2015;74:642–7.
14 Bashir M, Sherman KA, Solomon DH, *et al*. Cardiovascular disease risk in calcium pyrophosphate deposition disease: a nationwide study of Veterans. *Arthritis Care Res* 2021. doi:10.1002/acr.24783. [Epub ahead of print: 14 Sep 2021].
15 Tedeschi SK, Yoshida K, Huang W, *et al*. Confirming prior and identifying novel correlates of acute calcium pyrophosphate crystal arthritis. *Arthritis Care Res* 2021. doi:10.1002/acr.24770. [Epub ahead of print: 16 Aug 2021].
16 Tedeschi SK, Cai T, He Z, *et al*. Classifying pseudogout using machine learning approaches with electronic health record data. *Arthritis Care Res* 2021;73:442–8.
17 Kiyota Y, Schneeweiss S, Glynn RJ, *et al*. Accuracy of Medicare claims-based diagnosis of acute myocardial infarction: estimating positive predictive value on the basis of review of hospital records. *Am Heart J* 2004;148:99–104.
18 Kim SC, Solomon DH, Rogers JR, *et al*. Cardiovascular safety of tocilizumab versus tumor necrosis factor inhibitors in patients with rheumatoid arthritis: a Multi-Database cohort study. *Arthritis & Rheumatology* 2017;69:1154–64.
19 Kumamaru H, Judd SE, Curtis JR, *et al*. Validity of claims-based stroke algorithms in contemporary Medicare data: reasons for geographic and racial differences in

stroke (REGARDS) study linked with Medicare claims. *Circ Cardiovasc Qual Outcomes* 2014;7:611–9.
20 He M, Santiago Ortiz AJ, Marshall J, *et al*. Mapping from the International classification of diseases (ICD) 9th to 10th revision for research in biologics and biosimilars using administrative healthcare data. *Pharmacoepidemiol Drug Saf* 2020;29:770–7.
21 Culler SD, Kugelmass AD, Brown PP, *et al*. Trends in coronary revascularization procedures among Medicare beneficiaries between 2008 and 2012. *Circulation* 2015;131:362–70.
22 Wei W-Q, Teixeira PL, Mo H, *et al*. Combining billing codes, clinical notes, and medications from electronic health records provides superior phenotyping performance. *J Am Med Inform Assoc* 2016;23:e20–7.
23 Nakasian SS, Rassen JA, Franklin JM. Effects of expanding the look-back period to all available data in the assessment of covariates. *Pharmacoepidemiol Drug Saf* 2017;26:890–9.
24 Veronese N, Stubbs B, Solmi M, *et al*. Osteoarthritis increases the risk of cardiovascular disease: data from the osteoarthritis initiative. *J Nutr Health Aging* 2018;22:371–6.
25 Wang H, Bai J, He B, *et al*. Osteoarthritis and the risk of cardiovascular disease: a meta-analysis of observational studies. *Sci Rep* 2016;6:39672.
26 Radner H, Yoshida K, Mjaavatten MD, *et al*. Development of a multimorbidity index: impact on quality of life using a rheumatoid arthritis cohort. *Semin Arthritis Rheum* 2015;45:167–73.
27 Burt HM, Jackson JK, Rowell J. Calcium pyrophosphate and monosodium urate crystal interactions with neutrophils: effect of crystal size and lipoprotein binding to crystals. *J Rheumatol* 1989;16:809–17.
28 Poznyak AV, Melnichenko AA, Wetzker R, *et al*. NLPR3 inflammasomes and their significance for atherosclerosis. *Biomedicines* 2020;8:205.
29 Kim SY, Guevara JP, Kim KM, *et al*. Hyperuricemia and coronary heart disease: a systematic review and meta-analysis. *Arthritis Care Res* 2010;62:170–80.
30 Grayson PC, Kim SY, LaValley M, *et al*. Hyperuricemia and incident hypertension: a systematic review and meta-analysis. *Arthritis Care Res* 2011;63:102–10.
31 Savage CD, Lopez-Castejon G, Denes A, *et al*. NLRP3-Inflammasome activating DAMPs stimulate an inflammatory response in glia in the absence of priming which contributes to brain inflammation after injury. *Front Immunol* 2012;3:288.
32 Martinez Sanchis A, Pascual E. Intracellular and extracellular CPPD crystals are a regular feature in synovial fluid from uninflamed joints of patients with CPPD related arthropathy. *Ann Rheum Dis* 2005;64:1769–72.
33 Fuller A, Cai K, Diaz-Torne C, *et al*. Outcome domains reported by patients, caregivers, healthcare professionals and stakeholders for calcium pyrophosphate deposition (CPPD): a content analysis based on semi-structured qualitative interviews from the OMERACT CPPD Working group. *Semin Arthritis Rheum* 2021;51:650–4.
34 Wang C, Kane R, Levenson M, *et al*. Association between changes in CMS reimbursement policy and drug labels for Erythrocyte-Stimulating agents with outcomes for older patients undergoing hemodialysis covered by fee-for-service Medicare. *JAMA Intern Med* 2016;176:1818–25.
35 Nidorf SM, Fiolet ATL, Mosterd A, *et al*. Colchicine in patients with chronic coronary disease. *N Engl J Med* 2020;383:1838–47.
36 Tardif J-C, Kouz S, Waters DD, *et al*. Efficacy and safety of low-dose colchicine after myocardial infarction. *N Engl J Med* 2019;381:2497–505.

Clinical image: bone erosions in a young man

Léa Bastard ,[1] Benjamin Hagege,[1] Maxime Breban ,[1,2] Hélène Gouze [1]

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[1]Rheumatology Department, Hospital Ambroise-Pare, Boulogne-Billancourt, France
[2]Infection & Inflammation, UMR 1173, Inserm, UVSQ/ Paris Saclay, Montigny-le-Bretonneux, France

Correspondence to
Dr Hélène Gouze, Hospital Ambroise-Pare Rheumatology Department, Boulogne-Billancourt, Île-de-France, France; helene.gouze@aphp.fr

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A previously healthy 22-year-old man presented with bone and joint pain progressively increasing over 12 months, localised on forearms and legs. One year before, he had suffered from desquamating skin lesions on both hands, which had spontaneously regressed. He had lost 10 kg since his first skeletal symptoms with no other general signs such as fever. There was no heel pain, dactylitis, psoriasis, transit disorder or genital infection preceding the symptoms. Clinical palpation reproduced bone and joint pain. No signs of systemic disease were found.

Standard laboratory examinations showed an inflammatory syndrome with C reactive protein of 29 mg/L, erythrocyte sedimentation rate of 54 mm.

X-rays of the long bones showed several focal cortical and medullary bone lytic lesions with periosteal reaction visible in middle parts of radius, ulna and tibias. There was a significant uptake in both tibias, parietal and occipital bones and mandible that matched with osteolytic lesions on bone scintigraphy. Positron emission tomography/CT scanner revealed areas of uptake in all tibias, radius and ulna (figure 1).

Given this medical history and the radiological presentation, some hypotheses were discussed: SAPHO (synovitis, acne, pustulosis, hyperostosis, osteitis) syndrome, Langerhans cell histiocytosis, tumorous diseases. Another hypothesis was syphilitic infection, based on the following features: the previous history of skin lesions, the fluctuating nature of symptoms and finally the topography of bone lesions in ulnas and tibias, strongly suggestive of syphilis.

Diagnosis of syphilitic infection was, therefore, suspected, and laboratory tests revealed positive *Treponema pallidum* haemagglutination assay with levels of 24.62 (positive if ≥1) and veneral disease research laboratory (VDRL) test with ratio of 1/512, confirming the diagnosis of late syphilis with osteitis.

The patient received three doses of long-acting benzathin penicillin G intramuscularly (2.4 million units per injection) administrated at weekly intervals.[1] Clinical response was quickly achieved after the first intramuscular injection and VDRL test rapidly decreased and was of 1/16 at 8 months.

This clinical case highlights the importance of the new classification that distinguishes early syphilis from late syphilis arising after 1 year of evolution.[2] Although syphilitic osteitis is rare, this diagnosis must be considered, even in young adults, especially as the incidence of syphilis is currently raising.[3]

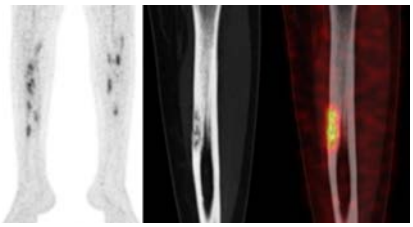

Figure 1 Positron emission tomography/CT scanner images revealing intense and diffuse bilateral bone hypermetabolism corresponding to focal lytic lesions in both tibias.

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Contributors LB and HG prepared the clinical image and the medical description of the case. All the authors were in charge of the patient during his hospitalisation, and BH was in charge of the patient follow-up and provided information on the patient's evolution. LB, HG, BH and MB discussed the manuscript and contributed to the final manuscript.

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ORCID iDs
Léa Bastard http://orcid.org/0000-0001-9060-8180
Maxime Breban http://orcid.org/0000-0002-6932-9395
Hélène Gouze http://orcid.org/0000-0002-5333-0316

REFERENCES

1 Forrestel AK, Kovarik CL, Katz KA. Sexually acquired syphilis: laboratory diagnosis, management, and prevention. *J Am Acad Dermatol* 2020;82:17–28.
2 Dupin N. [Syphilis]. *Rev Med Interne* 2016;37:735–42.
3 Ghanem KG, Ram S, Rice PA. The modern epidemic of syphilis. *N Engl J Med* 2020;382:845–54.

Progressive increase in time to referral and persistently severe clinical presentation over the years in autoantibody-negative patients with rheumatoid arthritis in the setting of an early arthritis clinic

Prompt identification of patients with rheumatoid arthritis (RA), ideally within a window of opportunity of approximately 12 weeks, increases potential for antirheumatic treatments to dampen the inflammatory process in a milder and more reversible stage of the disease, thus enabling more favourable outcomes.[1] Over the past 20 years, strategies aimed at reducing delays in RA referral and treatment have included the widespread diffusion of dedicated early arthritis clinics (EACs),[2] as well as the development of more sensitive classification criteria.[3] Still, the percentage of patients seen within the window of opportunity apparently remains low,[4] and the new RA criteria, heavily weighted on autoantibodies, may have further hindered the recognition and treatment of seronegative patients.[5]

Here, we analysed changes in the diagnostic delay and clinical presentation of patients with RA admitted to the EAC of the Division of Rheumatology of the San Matteo University Hospital, Pavia, Italy, from its institution in 2005 to 2017. Referral criteria to the EAC have remained stable over the years and include ≥3 swollen joints (SJs), or in case of <3 SJs, a positive squeeze test or morning stiffness >30 min.[6] From all patients with early arthritis (N=1553), we selected 668 patients fulfilling at enrolment at least the 1987 American College of Rheumatology (ACR) criteria for RA before December 2010 (n=345, 88.4% also fulfilling the 2010 criteria) and at least the 2010 ACR/European Alliance of Associations for Rheumatology criteria after January 2011 (n=323, 63.5% also fulfilling the 1987 criteria). In line with published studies[5] and with the prognostic value of the 2010 criteria,[3] application of the two sets of criteria was used as reference for RA. Time from first self-reported joint symptom to referral was compared across different time periods: (1) 2005–2007, (2) 2008–2010, (3) 2011–2013 and (4) 2014–2017. Clinical characteristics were collected according to standardised assessments. Data were analysed in the total population and after stratification for autoantibody status (double-negative for rheumatoid factor (RF) and anticitrullinated protein antibodies (ACPA) vs RF-positive and/or ACPA-positive).

In all, delay in the referral of patients classified as RA collectively increased from a mean (SD) of 20.8 (20.5) weeks before 2010 to 24.4 (20.6) weeks thereafter (p=0.02) (online supplemental table S1), and the proportion of patients identified within 12 weeks non-significantly decreased from 39.3% to 35.3%. Still, patients presented with progressively milder inflammatory markers despite unchanged joint tenderness and patient-reported outcomes (PROs) (online supplemental table S1). Trends were however remarkably different in different autoantibody subgroups. In RF/ACPA-positive patients, a stable proportion of 41%–44% were referred within 12 weeks (figure 1A), with only marginal differences of around ±10% in relation to disease activity (online supplemental figure S1A,B). At presentation, patients had less SJs and lower C reactive protein (CRP) levels; the mean decrease of SJs and CRP from 2005–2007 to 2014–2017 were −5.6 and −1.1 mg/dL, respectively (figure 1B and online supplemental table S2). The improvement in PROs was smaller but still significant over time (figure 1C and online supplemental table S2). In contrast, in autoantibody-negative patients, the proportion of patients identified within 12 weeks progressively decreased from 37.9% to 25.6% (p=0.08) (figure 1D). Of note, the reduction in the rate of early referral after 2010 was prominent in patients classified as RA solely

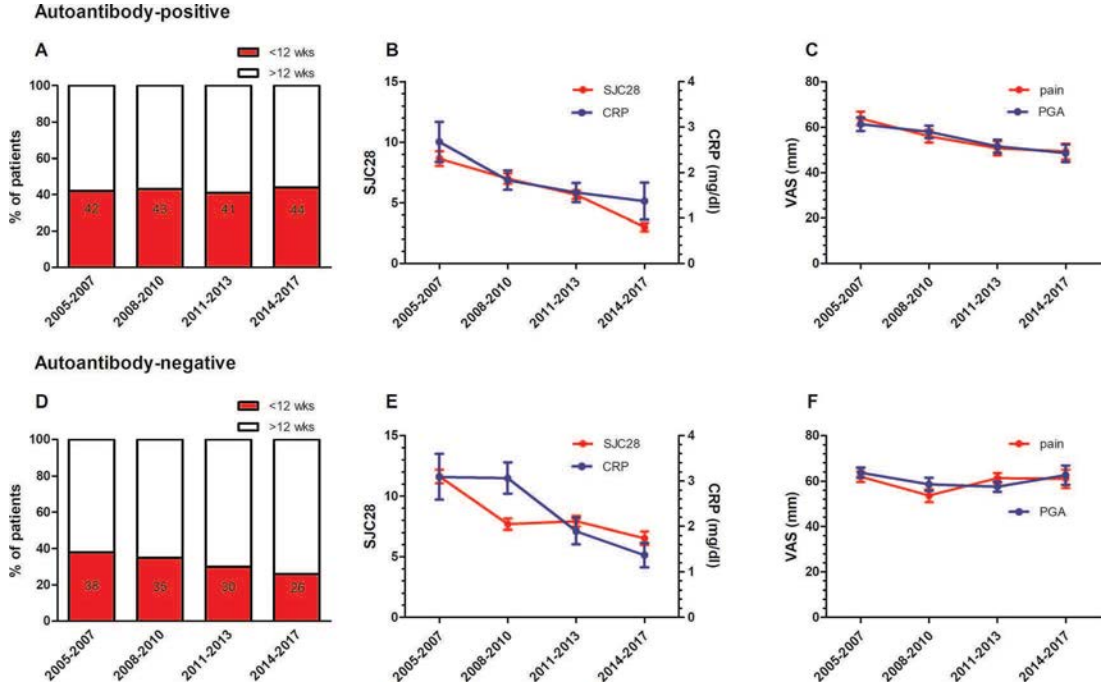

Figure 1 Time to referral and clinical presentation of autoantibody-positive and autoantibody–negative patients with early rheumatoid arthritis. Proportion of patients identified within 12 weeks from symptom onset among autoantibody-positive (A) and autoantibody-negative (D) RA in different time periods. Values of SJC28, CRP (B, E) and VAS for pain and PGA of disease activity (C, F) in autoantibody-positive (B, C) and autoantibody-negative (E, F) RA in different time periods. Values are expressed as mean and SE. CRP, C reactive protein; PGA, patient global assessment; RA, rheumatoid arthritis; SJC28, swollen joint; VAS, Visual Analogue Scale; wks, weeks.

on the basis of the 2010 criteria (only 25.4% seen within the window of opportunity), but numerically affected also patients meeting both sets of criteria (referred early in 29.8% of the cases). Decrease in early identification was observed particularly in patients with high disease activity (44.5% vs 31% before and after 2010, respectively, p=0.09), while patients with moderate inflammation were seen within the window of opportunity at stably low rates of 25%–29% (online supplemental figure 1C,D). Furthermore, in autoantibody-negative patients, the improvement over time of inflammatory features, especially of SJs, was significant but smaller compared with autoantibody-positives, and PROs such as pain and global assessment of disease activity remained severely impacting, with mean values of up to 60 mm even in recent times (figure 1E,F and online supplemental table S3).

Collectively, our data indicate that a large proportion of patients with RA still miss the opportunity of being identified early despite dedicated fast-track access to rheumatology care. Strategies promoting early referral should therefore include education campaigns at the level of the population and health professionals. Nonetheless, autoantibody-positive patients present with milder and less disabling disease in more recent years. In contrast, patients lacking serological markers such as autoantibodies, despite fulfilling the prognostic criteria for chronic persistent arthritis, are at increased risk of delayed identification and remain burdened by severe disease. Such changing phenotype of autoantibody-negative RA arises from a number of concomitant factors which have only partially been explored, including delayed referral from primary care,[7] requirement of more severe inflammation to fulfil the RA criteria[8] and lower sensitivity of current classification tools.[5] Interventions that specifically aid the early and accurate identification of autoantibody-negative patients with RA therefore appear an urgent need.

Ludovico De Stefano,[1,2] Bernardo D'Onofrio,[1,2] Garifallia Sakellariou,[2,3] Antonio Manzo,[1,2] Carlomaurizio Montecucco ,[1,2] Serena Bugatti [1,2]

[1]Division of Rheumatology, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy
[2]Department of Internal Medicine and Therapeutics, University of Pavia, Pavia, Italy
[3]Maugeri Clinical Research Institutes IRCCS, Pavia, Italy

Correspondence to Professor Serena Bugatti, Division of Rheumatology, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy; serena.bugatti@unipv.it

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ORCID iDs
Carlomaurizio Montecucco http://orcid.org/0000-0001-8263-3925
Serena Bugatti http://orcid.org/0000-0002-5396-7077

REFERENCES

1 Burgers LE, Raza K, van der Helm-van Mil AH. Window of opportunity in rheumatoid arthritis - definitions and supporting evidence: from old to new perspectives. *RMD Open* 2019;5:e000870.
2 Villeneuve E, Nam JL, Bell MJ, *et al*. A systematic literature review of strategies promoting early referral and reducing delays in the diagnosis and management of inflammatory arthritis. *Ann Rheum Dis* 2013;72:13–22.
3 Aletaha D, Neogi T, Silman AJ. Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League against rheumatism collaborative initiative. *Ann Rheum Dis* 2010;2010:1580–8.
4 Raza K, Stack R, Kumar K, *et al*. Delays in assessment of patients with rheumatoid arthritis: variations across Europe. *Ann Rheum Dis* 2011;70:1822–5.
5 Boeters DM, Gaujoux-Viala C, Constantin A, *et al*. The 2010 ACR/EULAR criteria are not sufficiently accurate in the early identification of autoantibody-negative rheumatoid arthritis: results from the Leiden-EAC and ESPOIR cohorts. *Semin Arthritis Rheum* 2017;47:170–4.
6 Balduzzi S, Scirè CA, Sakellariou G, *et al*. In early inflammatory polyarthritis more intensive management according to the 2010 ACR/EULAR criteria leads to higher rates of clinical remission: comparison of two cohorts treated according to different treat-to-target protocols. *Clin Exp Rheumatol* 2017;35:401–5.
7 Pratt AG, Lendrem D, Hargreaves B, *et al*. Components of treatment delay in rheumatoid arthritis differ according to autoantibody status: validation of a single-centre observation using national audit data. *Rheumatology* 2016;55:1843–8.
8 Nordberg LB, Lillegraven S, Lie E, *et al*. Patients with seronegative RA have more inflammatory activity compared with patients with seropositive RA in an inception cohort of DMARD-naïve patients classified according to the 2010 ACR/EULAR criteria. *Ann Rheum Dis* 2017;76:341–5.

Cross-reactivity of anti-modified protein antibodies is also present in predisease and individuals without rheumatoid arthritis

The presence of anti-citrullinated protein antibodies (ACPAs), anti-carbamylated protein antibodies (anti-CarPAs) and anti-acetylated protein antibodies (AAPAs) is a hallmark of rheumatoid arthritis (RA). ACPA and anti-CarPA can already be detected years before RA onset.[1] Moreover, it has been shown that the citrullinated epitope recognition profile of ACPA expands before RA develops. Recently, it has become clear that ACPA can display cross-reactivity to other post-translational modifications (PTMs), more specifically homocitrulline and

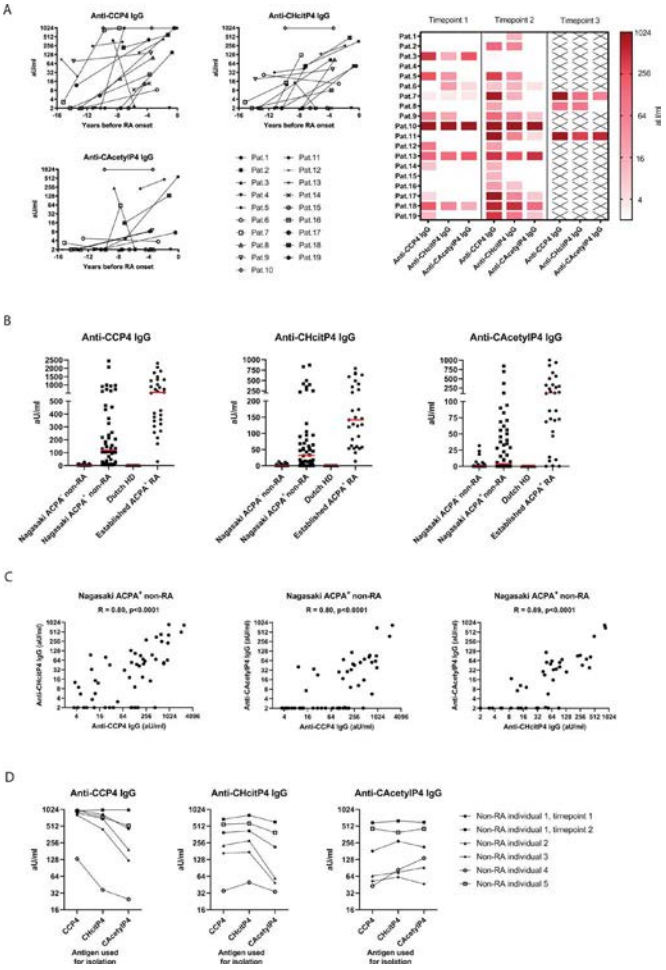

Figure 1 (A) ACPA, anti-CarP and AAPA IgG levels, using the CCP4, CHcitP4 and CAcetylP4 peptides as antigen, in arbitrary units per ml (aU/mL) over time of 19 patients with RA before disease onset. Left graphs show the data in years before onset. The heatmap on the right shows a summary of the AMPA IgG levels per time point. (B) ACPA, anti-CarP and AAPA IgG levels in aU/ml of Japanese ACPA-non-RA samples (n=197), Japanese AcpA +non RA samples (n=54), Dutch healthy donors (n=30) and established patients with RA (n=29). (C) Correlations of ACPA, anti-CarP and AAPA levels in aU/mL in Japanese ACPA +non RA samples. R=correlation coefficient. (D) ACPA, anti-CarP and AAPA IgG levels in aU/mL of six samples from Japanese AcpA +non RA samples after antibody isolation using CCP4, CHcitP4 or CAcetylP4 peptides. ACPA, anticitrullinated protein antibody; AAPA, antiacetylated protein antibody; anti-CarPA, anti-carbamylated protein antibodies; RA, rheumatoid arthritis.

acetyllysine, as shown at both the monoclonal and polyclonal antibody level.[2 3] B cell receptor analysis of ACPA-expressing B cells from patients with RA has shown that ACPAs have undergone extensive somatic hypermutation and that this can facilitate epitope spreading to multiple citrullinated epitopes.[4] Given the association of ACPA epitope spreading with progression to disease, it is relevant to obtain more insights when cross-reactivity to other PTMs is introduced. Furthermore, insights in whether cross-reactivity is also present in ACPA-positive subjects without RA or confined to subjects that will—or have developed RA will also help to better understand the evolution of anti-modified protein antibody (AMPA) responses. Therefore, we analysed cross-reactivity of the ACPA response in pre-disease samples and ACPA-positive individuals without RA. To this end, ACPA, anti-CarPA and AAPA in different cohorts were measured using modified peptides as described in online supplemental materials. First, we analysed the AMPA-IgG response in samples from 19 different Swedish subjects who later developed RA. As expected, ACPA could be detected years before disease onset with a rise in antibody level over time (figure 1A). We detected a similar pattern for anti-CarPA and AAPA. Interestingly, for most patients with detectable ACPA, anti-CarPA and/or AAPA, these antibodies could be detected at the same timepoint, indicating their simultaneous appearance years before disease onset. Next, we analysed AMPA levels in samples from ACPA-positive and ACPA-negative Japanese individuals without RA, derived from the community-based Nagasaki Island study (figure 1B, online supplemental figure S1).[5] Intriguingly, a strong correlation between levels of the different individual AMPA-reactivities was observed, pointing to cross-reactivity of the antibodies (figure 1C). To experimentally confirm cross-reactivity, we selected six samples from ACPA-positive non-RA individuals with high AMPA values, isolated ACPA, anti-CarPA and AAPA and determined the reactivity of the isolated antibodies to the three different PTMs. Isolated ACPAs were highly reactive to the homocitrullinated and acetylated antigen and vice versa, showing that AMPA in individuals without RA are also cross-reactive towards different PTMs (figure 1D). These results were confirmed on post-translationally modified fibrinogen and FCS (online supplemental figure S2). Interestingly, the reactivity to citrullinated/homocitrullinated peptides was higher when AMPA were isolated with a citrullinated or homocitrullinated antigen than with an acetylated antigen. This suggests cross-reactivity between ACPA and anti-CarPA is stronger than between either of them and AAPA. Together, our data show that ACPA, anti-CarPA and AAPA already coexist before disease onset. Moreover, ACPA can be cross-reactive towards homocitrulline and acetyllysine in ACPA-positive individuals without RA. These results indicate that cross-reactivity towards different PTMs emerges when AMPA responses become detectable and provide evidence that cross-reactivity towards different PTMs is an intrinsic characteristic of AMPA responses. This finding is in line with the observation that (germline) ACPA-IgM can be cross-reactive towards other PTMs as well.[6] Although cross-reactivity seems to be an intrinsic feature of AMPA, it is tempting to speculate that the most cross-reactive B cells are selected during progression towards RA, explaining the increase of the ACPA epitope recognition profile in time towards disease onset. Although cross-reactivity is already present before disease onset, the further increase in AMPA cross-reactivity and level could be a valuable biomarker in predicting transition towards disease.

Sanne Reijm ,[1] **Astrid S Brehler,**[1] **Solbritt Rantapää-Dahlqvist** ,[2] **Atsushi Kawakami,**[3] **Takahiro Maeda,**[4] **Shin-ya Kawashiri,**[5] **Mami Tamai,**[5] **Diane van der Woude,**[1] **René E M Toes** [1]

[1]Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands
[2]Department of Public Health and Clinical Medicine/Rheumatology, Umeå Universitet, Umea, Sweden
[3]Department of Immunology and Rheumatology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan
[4]Department of Community Medicine, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan
[5]Department of Immunology and Rheumatology Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan

Correspondence to Sanne Reijm, Department of Rheumatology, Leiden University Medical Center, Leiden 2333 ZA, The Netherlands; s.reijm@lumc.nl

Handling editor Josef S Smolen

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ORCID iDs
Sanne Reijm http://orcid.org/0000-0002-4424-4256
Solbritt Rantapää-Dahlqvist http://orcid.org/0000-0001-8259-3863
René E M Toes http://orcid.org/0000-0002-9618-6414

REFERENCES

1 Shi J, van de Stadt LA, Levarht EWN, *et al*. Anti-carbamylated protein (anti-CarP) antibodies precede the onset of rheumatoid arthritis. *Ann Rheum Dis* 2014;73:780–3.
2 Kissel T, Reijm S, Slot LM, *et al*. Antibodies and B cells recognising citrullinated proteins display a broad cross-reactivity towards other post-translational modifications. *Ann Rheum Dis* 2020;79:472–80.
3 Sahlström P, Hansson M, Steen J, *et al*. Different hierarchies of Anti-Modified protein autoantibody reactivities in rheumatoid arthritis. *Arthritis Rheumatol* 2020;72:1643–57.
4 Kongpachith S, Lingampalli N, Ju C-H, *et al*. Affinity maturation of the Anti-Citrullinated protein antibody paratope drives epitope spreading and polyreactivity in rheumatoid arthritis. *Arthritis Rheumatol* 2019;71:507–17.
5 Kawashiri S-Y, Tsuji Y, Tamai M, *et al*. Effects of cigarette smoking and human T-cell leukaemia virus type 1 infection on anti-citrullinated peptide antibody production in Japanese community-dwelling adults: the Nagasaki islands study. *Scand J Rheumatol* 2021;50:295–8.
6 Reijm S, Kissel T, Stoeken-Rijsbergen G, *et al*. Cross-Reactivity of IgM anti-modified protein antibodies in rheumatoid arthritis despite limited mutational load. *Arthritis Res Ther* 2021;23:230.

Sequential interleukin-17/interleukin-23 inhibition in treatment-refractory psoriatic arthritis

Treatment of psoriatic arthritis (PsA) has considerably improved by the introduction of biological disease modifying antirheumatic drugs.[1 2] Monoclonal antibodies targeting tumour necrosis factor alpha (TNFa), interleukin-17 (IL-17) and IL-23 have shown efficacy in psoriatic joint and skin disease.[3] While most of PsA patients respond to either one of these treatments, a subset of patients is highly resistant to all three cytokine blocking modalities and shows refractory active disease. This subset of patients represents a challenge that requires new therapeutic concepts.

Combined cytokine inhibition might represent an attractive opportunity for such patients: Within the IL-17 family, this concept is followed by dual inhibition of IL-17A and IL-17F by bimekizumab,[4] while evidence for inhibition across cytokine families is sparse to date. Dual inhibition of TNF and IL-17 has not shown additive efficacy over single TNF inhibition when used by PsA patients failing on methotrexate.[5] In resistant PsA patients, combination of ustekinumab with TNF inhibitors has been used with success in two small case series,[6 7] however, infection rate was high, suggesting that such treatment should be used with caution. These data indicate limitations of combined cytokine blockade with respect to safety and efficacy in PsA.

Herein, we used an alternating treatment regimen cycling between IL-23 and IL-17 inhibitors in three PsA patients failing on single inhibition of TNF (adalimumab 40 mg/2 weeks), IL-23 (guselkumab 100 mg/2 months) and IL-17 (secukinumab 300 mg/month). This approach conceptualises observations that IL-23 and IL-17 inhibition share a low infection risk,[8] can act independently from each other[9] and that IL-23 inhibition may have long-standing effects that may sensitise patients to IL-17 inhibition.[10] Alternating IL-23/IL-17 treatment was done by administering guselkumab (100 mg), followed by secukinumab (150 mg) after 2 months, followed by guselkumab after 1 month and so on. Based on this regimen also the overall drug costs are not higher than with single cytokine inhibition.

All three patients (figure 1A) had severe PsA with moderate-to-severe psoriasis (Psoriasis Area and Severity Index (PASI): 12.1±2.9), very highly active arthritis (Disease Activity in Psoriatic Arthritis (DAPSA): 33.6±6.6) and active enthesitis (Spondyloarthritis Research Consortium of Canada Index (SPARCC): 5.0±1.0). None of the patients were reaching sufficient and comprehensive control of disease after 6 months of adalimumab (PASI: 7.0±2.2; DAPSA: 14.3±4.0; SPARCC: 2.3±0.5), after 6 months of secukinumab (3.8±2.1; 20.3±3.2; 1.3±1.1) or after 6 months of guselkumab (2.9±2.4; 21.3±3.5; 1.3±1.1) treatment (figure 1B). While patients improved in their symptoms and showed partial response to single agents, none of them reached minimal disease activity (MDA) state or an acceptable symptom state. Based on this, alternating IL-23/IL-17 treatment was started according to the regimen described above with switching to secukinumab while being already on guselkumab as the last treatment in all patients. Alternating treatment lead to a continuous improvement of the activity in all domains (joints, skin, entheses) with very low values after 6 months (0.5±0.5; 4.3±1.5; 0.3±0.5) (figure 1C). Improvement was consistent among all different components of arthritis (figure 1D). All three patients reached MDA state

A

Measure	Treatments	1	2	3
Age		35	41	39
Sex		M	F	M
Duration		2.5	3.0	2.2
DAPSA	Baseline	37	38	26
	TNFi	19	12	12
	IL-17i	24	18	19
	IL-23i	25	21	18
	IL-17/23^{alt}	6	4	3
SPARCC	Baseline	6	4	5
	TNFi	3	2	2
	IL-17i	2	2	0
	IL-23i	2	2	0
	IL-17/23^{alt}	1	0	0
PASI	Baseline	13.0	8.9	14.6
	TNFi	5.9	10.2	5.0
	IL-17i	2.3	6.3	2.9
	IL-23i	1.9	5.8	1.2
	IL-17/23^{alt}	0.5	1	0

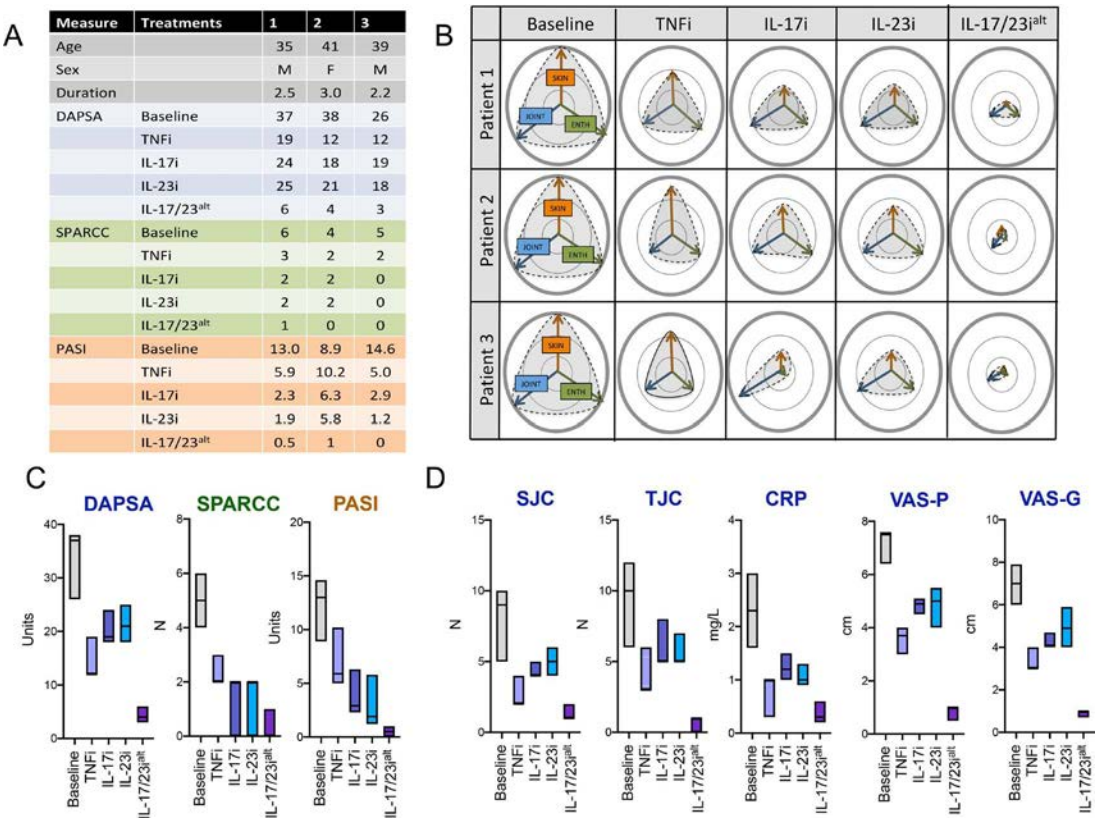

Figure 1 Effects of alternating interleukin-17 (IL-17)/IL-23 inhibition on the key domains of psoriatic arthritis. (A) Table showing demographic and clinical characteristics of the patients. (B) Circles showing relative responses to 6-month inhibition of tumour necrosis factor alpha (TNFi) with adalimumab, inhibition of IL-17 (IL-17i) with secukinumab, IL-23i with guselkumab and alternating inhibition of IL-17 and IL-23 (IL-1723i^{alt}) on the skin (red vector), joints (blue vector) and entheses (green vector). Rows indicate the three patients. First column shows the baseline. Outer circle shows 100% of baseline activity, middle circle 50% and inner circle 10% of baseline activity. (C) Responses observed in the joints (Disease activity in Psoriatic Arthritis; DAPSA), the entheses (Spondyloarthritis Research Consortium of Canada index, SPARCC) and the skin (Psoriasis Area and Severity Index; PASI) after 6-month treatment with the aforementioned agents. (D) Swollen joint count (SJC), tender joint count (TJC), C reactive protein (CRP) level, patient pain on a Visual Analogue Scale (VAS-P) and patient global disease activity on a visual analogue scale (VAS-G) after 6-month treatment with the aforementioned agents.

and were continued on this regimen. No infections and no other side effects occurred over the 6 months of treatment.

These data suggest that IL-17 and IL-23 inhibition can add each other and can improve the treatment of partially resistant PsA, even if not used simultaneously but in an alternating mode. Additive action of IL-17 and IL-23 inhibition is not entirely surprising as the cytokine has different cellular sources (T cells and dendritic cells, respectively) and also distinct cellular targets (neutrophils and T cells, respectively).[8] Sequential targeting of IL-23/IL-17 may therefore allow a more comprehensive interference with cellular activation in PsA. Whether such treatment allows to reset the altered immune response in PsA and in consequence restores responsiveness to single cytokine blockade is currently unknown. Of note, these three cases do not allow a general conclusion whether such approach is efficacious and safe in treatment-resistant PsA, but suggest that alternating therapy with IL-17- and IL-23 inhibitors is feasible in principle. Due to the small sample size and the short follow-up, however, more information on the safety of such approaches is needed.

David Simon [1,2] **Filippo Fagni**,[1,2] **Georg Schett** [1,2]

[1]Department of Internal Medicine 3- Rheumatology and Immunology, Friedrich-Alexander-University Erlangen-Nürnberg and Universitätsklinikum Erlangen, Erlangen, Germany, Erlangen, Germany

[2]Deutsches Zentrum Immuntherapie, Friedrich-Alexander-University Erlangen-Nürnberg and Universitätsklinikum Erlangen, Erlangen, Germany

Correspondence to Professor Georg Schett, Rheumatology, Friedrich-Alexander-Universitat Erlangen-Nurnberg, Erlangen, Bayern, Germany; georg.schett@uk-erlangen.de

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ORCID iDs
David Simon http://orcid.org/0000-0001-8310-7820
Georg Schett http://orcid.org/0000-0001-8740-9615

REFERENCES

1 Ritchlin CT, Colbert RA, Gladman DD. Psoriatic arthritis. *N Engl J Med* 2017;376:957–70.
2 Gossec L, Baraliakos X, Kerschbaumer A, *et al*. EULAR recommendations for the management of psoriatic arthritis with pharmacological therapies: 2019 update. *Ann Rheum Dis* 2020;79:700.1–12.
3 Schett G, McInnes IB, Neurath MF. Reframing immune-mediated inflammatory diseases through signature cytokine hubs. *N Engl J Med* 2021;385:628–39.
4 Gordon KB, Foley P, Krueger JG, *et al*. Bimekizumab efficacy and safety in moderate to severe plaque psoriasis (BE READY): a multicentre, double-blind, placebo-controlled, randomised withdrawal phase 3 trial. *Lancet* 2021;397:475–86.
5 Mease PJ, Genovese MC, Weinblatt ME, *et al*. Phase II study of abt-122, a tumor necrosis factor- and Interleukin-17A-Targeted dual variable domain immunoglobulin, in patients with psoriatic arthritis with an inadequate response to methotrexate. *Arthritis Rheumatol* 2018;70:1778–89.
6 Gniadecki R, Bang B, Sand C. Combination of antitumour necrosis factor-α and anti-interleukin-12/23 antibodies in refractory psoriasis and psoriatic arthritis: a long-term case-series observational study. *Br J Dermatol* 2016;174:1145–6.
7 De Marco G, McGonagle D, Mathieson HR, *et al*. Combined inhibition of tumour necrosis factor-alpha and interleukin-12/23 for long-standing, refractory psoriatic disease: a differential role for cytokine pathways? *Rheumatology* 2018;57:2053–5.
8 Cui L, Chen R, Subedi S, *et al*. Efficacy and safety of biologics targeting IL-17 and IL-23 in the treatment of moderate-to-severe plaque psoriasis: a systematic review and meta-analysis of randomized controlled trials. *Int Immunopharmacol* 2018;62:46–58.
9 Chizzolini C, Chicheportiche R, Alvarez M, *et al*. Prostaglandin E2 synergistically with interleukin-23 favors human Th17 expansion. *Blood* 2008;112:3696–703.
10 Cheuk S, Wikén M, Blomqvist L, *et al*. Epidermal Th22 and Tc17 cells form a localized disease memory in clinically healed psoriasis. *J Immunol* 2014;192:3111–20.

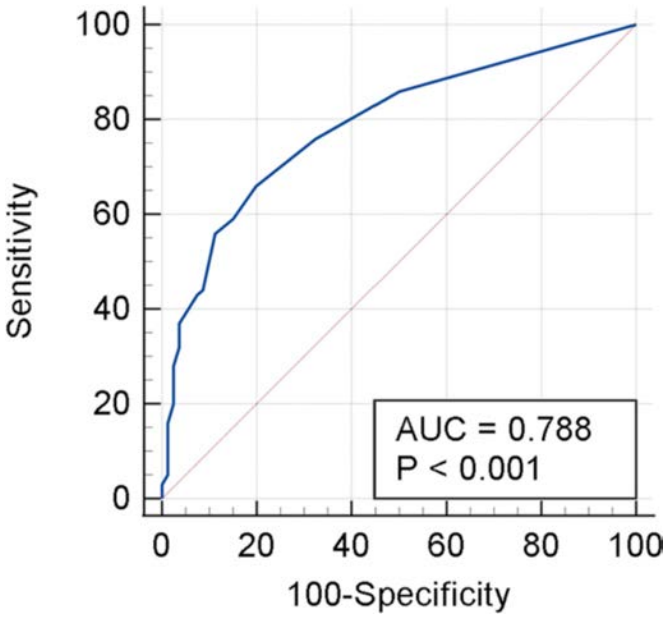

Figure 1 Receiver operating characteristics curve of delta physician global assessment for predicting flare. AUC, area under the curve.

Determination of the minimal clinically important difference (MCID) of the physician global assessment (PGA) in SLE

Accurate and reliable measurement of systemic lupus erythematosus (SLE) disease activity is critical for clinical and translational research. The Physician Global Assessment (PGA) is a well-accepted instrument that measures SLE disease activity. It is an anchored, visual analog scale (VAS) ranging from 0 to 3 capturing the physician's overall impression of a patient's disease activity. Importantly, it is feasible, valid and sensitive to change.[1] The PGA is used in SLE clinical trials and observational cohort studies, supplementing other disease activity indices such as the SLE disease activity index (SLEDAI) and British Isles Lupus Assessment Group (BILAG). It is incorporated into responder indices (the SLE Responder Index (SRI) and BILAG-Based Composite Lupus Assessment (BICLA)), and is also part of the SELENA-SLEDAI Flare Index.[2 3] Additionally, the PGA is a component of the Lupus Low Disease Activity State (LLDAS) and the Definition of Remission in SLE (DORIS).[4 5] As neither a BICLA nor SRI response can be achieved if the PGA increases (reflecting worsening disease activity) by greater than 0.3, an increase of 0.3 has been considered meaningful.[2 3] However, the minimal clinically important difference (MCID) for the PGA has not been assessed. Our objective was to determine the MCID of the PGA.

Scoring of the physician global assessment (PGA) was performed with the usual instructions 'How do you rate your patient's current disease activity?' using an anchored VAS of 0–3 with 0=none, 1=mild, 2=moderate and 3=most active disease imaginable.[6] PGAs were scored prospectively by three physicians on consecutive patient visits. The delta PGA (ΔPGA) was the difference between a visit PGA and the previous (baseline) visit's PGA. A flare visit was defined as a one in which therapy was escalated (initiation or increase of corticosteroids and/or disease modifying antirheumatic drugs or biologic agents) based on disease activity. All other visits were termed non-flare visits. We constructed a receiver operating characteristics (ROC) curve to visualise the performance of the Δ PGA for predicting flare and determined the minimal clinically important difference (MCID) for flare by calculating Youden's index for the ΔPGA in 0.1 increments.

We recorded the ΔPGA and therapeutic decisions across 126 paired visits in 66 subjects (mean age: 39.9 years (SD=11.5 years); 84.8% women; 25.8% Hispanic/Latino ethnicity; 30.3% white, 53.0% black, 7.6% Asian, 9.1% other race). The baseline PGA was between 0.0 and 0.9 in 78 visits, 1.0 and 1.9 in 36 visits and 2.0 and 3.0 in 12 visits. Flare occurred in 86 visits (68.3%). Of these 86 flare visits, baseline PGA scores were between 0.0 and 0.9 in 44 (51.2%), 1.0 and 1.9 in 30 (34.9%) and 2.0 and 3.0 in 12 (13.9%). Interestingly, flares occurred in 44 of 78 (56.4 %) visits with a baseline PGA between 0 and 0.9, 30 of 36 (83.3%) with a baseline PGA between 1.0 and 1.9 and 12 of 12 (100%) with a baseline PGA between 2.0 and 3.0, suggesting a lower flare rate among those with lower baseline PGA scores.

The ROC curve for the performance of ΔPGA in predicting flare is shown in figure 1. The area under the curve was 0.788 (SE=0.03), p<0.001. A ΔPGA of 0.3 was associated with the highest Youden's index (0.460), corresponding to a sensitivity of 66.0% and specificity of 80.0% for predicting flare.

Preliminary results from this small observational study suggest that the MCID for an increase in disease activity assessed by the PGA is 0.3. Notably, this value is consistent with what has been considered a meaningful change in the PGA by the SRI and BICLA responder indices. However, the sensitivity and specificity for an MCID using our definition of flare was suboptimal.

Additional studies involving a greater number of physicians and patient visits, as well as consideration of alternative definitions of a clinically significant change in disease activity, are necessary to confirm this MCID for the PGA.

Erik W Anderson , Meggan Mackay, Giovanni Franchin, Cynthia Aranow

The Feinstein Institute for Medical Research, Manhasset, New York, USA

Correspondence to Dr Cynthia Aranow, The Feinstein Institute for Medical Research, Manhasset, New York, USA; caranow@northwell.edu

Handling editor Josef S Smolen

Twitter Erik W Anderson @erikanderson_md

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ORCID iDs
Erik W Anderson http://orcid.org/0000-0003-3154-9648
Cynthia Aranow http://orcid.org/0000-0001-9299-0053

REFERENCES

1 Chessa E, Piga M, Floris A, *et al*. Use of physician global assessment in systemic lupus erythematosus: a systematic review of its psychometric properties. *Rheumatology* 2020;59:3622–32.
2 Furie RA, Petri MA, Wallace DJ, *et al*. Novel evidence-based systemic lupus erythematosus responder index. *Arthritis Rheum* 2009;61:1143–51.
3 Furie R, Morand EF, Bruce IN, *et al*. What does it mean to be a British Isles lupus assessment group-based composite lupus assessment Responder? Post hoc analysis of two phase III trials. *Arthritis Rheumatol* 2021;73:2059–68.
4 Franklyn K, Lau CS, Navarra SV, *et al*. Definition and initial validation of a lupus low disease activity state (LLDAS). *Ann Rheum Dis* 2016;75:1615–21.
5 van Vollenhoven RF, Bertsias G, Doria A. DORIS definition of remission in SLE: final recommendations from an international Task force. *Lupus Sci Med* 2021;2021:8.
6 Petri M, Kim MY, Kalunian KC, *et al*. Combined oral contraceptives in women with systemic lupus erythematosus. *N Engl J Med* 2005;353:2550–8.

SGLT2 inhibitors in lupus nephropathy, a new therapeutic strategy for nephroprotection

Systemic lupus erythematosus (SLE) is a chronic autoimmune condition characterised by heterogeneous clinical features. The patients with SLE are known to have an increased risk of cardiovascular events, due to both traditional and disease-specific risk factors, including inflammation, endothelial dysfunction, accelerated atherosclerosis and lupus nephritis (LN). Since chronic kidney disease (CKD) is per se one of the strongest CV risk factors, any manoeuvres to prevent CKD progression, including reduction of albuminuria and prevention of estimated glomerular filtration decline, will likely have profound influences on patient outcomes.[1 2]

All patients with LN have by definition CKD, since they display albuminuria to varying degrees. While albuminuria is a classical sign of renal damage, a substantial portion of patients will also have structural and functional impairment of their kidney function as hallmark of CKD, that is, glomerular hyperfiltration and albuminuria. In the past, renin–angiotensin–aldosterone system inhibitors (RAASi) has already conferred nephroprotective potential in patients with LN; however, a substantial residual renal risk remains in all forms of CKD. In the last few years, novel treatment strategies are therefore required to further decrease proteinuria and to slow kidney function decline.[3]

Sodium–glucose cotransporter-2 inhibitors (SGLT2i) have recently been demonstrated to exert profound cardio and nephroprotection in large cardiovascular outcome trials. An initial drop of eGFR after SGLT-2i administration (2–5 mL/min) is detected during the first weeks of treatment reflecting a reduction of intraglomerular pressure. A similar effect was observed with mineralocorticoid receptor blockers and ACE inhibitor.[4] SGLT2i inhibits the sodium proton exchanger, further increasing the delivery of sodium to the loop of Henle, which results in activation of TGF feedback response with consequent attenuation of glomerular hyperfiltration. They reduce progression of CKD including albuminuria and improve outcomes in heart failure patients with and without type 2 diabetes on top of angiotensin-blocking agents.[5] Since many aetiologies of non-diabetic nephropathy are characterised by intraglomerular hypertension, we hypothesise that SGLT2i acutely decrease GFR and proteinuria in patients without diabetes at risk of progressive kidney function loss via a glucose independent haemodynamic mechanism. Furthermore, distinct complications of SLE may also seem to be amenable to the therapeutic potential with SGLT2i such as the increased occurrence of pulmonary hypertension, metabolic syndrome and increased blood pressure.[6] Patients with LN were excluded from such studies due to potential necessities of acute immunosuppression.[7]

The aim of this study was to analyse the effect of SGLT2i in patients with LN in chronic and stable treatment with immunosuppression and residual proteinuria.

Five patients with histologically confirmed LN on immunosuppressive therapy with mean proteinuria of 2.2 g/day had empaglifozin 10 mg/day added. Within 8 weeks of starting treatment, the patients experienced a dramatic decrease in proteinuria (49.9%) with minimal change in glomerular filtration rate (table 1, online supplemental figure 1).

This pilot trial evaluates the antiproteinuric and nephroprotective effect of SGLT2i in patients with LN. Landmark studies have unequivocally demonstrated the renoprotective effect of SGLT2i in addition to the standard of care (RAASi) in different chronic proteinuric nephropathies. For this reason, we believe that these drugs combined with RAASi may be an effective alternative in the management of residual proteinuria in patients with lupus nephropathy with adequate immunosuppression due to their nephroprotective and cardioprotective effects. Prospective randomised studies are needed to demonstrate the potential beneficial effect of SGT2 inhibitors in patients with LN.

Table 1 Main effects of sodium–glucose cotransporter-2 inhibitors (empaglifozin 10 mg) lupus nephritis

N	Age/sex	Classification LN (ISN/RPS 2003)	IMS (doses mg/day)	RAASi (doses mg/day)	GFR baseline (mL/min/1.73 m^2)	GFR 8 weeks (ml/min/1.73 m^2)	Proteinuria baseline (g/day)	Proteinuria 8 weeks (g/day)	Serum albumin baseline (g/dL)	Serum albumin 8 weeks (g/dL)
1	63/F	V	S 2.5+MPA 980	Enalapril 20+SPR 25	53	44	1.8	0.9	4.2	4.1
2	59/F	IIIA	MPA 1600	Telmisartan 80+SPR 25	65	60	1.9	0.8	4.2	4.5
3	46/F	V	S 2.5+MMF 1250	Irbesartan 150+SPR 25	89	74	0.62	0.27	3.9	4.3
4	32/F	1-IVAG+V 2-IVAG	S 5+MPA 720	Telmisartan 80	34	30	5.96	3.7	2.7	3.5
5	46/F	1-V 2-IVS (A,C)+V	S 5+MPA 1080	Enalapril 10+SPR 25	94	90	0.76	0.39	3.8	4.2

F, female; GFR, glomerular filtration rate; IMS, immunossupression; LN, lupus nephritis; MMF, mycofenolate mofetil; MPA, mycophenolic acid; RAASi, renin-angiotensin-aldosterone inhibitors; S, steroids; SPR, spironolactone.

Enrique Morales ,[1] **Maria Galindo**[2]

[1]Nephrology, Hospital Universitario 12 de Octubre, Madrid, Spain
[2]Rheumatology, Hospital Universitario 12 de Octubre, Madrid, Spain

Correspondence to Dr Enrique Morales, Nephrology, Hospital Universitario 12 de Octubre, Madrid, Spain, Spain; emoralesr@senefro.org

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ORCID iD
Enrique Morales http://orcid.org/0000-0002-5824-6973

REFERENCES

1 Anders H-J, Saxena R, Zhao M-H, *et al*. Lupus nephritis. *Nat Rev Dis Primers* 2020;6:7.
2 Morales E, Galindo M, Trujillo H, *et al*. Update on lupus nephritis: looking for a new vision. *Nephron* 2021;145:1–13.
3 Parikh SV, Almaani S, Brodsky S, *et al*. Update on lupus nephritis: core curriculum 2020. *Am J Kidney Dis* 2020;76:265–81.
4 Trujillo H, Caravaca-Fontán F, Caro J, *et al*. The forgotten Antiproteinuric properties of diuretics. *Am J Nephrol* 2021;52:435–49.
5 Heerspink HJL, Stefánsson BV, Correa-Rotter R. DAPA-CKD trial committees and Investigators. dapagliflozin in patients with chronic kidney disease. *N Engl J Med* 2020;383:1436–46.
6 Säemann M, Kronbichler A. Call for action in ANCA-associated vasculitis and lupus nephritis: promises and challenges of SGLT-2 inhibitors. *Ann Rheum Dis* 2022;81:614–7.
7 Wheeler DC, Stefánsson BV, Jongs N, *et al*. Effects of dapagliflozin on major adverse kidney and cardiovascular events in patients with diabetic and non-diabetic chronic kidney disease: a prespecified analysis from the DAPA-CKD trial. *Lancet Diabetes Endocrinol* 2021;9:22–31.

Humoral immune-response to a SARS-CoV-2-BNT162b2 booster in inflammatory arthritis patients who received an inactivated virus vaccine

CoronaVac, an inactivated SARS-CoV-2 vaccine, has been administered in over 100 countries worldwide, but its immunity wanes quickly over time.[1] In consequence, boosters are being recommended.

We evaluated the immunogenicity of an mRNA vaccine booster (BNT162b2) in inflammatory arthritis (IA) patients with biologic treatments previously vaccinated with CoronaVac. Consenting adults with IA followed at Red Salud UC-CHRISTUS (Chile), who were on anti-TNF, anti-IL6 or anti-IL17 biologics, vaccinated with CoronaVac (0, 28), were eligible. Those with a SARS-CoV-2 infection history were excluded. Humoral response was assessed by measuring IgG SARS-CoV-2 total antibody (Tab) and neutralising antibody (Nab) within 7 days and 4 weeks after the booster. DMARDs were not discontinued.

The primary outcome was the proportion of participants with positive SARS-CoV-2 Nab 4 weeks after the BTN162b2 booster. A neutralisation of 30% or more at a 1:10 dilution was considered positive.[2] Dichotomous and continuous variables were compared using the McNemar or Wilcoxon signed-rank test.

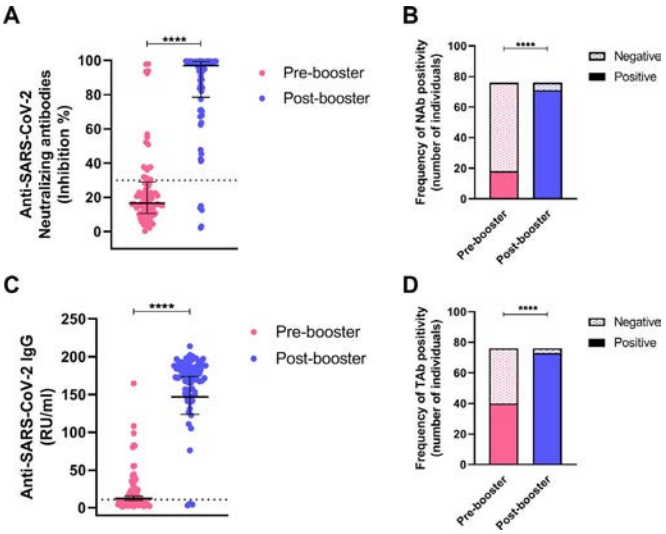

Figure 1 Humoral response against SARS-CoV-2 4 weeks after the booster dose. Distribution for (A) neutralising activity (median (IQR) of percentage of inhibition), (B) neutralising antibodies positivity (≥30% of inhibition rate), (C) total IgG anti S1 GMC (95% CI),RU/mL, (D) frequency of total IgG anti S1 positivity (≥11 relative units per mL, RU/mL).

Confounding and effect modifiers of covariates were explored using binary regression models.

Seventy-six individuals were included. Mean age was 51.9 (SD 11.3) and 73.6% were female. Mean years since diagnosis were 6.2 years (SD 7.4), 74% had rheumatoid arthritis (RA), 24% psoriatic arthritis, 1% juvenile idiopathic arthritis and 1% ankylosing spondylitis. Overall, 45% used low dose prednisone, 36% methotrexate, 21% leflunomide and 14% sulfasalazine. The median (IQR) number of days between the second CoronaVac dose and the BNT162b2 booster was 157 (143-170). At baseline, 18 participants (24%) had Nab and 40 (53%) had positiveTab. Age was inversely and independently associated with the probability of having detectable Nab at baseline (p value 0.006). In the same model, neither gender, prednisone, methotrexate nor time between CoronaVac vaccination and the booster predicted baseline Nab serostatus. Four weeks after receiving a BNT162b2 booster, 71 (94%) and 73 (96%) individuals had positive Nab and Tab, respectively. The median (IQR) neutralising activity rose from 17% (11%–29%) to 97% (80%–99%) after boosting (figure 1). Five participants (6.8%) remained Nab-seronegative; all had RA, received steroids and four of them used methotrexate. Sixty-two per cent of patients reported adverse events, all mild.

Multivariate analysis found no association among age, gender, prednisone, methotrexate and postbooster Nab levels. Response was not correlated to the time elapsed since the second dose of CoronaVac.

No COVID-19 cases were reported after the booster (follow-up 1–3 months).

After an mRNA vaccine booster, our sample of IA patients using biologics and vaccinated with CoronaVac significantly improved their humoral immune response against SARS-CoV-2. Little is known about response to vaccine boosters following inactivated vaccines. One study compared BNT162b2 versus CoronaVac in 80 healthy individuals with low antibody response to CoronaVac. Nab seroconversion was observed in 96.8% and 57.7% of participants receiving BNT162b2 or CoronaVac boosters, respectively.[3] A recent study of a homologous booster of CoronaVac in patients with rheumatic disease showed 81.4% of subjects produced Nab.[4] Booster doses have also been explored in rheumatologic patients vaccinated with mRNA vaccines. A small study in RA patients unresponsive to BNT162b2 showed that after a homologous booster plus DMARDS discontinuation, 15 of 17 participants reached adequate Tab titers.[5]

Similarly, 16 out of 18 autoimmune disease patients vaccinated with mRNA vaccines and boosted with mRNA or viral-vectored vaccines increased Tabs.[6]

Our study limitations include the lack of assessment of cellular response and the short postbooster clinical follow-up. Results should not be generalised to patients receiving other biologics.

In conclusion, our study suggests that IA patients on biologic drugs receiving inactivated COVID-19 vaccines should receive a booster dose. A mix-and-match approach with mRNA vaccines is well tolerated and highly immunogenic.

Josefina Durán ,[1] **Paula Isabel Burgos,**[1] **Nicole Le Corre,**[2] **Cinthya Ruiz Tagle,**[3] **Constanza Martinez-Valdebenito,**[2] **Mauricio Castro,**[4] **Valentina Metcalfe,**[5] **Paula Niemann,**[5] **M Elvira Balcells**[3]

[1]Department of Rheumatology, Pontificia Universidad Católica de Chile, Santiago, Chile
[2]Department of Pediatric Immunology and Infectious Diseases, Pontificia Universidad Católica de Chile, Santiago, Chile
[3]Department of Infectious Diseases, Pontificia Universidad Católica de Chile, Santiago, Chile
[4]Department of Statistics, Pontifiicia Universidad Católica de Chile, Santiago, Chile
[5]Pontificia Universidad Católica de Chile, Santiago, Chile

Correspondence to Dr Josefina Durán, Rheumatology, Pontificia Universidad Católica de Chile, Santiago, Chile; jgduran@uc.cl

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ORCID iD
Josefina Durán http://orcid.org/0000-0001-7376-7729

REFERENCES

1 Mallapaty S. China's COVID vaccines have been crucial - now immunity is waning. *Nature* 2021;598:398–9.

2 Tan CW, Chia WN, Qin X, *et al*. A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2-spike protein-protein interaction. *Nat Biotechnol* 2020;38:1073–8.
3 CKP M, Cheng SMS, Chen C. A RCT using CoronaVac or BNT162b2 vaccine as a third dose in adults vaccinated with two doses of CoronaVac. *Am J Respir Crit Care Med* 2022. doi:101164/rccm202111-2655LE
4 Aikawa NE, Kupa LdeVK, Medeiros-Ribeiro AC, *et al*. Increment of immunogenicity after third dose of a homologous inactivated SARS-CoV-2 vaccine in a large population of patients with autoimmune rheumatic diseases. *Ann Rheum Dis* 2022;81:1036–43.
5 Schmiedeberg K, Vuilleumier N, Pagano S, *et al*. Efficacy and tolerability of a third dose of an mRNA anti-SARS-CoV-2 vaccine in patients with rheumatoid arthritis with absent or minimal serological response to two previous doses. *Lancet Rheumatol* 2022;4:e11–13.
6 Connolly CM, Teles M, Frey S, *et al*. Booster-dose SARS-CoV-2 vaccination in patients with autoimmune disease: a case series. *Ann Rheum Dis* 2022;81:291–3.

More evidences on which biologic and which pathway is key in severe-critical COVID-19 pneumonia

I read with great interest the paper by Della Torre *et al* on the effects of sarilumab in severe acute respiratory syndrome coronavirus 2 severe-critical pneumonia. They show that sarilumab treated and standard of care (SOC) treated patients present a mortality rate which is statistically not different (n 28 SARI=7%vs n 28 SOC=18%; p=NS).[1] These data confirm previous data from the same group; when analysing patients treated with tocilizumab, they showed no statistically significant differences (n 33 SOC=33%, mortality vs tocilizumab (TOCI) n 32=16%, p=NS).[2] These data seem to suggest that interleukin (IL)-6 is not the main target. Indeed out of more than 20 studies reported so far in the literature, only half reported clinically significant results (paper submitted). The various studies have so many bias and differences that a definite conclusion is impossible. However, since the approach with biologics has a strong rationale in controlling the cytokine release syndromes in the severe-critical phases of the disease and data on bronchoalveolar lavage cells, and on single-cell analysis suggest that some targets (IL-6, IL-8, interferon γ (IFNγ), Il1β, IFNα/β) are certainly more expressed than others,[3] it is and will be of crucial importance the definition of a possible hierarchy in the intervention, especially because targeting one molecule, and less others, may lead to control several other manifestations of the disease, such as the increased coagulation abnormalities[4 5] and the cardiac ECG abnormalities present in several of these patients.[6] The issue is then of clear biological but also of clinical relevance.[7] The San Raffaele group published two other important studies with different biologics, anakinra and mavrilimumab in severe-critical patients. In these two studies, the results were more favourable. In the anakinra (targeting IL1) study, they showed that the death rate with the SOC (n 16 patients) was 44% versus 10%, in the anakinra treated (high dose), p=0.009.[8] In the mavrilimumab (targeting granulocyte macrophage- colony stimulating factor receptor (GM-CSF-R)) study, they had a mortality rate of 26% (n 26 SOC), versus 0% in the mavrilimumab (MAVRI) (n 13) subset (Fisher's exact test=0.08).[9] All the studies had a 28-day follow-up as a censor-day time (table 1).

It is clear that the numbers are low and bias are high, yet they are hypothesis generating. However, the observation that different mortality rates are seen in the SOC groups (pretty similar in numbers) can be explained only if the patients are different. Given that they are different, could the AA provide a comparison of the entire cohort of SOC-treated patients (n 103) versus each single biologic to understand whether they show differences in terms of major outcome and how much is the difference considering the various biologics tested against the whole SOC cohort? The other possible alternatives, that is, that the 33 SOC patients in the TOCI trials represent the whole cohort, would be hard to understand because of the higher mortality rate, and the other possibility that some patients belong to one study and other patients to the other study again would raise the need to really understand which is the number of the overall cohort of SOC and the mortality rate in the SOC cohort. The analysis of the entire data set of patients treated with the SOC raises other possible bias, when making comparisons, yet it could offer the opportunity to better interpret the real value efficacy of each single biologic targeting different pathways.

Table 1 Major outcome with the various biologics in severe-critical acute respiratory syndrome coronavirus 2 pneumonia in the San Raffaele studies

Authors biologics	N of SOC treated patients	Death rate (%)	N of biologic treated patients	Death rate (%)	P value
Della Torre *et al*[1] *Sarilumab*	28	18	28	7	NS
Campochiaro *et al*[2] *Tocilizumab*	33	33	32	16	NS
Cavalli *et al*[8] *Anakinra*	16	44	29 (high dose)	10	0.009
De Luca *et al*[9] *Mavrilimumab*	26	27	13	0	Fisher's exact t =0.08

Gianfranco Ferraccioli

Department of Rheumatology, Policlinico Universitario Agostino Gemelli, Universita' Cattolica del Sacro Cuore, Roma, Lazio, Italy

Correspondence to Professor Gianfranco Ferraccioli, Department of Rheumatology, Policlinico Universitario Agostino Gemelli, Universita' Cattolica del Sacro Cuore, Roma 00168, Lazio, Italy; gianfranco.ferraccioli@unicatt.it

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ORCID iD

Gianfranco Ferraccioli http://orcid.org/0000-0002-6884-4301

REFERENCES

1 Della-Torre E, Campochiaro C, Cavalli G, *et al*. Interleukin-6 blockade with sarilumab in severe COVID-19 pneumonia with systemic hyperinflammation: an open-label cohort study. *Ann Rheum Dis* 2020;79:1277–85.
2 Campochiaro C, Della-Torre E, Cavalli G, *et al*. Efficacy and safety of tocilizumab in severe COVID-19 patients: a single-centre retrospective cohort study. *Eur J Intern Med* 2020;76:43–9.
3 Liao M, Liu Y, Yuan J, *et al*. Single-Cell landscape of bronchoalveolar immune cells in patients with COVID-19. *Nat Med* 2020;26:842–4.
4 Moore JB, June CH. Cytokine release syndrome in severe COVID-19. *Science* 2020;368:473–4.
5 van der Poll T, Levi M, Hack CE, *et al*. Elimination of interleukin 6 attenuates coagulation activation in experimental endotoxemia in chimpanzees. *J Exp Med* 1994;179:1253–9.
6 Erre GL, Ferraccioli ES, Piga M, *et al*. Antimalarial use and arrhythmias in COVID-19 and rheumatic patients: a matter of dose and inflammation? *Ann Rheum Dis* 2021;80:e29.
7 Gremese E, Ferraccioli ES, Alivernini S, *et al*. Basic immunology may lead to translational therapeutic rationale:SARS.CoV2 and rheumatic diseases.. *Europ.J.Clin.Invest. 2020,* https://doi.org/10.1111/eci.13342 2020.
8 Cavalli G, De Luca G, Campochiaro C, *et al*. Interleukin-1 blockade with high-dose anakinra in patients with COVID-19, acute respiratory distress syndrome, and hyperinflammation: a retrospective cohort study. *Lancet Rheumatol* 2020;2:e325–31.
9 De Luca G, Cavalli G, Campochiaro C, *et al*. Gm-Csf blockade with mavrilimumab in severe COVID-19 pneumonia and systemic hyperinflammation: a single-centre, prospective cohort study. *Lancet Rheumatol* 2020.

Response to: 'More evidences on which biologic and which pathway is key in severe-critical COVID-19 pneumonia' by Ferraccioli

We thank Prof Ferraccioli for his positive comments on our open-label trials with sarilumab, tocilizumab, anakinra and mavrilimumab in patients with severe hyperinflamed COVID-19[1–4] and for remarking the unprecedented opportunity offered by our studies to better understand the relative contribution of different targetable inflammatory pathways to the pathogenesis of severe COVID-19.[5] The study designs we adopted should be definitely interpreted in light of the scientific data that progressively became available and of the course of the pandemic wave that struck Northern Italy and our Institution. Between 24 February and 22 May 2020, San Raffaele Hospital (Milan, Italy) admitted more than 1000 patients with COVID-19.[6–8] Intriguingly, the clinical phenotype of admitted patients changed over time and the severity of the disease progressively varied in parallel with outbreak exhaustion.[6]

When our hospital was first hit by the pandemic, accumulating evidence from China was pointing at interleukin (IL)-6 as a master regulator of the cytokine storm occurring in severe COVID-19.[9] Tocilizumab was, therefore, first used as a potential treatment[2] and subsequently replaced by intravenous sarilumab due to sudden shortage of the drug.[1] As described in our studies, however, none of the two IL-6 receptor antagonists convincingly impacted disease outcomes, prompting the search for alternative therapeutic strategies.[1 2] Blockade of IL-1 and of granulocyte–macrophage colony-stimulating factor (GM-CSF) was deemed potential rational approaches based on the upstream position of these molecules in the inflammatory cascade and on the ready availability of selective inhibitors.[9 10] In particular, due to its remarkable safety profile, intravenous anakinra was administered mainly to patients affected by severe acute respiratory distress syndrome managed outside intensive care unit (baseline Pao_2:Fi_{o2} <100 mm Hg in 86% of cases).[3] On the contrary, mavrilimumab was administered at later stages of the COVID-19 outbreak when admitted patients were generally less compromised (baseline Pao_2:Fio_2 >100 mm Hg in 92% of cases).[4] Hence, because our studies were not conducted in parallel and patients enrolled largely differed in terms of age and severity, mortality rates were also different among the four groups of matched controls treated with standard of care. Accordingly, although an oversimplistic comparison between the weighted mortality rate of patients treated with standard of care (30%) and with anticytokine therapies (10%) would suggest better outcomes in the latter group, our studies were not designed to clarify the relative efficacy of each single biologic agent.[1–4] The dilemma of whether to preferentially target IL-1, IL-6 or GM-CSF in severe COVID-19 remains, therefore, to be solved.

Yet, our pioneering experience returned three major pathophysiological insights. First, mechanisms inherent to IL-6 pathway are likely not the only drivers of severe COVID-19 as serum IL-6 levels were not associated with disease mortality, lung consolidation or respiratory failure in our patients.[1 6] Accordingly, IL-6 blocking strategies with either tocilizumab or sarilumab were not associated with clinical improvement in patients with critical COVID-19 compared with local standard of care.[1 6] Further evidence of the apparent inefficacy of anti-IL-6 treatments in severe disease is, indeed, provided by the early termination of a phase 3 randomised-controlled trial of intravenous sarilumab 400 mg conducted in the USA: in this trial involving 194 patients with severe COVID-19 sarilumab did not provide any additional benefit compared with placebo in mechanically ventilated patients and was associated with a negative trend in not mechanically ventilated subjects.[11]

Second, targeting IL-1 or GM-CSF seems a more promising approach since upstream blockade of the inflammatory cascade may allow a better control of cytokine storm-induced organ damage with a better safety profile. Third, the sarilumab study revealed for the first time that the degree of lung consolidation predicted disease response to a biologic treatment, a finding that may be of relevance for designing further clinical trials.[1] In this sense, intercepting rampant inflammation before the establishment of lung damage remains imperative to avoid COVID-19 progression to stages where even biologic agents might not be effective. Indeed, preliminary evidence of the effective early administration of anti-inflammatory molecules targeting the inflammasome activation such as colchicine seems to support the rationale of this approach.[12]

The results of ongoing randomised placebo-controlled trials comparing IL-1 and IL-6 blocking strategies on larger number of patients with COVID-19 are eagerly awaited to possibly substantiate our observations and to definitively rank the efficacy of different anticytokine therapies.[13] A retrospective comparison of our entire cohort of patients treated with standard of care versus each single biologic agent is also currently under preparation and will be based on a rigorous case–control matching in order to contain analytical biases and to retrieve informative results.

Emanuel Della-Torre [1,2] **Corrado Campochiaro** [1,2] **Giulio Cavalli**,[1,2] **Giacomo De Luca**,[1,2] **Fabio Ciceri**,[2,3] **Alberto Zangrillo**,[2,4] **Lorenzo Dagna** [1,2]

[1]Unit of Immunology, Rheumatology, Allergy and Rare Diseases (UnIRAR), San Raffaele Scientific Institute, Milan, Italy
[2]Vita-Salute San Raffaele University, San Raffaele Scientific Institute, Milan, Italy
[3]Hematology and Bone Marrow Transplant Unit, San Raffaele Scientific Institute, Milan, Italy
[4]Department of Anesthesia and Intensive Care, San Raffele Scientific Institute, Milan, Italy

Correspondence to Dr Emanuel Della-Torre, Università Vita-Salute San Raffaele, San Raffaele Scientific Institute, Milan 20132, Italy; dellatorre.emanuel@hsr.it

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ORCID iDs
Emanuel Della-Torre http://orcid.org/0000-0002-9192-4270
Corrado Campochiaro http://orcid.org/0000-0001-6806-3794
Lorenzo Dagna http://orcid.org/0000-0002-7428-315X

REFERENCES

1 Della-Torre E, Campochiaro C, Cavalli G, *et al*. Interleukin-6 blockade with sarilumab in severe COVID-19 pneumonia with systemic hyperinflammation: an open-label cohort study. *Ann Rheum Dis* 2020;79:1277–85.
2 Campochiaro C, Della-Torre E, Cavalli G, *et al*. Efficacy and safety of tocilizumab in severe COVID-19 patients: a single-centre retrospective cohort study. *Eur J Intern Med* 2020;76:43–9.
3 Cavalli G, De Luca G, Campochiaro C, *et al*. Interleukin-1 blockade with high-dose anakinra in patients with COVID-19, acute respiratory distress syndrome, and hyperinflammation: a retrospective cohort study. *Lancet Rheumatol* 2020;2:e325–31.
4 De Luca G, Cavalli G, Campochiaro C, *et al*. Gm-Csf blockade with mavrilimumab in severe COVID-19 pneumonia and systemic hyperinflammation: a single-centre, prospective cohort study. *Lancet Rheum* 2020.
5 Ferraccioli G. More evidences on which biologic and which pathway is key in severe-critical COVID-19 pneumonia. *Ann Rheum Dis* 2022;81:e157.
6 Ciceri F, Castagna A, Rovere-Querini P, *et al*. Early predictors of clinical outcomes of COVID-19 outbreak in Milan, Italy. *Clin Immunol* 2020;217:108509.
7 Zangrillo A, Beretta L, Scandroglio AM, *et al*. Characteristics, treatment, outcomes and cause of death of invasively ventilated patients with COVID-19 ARDS in Milan, Italy. *Crit Care Resusc* 2020
8 Zangrillo A, Beretta L, Silvani P, *et al*. Fast reshaping of intensive care unit facilities in a large metropolitan hospital in Milan, Italy: facing the COVID-19 pandemic emergency. *Crit Care Resusc* 2020.
9 Aziz M, Fatima R, Assaly R. Elevated interleukin-6 and severe COVID-19: a meta-analysis. *J Med Virol* 2020.
10 Bonaventura A, Vecchié A, Wang TS, *et al*. Targeting GM-CSF in COVID-19 pneumonia: rationale and strategies. *Front Immunol* 2020;11.
11 . Available: http://www.sanofi.com/en/media-room/press-releases/2020/2020-07-02-22-30-00
12 Della-Torre E, Della-Torre F, Kusanovic M, *et al*. Treating COVID-19 with colchicine in community healthcare setting. *Clin Immunol* 2020;217:108490.
13 . Available: https://clinicaltrials.gov/ct2/results?cond=Covid19&term=tocilizumab&type

Correspondence on: 'Paediatric multisystem inflammatory syndrome temporally associated with SARS-CoV-2 mimicking Kawasaki disease (Kawa-COVID-19): a multicentre cohort' by Pouletty *et al*

We read with interest the article by Pouletty *et al*,[1] in which the authors describe a multicentre compilation of patients with Kawasaki disease (KD) in France, associated with the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Other colleagues in Europe and USA have recently reported similar experiences.[2–5]

We report a prospective case series of paediatric patients that fulfilled clinical diagnostic criteria of KD during the SARS-CoV-2 pandemic in a paediatric referral centre in Barcelona, Spain. KD was defined according to the 2017 criteria of the American Heart Association.[6] Assessment of SARS-CoV-2 infection was made by means of quantitative real-time PCR assay (GeneFinder COVID-19 Plus, Elitech; Puteaux, France) in nasopharyngeal samples; stools were tested in patients with diarrhoea. SARS-CoV-2 IgG qualitative determination (SARS-CoV-2 IgG chemiluminescent microparticle immunoassay; Abbot, Chicago, Illinois) was performed during admission. Statistical analyses were performed using SPSS V.25 (IBM). Informed consent was obtained from parents or legal guardians, as was informed assent in patients aged >12 years.

From March 23 to May 14, twelve previously healthy patients with KD were admitted to our institution (table 1). The yearly number of patients with KD diagnosed in our centre is around 10–12. Prior to diagnosis, several patients reported gastrointestinal symptoms (10/12, 83.3%; vomiting, diarrhoea and abdominal pain) and neurological symptoms (5/12, 41.6%; irritability, headache, decreased consciousness and febrile seizures). Only patient 10 was referred with respiratory symptoms (cough) and had an abnormal chest X-ray showing pneumonia at presentation. Lymphopenia and thrombocytopenia were observed at diagnosis in eight and five patients, respectively. Inflammatory markers (C-reactive protein, ferritin, erythrocyte sedimentation rate and procalcitonin) were elevated in most patients, as were N-terminal pro-brain natriuretic peptide levels (median (range): 2930 (178–7994) ng/L).[7]

At or during admission, 6/12 (50%) patients showed microbiological and/or serological evidence of SARS-CoV-2 infection. As compared with children in whom SARS-CoV-2 infection was not demonstrated, the former had statistically significant lower platelet counts, and higher levels of inflammatory markers (C-reactive protein, procalcitonin and ferritin) and N-terminal pro-brain natriuretic peptide at diagnosis (table 2).

Ultrasound or clinical signs of cardiac involvement were noted at admission only in patient 8 (left and right coronary aneurysm, +4.7 and +4.8 Z-score). Coronary aneurysms were observed later in two further patients: on day 14 in patient 1 (left coronary aneurysm, 4.2 Z-score) and on day 12 in patient 10 (left

Table 1 Clinical and laboratory features of 12 patients with Kawasaki disease who presented during the SARS-CoV-2 pandemic

Patient No	1	2	3	4	5	6	7	8	9	10	11	12
	Complete KD							Incomplete KD				
Age, ethnicity	Infant, Latin	Infant, Caucasian	Infant, Caucasian	Toddler, Caucasian	Toddler, Caucasian	Toddler, Caucasian	Child, Caucasian	Infant, Asian	Toddler, Caucasian	Toddler, Black	Adolescent, Caucasian	Adolescent, Caucasian
Symptoms prior to diagnosis												
Duration (days)	6	4	5	5	7	5	4	7	7	4	7	7
Respiratory	No	No	No	No	Yes	No	No	No	No	Yes	No	No
Gastrointestinal	No	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Neurological	No	Yes	No	Yes	No	No	Yes	Yes	No	Yes	No	No
KD signs at diagnosis												
Conjunctivitis	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	Yes	Yes
Erythema mouth/pharynx	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No	No
Polymorphous rash	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
Lymphadenopathy	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	No	Yes	Yes
Erythema of the palms/soles	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No
Contact with SARS-CoV-2 case	No	No	No	No	No	No	No	No	No	Yes	No	Yes
SARS-CoV-2 RT-PCR in respiratory sample/stools	NEG/ND	NEG/POS	NEG/NEG	NEG/NEG	NEG/ND	NEG/NEG	NEG/NEG	NEG/NEG	NEG/ND	POS/ND	POS/ND	POS/ND
SARS-CoV-2 IgG	NEG	POS	NEG	NEG	NEG	POS	POS	NEG	NEG	POS	POS	POS
CRP (normal <15 mg/L)	184	73	46	42	33	165	173	128	133	229	276	241
PCT (normal <0.5 ng/mL)	0.08	3.1	0.11	0.21	0.14	6.75	6.7	0.51	0.07	4.65	3.97	2.11
Ferritin (normal <120 ng/mL)	114	358	66	450	96	67	604	219	142	563	>2000	1424
ESR (normal <15 mm/hour)	83	14	43	2	23	63	9	2	46	19	39	76
NT pro-BNP (normal <200 ng/L)	1416	647	178	291	457	7994	4510	3628	344	4840	2930	5500
Platelet count (normal 150–400×10^9/L)	449	129	373	129	509	296	149	249	456	170	117	76
Lymphopenia	No	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	Yes	Yes
Treatment	IVIG followed by IVIG+mPDN	IVIG+mPDN	IVIG	IVIG	IVIG	IVIG	IVIG+mPDN	IVIG+mPDN	IVIG followed by IVIG+mPDN	IVIG	mPDN	None
Length of stay (days)	5	6	5	2	3	4	8	12	8	10	3	6

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IVIG, intravenous immunoglobulin; KD, Kawasaki disease; mPDN, methylprednisolone; ND, not done; NEG, negative; NT pro-BNP, N-terminal pro-brain natriuretic peptide; PCT, procalcitonin; POS, positive; RT-PCR, real-time PCR; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Table 2 Differences between patients with Kawasaki disease with and without SARS-CoV-2 infection

	Positive SARS-CoV-2 infection RT-PCR or serology (n=6)	Negative SARS-CoV-2 infection RT-PCR or serology (n=6)	P value
Female sex	3 (50.0)	3 (50.0)	1
Age (years)	5.3 (2.5–11.2)	2.1 (1.2–3.1)	0.078
Complete Kawasaki disease	3 (50.0)	4 (66.7)	0.55
Symptoms prior to diagnosis			
Respiratory	1 (16.7)	1 (16.7)	1
Gastrointestinal	6 (100)	4 (66.7)	0.45
Neurological	3 (50.0)	2 (33.3)	0.5
Development of aneurysms	1 (16.7)	2 (33.3)	0.5
Lab values at admission			
C-reactive protein (mg/L)	180 (119–252)	87 (40–146)	0.029
Procalcitonin (ng/mL)	4.65 (3.53–6.72)	0.12 (0.08–0.28)	0.02
Ferritin (ng/mL)	563 (212–1302)	127 (89–277)	0.065
ESR (mm/hour)	14 (7–41)	33 (2–55)	0.12
NT pro-BNP (ng/L)	4510 (1788–6417)	400 (263–1969)	0.015
Platelet counts (x10^9/L)	149 (114–233)	408 (219–469)	0.016
Lymphopenia	5 (83.3)	3 (50.0)	0.54

Data are expressed with n (%) or with medians and IQRs.
ESR, erythrocyte sedimentation rate; NT pro-BNP, N-terminal pro-brain natriuretic peptide; RT-PCR, real-time PCR; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

coronary aneurysm, +3.6 Z-score). Patient 10 was a toddler who developed decreased consciousness, hypotension and clinical signs of hypoperfusion consistent with KD shock syndrome on day 3 of fever. Transfer to the paediatric intensive care unit was required and vasoactive support (for 48 hours) and non-invasive mechanical ventilation (for 3 days) were implemented, together with intravenous immunoglobulins (IVIG), methylprednisolone, hydroxychloroquine and azithromycin.

All patients were treated within 10 days of symptoms onset; only patient 12, with a self-limited incomplete KD, did not receive any immunomodulatory treatment. Median (range) duration of admission was 4 (2–12) days and all patients were discharged without incidents. Follow-up is ongoing in all cases.

We describe a higher than expected incidence of KD within a very short time frame (7 weeks) in Catalonia, compared with a historical series,[8] with half of the cases being associated with SARS-CoV-2 infection, in line with the experience of other authors.[2–5] Surprisingly, SARS-CoV-2-related KD cases were not reported in China, where the pandemic began and where the incidence of KD (40.9–55.1 per 100 000 children <5 years) is higher than in European countries.[9]

KD seems to be caused by a complex interaction between genetic and immunity factors, triggered by infections.[6 10] Several pathogens have been found to be involved in the pathogenesis of KD, including coronaviruses.[11] As compared with 'classical' KD, SARS-CoV-2-related KD cases differ in several clinical characteristics: patients are older[2–5]; present more often with respiratory, gastrointestinal or neurological symptoms[3]; and develop a more severe disease in terms of cardiovascular involvement.[3–5] The incidence of coronary aneurysms in KD treated with IVIG ranges from 4% to 6%,[12] as compared with 25% in our series. Also, higher rates of leucopenia, lymphopenia and thrombocytopenia, as well as increased inflammation, have been reported.[1 3–5] Interestingly, in our series, significant differences in these lab values were observed between patients with and those without confirmed SARS-CoV-2 infection. While preliminary, these differences point at an association between SARS-CoV-2 infection and the pathogenesis of KD, beyond the temporal sequence.

Given the lack of evidence-based treatments for COVID-19, we treated the patients in our series according to available KD treatment guidelines,[6] mainly IVIG (n=10) and steroids (n=6). Outcomes were good in all cases. We did not need to use other immunomodulatory drugs, such as anakinra or infliximab, as other authors have reported doing.[2–5]

Our study is limited by low numbers, the short follow-up period and its observational design. Nevertheless, our series is in line with recent observational data that describe an association between SARS-CoV-2 infection and a paediatric inflammatory multisystem syndrome that shares a number of clinical and analytical features with KD in children. Further studies are needed to confirm the more pronounced inflammatory response we observed in those cases in which a SARS-CoV-2 infection was demonstrated.

Rosa Pino,[1] Ana Carolina Izurieta,[1] María Ríos-Barnés,[2] Sílvia Ricart,[1,3] Mariona F De Sevilla,[1,4] Laura Monfort,[1] Cristian Launes,[1,4] Iván Cano,[1] Laura Lecina,[1] Judith Sánchez Manubens,[5,6] Juan Manuel Mosquera,[5] Iolanda Jordan,[4,7] Joan Sánchez de Toledo,[8,9] Manuel Monsonís,[10] Cristina Esteva,[4,11] Carmen Muñoz-Almagro,[4,11] Victòria Fumadó,[2,3] Clàudia Fortuny,[2,4] Juan José Garcia Garcia,[2,4] Antoni Noguera-Julian ,[2,4] Jordi Anton,[3,5] On behalf of the Kids Corona Project

[1]Hospital Medicine Unit, Pediatrics Department, Hospital Sant Joan de Déu, Barcelona, Spain
[2]Infectious Diseases and Systemic Inflammatory Response in Pediatrics, Infectious Diseases Unit, Hospital Sant Joan de Déu, Barcelona, Spain
[3]Faculty of Medicine, Universitat de Barcelona, Barcelona, Spain
[4]CIBERESP, Madrid, Spain
[5]Pediatric Rheumatology Unit, Hospital Sant Joan de Déu, Barcelona, Spain
[6]Pediatric Rheumatology, Consorcio Corporación Sanitaria Parc Taulí, Sabadell, Barcelona, Spain
[7]Pediatric Intensive Care Department, Hospital Sant Joan de Déu, Barcelona, Spain
[8]Pediatric Cardiology Department, Hospital Sant Joan de Déu, Barcelona, Spain
[9]Department of Critical Care Medicine, Children's Hospital of Pittsburgh of University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA
[10]Department of Microbiology, Hospital Sant Joan de Déu, Barcelona, Spain
[11]Department of Molecular Microbiology, Hospital Sant Joan de Déu, Barcelona, Spain

Correspondence to Professor Antoni Noguera-Julian, Hospital Sant Joan de Déu, Passeig Sant Joan de Déu 2, 08950 Esplugues, Barcelona, Spain; ton@sjdhospitalbarcelona.org

Contributors RP, IJ, VF, CF, JJGG, ANJ and JA designed the study. RP, ACI, MRB, SR, MFDS, LM, CL, IC, LL, JSM, JMM, JSdT and VF collected the clinical data. MM, CE and CMA supervised the microbiological studies. RP, ANJ and JA analysed the data and wrote the paper. All authors have reviewed and approved the version submitted for publication.

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ORCID iD
Antoni Noguera-Julian http://orcid.org/0000-0001-7485-0583

REFERENCES

1 Pouletty M, Borocco C, Ouldali N, *et al*. Paediatric multisystem inflammatory syndrome temporally associated with SARS-CoV-2 mimicking Kawasaki disease (Kawa-COVID-19): a multicentre cohort. *Ann Rheum Dis* 2020;79:999–1006.
2 Verdoni L, Mazza A, Gervasoni A, *et al*. An outbreak of severe Kawasaki-like disease at the Italian epicentre of the SARS-CoV-2 epidemic: an observational cohort study. *The Lancet* 2020;395:1771–8.
3 Whittaker E, Bamford A, Kenny J, *et al*. Clinical characteristics of 58 children with a pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2. *JAMA* 2020. doi:10.1001/jama.2020.10369. [Epub ahead of print: 08 Jun 2020].
4 Feldstein LR, Rose EB, Horwitz SM, *et al*. Multisystem inflammatory syndrome in U.S. children and adolescents. *N Engl J Med* 2020. doi:10.1056/NEJMoa2021680. [Epub ahead of print: 29 Jun 2020].
5 Dufort EM, Koumans EH, Chow EJ, *et al*. Multisystem inflammatory syndrome in children in New York state. *N Engl J Med* 2020. doi:10.1056/NEJMoa2021756. [Epub ahead of print: 29 Jun 2020].
6 McCrindle BW, Rowley AH, Newburger JW, *et al*. Diagnosis, treatment, and long-term management of Kawasaki disease: a scientific statement for health professionals from the American heart association. *Circulation* 2017;135:927–99.
7 Gao L, Jiang D, Wen X-S, *et al*. Prognostic value of NT-proBNP in patients with severe COVID-19. *Respir Res* 2020;21:83.
8 Sánchez-Manubens J, Antón J, Bou R, *et al*. Incidence, epidemiology and clinical features of Kawasaki disease in Catalonia, Spain. *Clin Exp Rheumatol* 2016;34:S139–44.
9 Du Z-D, Zhao D, Du J, *et al*. Epidemiologic study on Kawasaki disease in Beijing from 2000 through 2004. *Pediatr Infect Dis J* 2007;26:449–51.
10 Yim D, Curtis N, Cheung M, *et al*. Update on Kawasaki disease: epidemiology, aetiology and pathogenesis. *J Paediatr Child Health* 2013;49:704–8.
11 Esper F, Shapiro ED, Weibel C, *et al*. Association between a novel human coronavirus and Kawasaki disease. *J Infect Dis* 2005;191:499–502.
12 Newburger JW, Takahashi M, Burns JC, *et al*. The treatment of Kawasaki syndrome with intravenous gamma globulin. *N Engl J Med* 1986;315:341–7.

Response to: 'Correspondence on 'Paediatric multisystem inflammatory syndrome temporally associated with SARS-CoV-2 mimicking Kawasaki disease (Kawa-COVID-19): a multicentre cohort' by Pouletty *et al*' by Pino *et al*

In their correspondence, Pino *et al*[1] reported a cohort of 12 children with Kawasaki disease (KD) during the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) epidemic in Barcelona, Spain. Among them, six had a positive SARS-CoV-2 infection confirmed by RT-PCR or serology while six had not. Interestingly, in line with our findings[2] and reports from other settings,[3–7] patients with multisystem inflammatory syndrome temporally associated with SARS-CoV-2 infection mimicking KD (Kawa-COVID-19) exhibited several differences as compared with classical KD, such as older age, higher inflammatory parameters, more frequent cytopenia and cardiac involvement, including myocarditis, often requiring haemodynamic support.[1 2] These important discrepancies led to consider Kawasaki syndrome associated with SARS-CoV-2 infection as a distinct entity (Kawa-COVID-19,[2] or multisystem inflammatory syndrome in children (MIS-C) associated with COVID-19,[8] or or paediatric inflammatory multisystem syndrom temporally associated with SARS-CoV-2 (PIMS-TS[9]). However, the possibility of a common pathway shared with classic KD has led to administer similar therapeutics to KD, including intravenous immunoglobulins (IVIG) and corticosteroids.[3–7] If a substantial proportion of children were resistant to the first dose of IVIG, the large majority had a favourable short-term evolution with a second dose of IVIG±corticosteroids, as described by Pino *et al* and in our cohort.[1 2]

The prognosis of KD is graved by its cardiac involvement,[10] especially with coronary aneurysms, which are specific of KD and could occur several weeks after onset of disease. Therefore, a close surveillance is recommended during the months following KD diagnosis.[10] Although only dilatations without aneurysms have been described at diagnosis by Pino *et al* and in our study, such complications have been described elsewhere in Kawa-COVID-19.[3 11] This coronary involvement may be more frequent in patients with first-line IVIG resistance,[10 12] raising concerns on the evolution of children with Kawa-COVID-19. To date, the middle-term evolution of these patients is unknown.

In table 1, we described the clinical, biological and cardiac evolution of eight children, who developed a Kawa-COVID-19 in our tertiary hospital located in Paris, France. SARS-CoV-2 infection was confirmed in all of them either by nasopharyngeal SARS-CoV-2 RT-PCR or by SARS-CoV-2 serology (table 1). They had initial severe presentation with six myocarditis and required haemodynamic support in five cases. One month after the diagnosis, clinical and biological assessments were normal in all cases, without any persistent inflammatory syndrome, and all had normal cardiac ultrasounds (table 1).

These preliminary findings need to be confirmed with larger multicentre cohorts and a more prolonged follow-up, but suggest that despite an initial severe presentation with potentially life threatening cardiac involvement, the middle-term evolution of this specific entity may be reassuring. Finally, one of the main challenges of Kawa-COVID-19 may be the need for a long-term follow-up and cardiac assessment to better evaluate incidence and risk factors of coronary involvement and/or other cardiac dysfunctions and maybe deciphering physiological pathways responsible for this specific organ failure.

Naim Ouldali,[1,2,3] Marie Pouletty,[1,2] Johanna Lokmer,[4] Cherine Benzouid,[4] Constance Beyler,[4] Anna Deho,[5] Ulrich Meinzer,[1,2,6,7] Albert Faye,[1,2,3] Isabelle Melki ,[1,2,8,9] Great Paris Region (GPR) Kawa-COVID-19 consortium

[1]General Paediatrics, Department of Infectious Disease and Internal Medicine, Robert Debré University Hospital, AP-HP, Paris, France, Reference centre for Rheumatic, AutoImmune and Systemic diseases in children (RAISE), Paris, France
[2]Université de Paris, UFR de Médecine Paris Nord, Paris, France
[3]INSERM UMR 1123, ECEVE, Paris, France
[4]Cardiopaediatric Unit, Robert Debré University Hospital, AP-HP, Paris, France
[5]Paediatric Intensive Care Unit, Robert Debré University Hospital, AP-HP, Paris, France
[6]Center for Research on Inflammation, INSERM, UMR1149, Paris, France
[7]Biology and Genetics of Bacterial Cell Wall Unit, Pasteur Institute, Paris, France
[8]Laboratory of Neurogenetics and Neuroinflammation, Imagine Institute, Paris, France

Table 1 Evolution of children with Kawa-COVID-19 1 month after disease onset in one Great Paris Region tertiary centre, n=8

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
Age (years)	15	12	11	11	13	6	14	10
Sex	Male	Female	Male	Female	Female	Male	Female	Female
Type of Kawasaki disease	Incomplete	Incomplete	Complete	Complete	Complete	Incomplete	Complete	Incomplete
SARS-CoV-2 nasopharyngeal RT-PCR	Negative	Positive	Positive	Positive	Positive	Negative	Positive	Negative
SARS-CoV-2 serology	IgG+	IgG+	IgG+	Negative	IgG+	IgG+	IgG+	IgG+
Cardiac involvement	Myocarditis and coronary dilatation (Z score=4)	None	None	Myocarditis	Myocarditis and coronary dilatation (Z score=4)	Myocarditis	Myocarditis	Myocarditis
Haemodynamic support	Yes	No	No	No	Yes	Yes	Yes	Yes
Ferritinaemia at diagnosis (microG/L)	1221	2500	768	118	1208	222	207	917
Maximal CRP level (mg/L)	309	258	179	119	352	369	316	444
Treatments	IVIG +mPDN	IVIG +mPDN	No	IVIG	IVIG +tocilizumab	IVIG +mPDN	IVIG	IVIG +mPDN
Evolution after 1 month								
Clinical assessment	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
CRP level (mg/L)	<10	<10	<10	<10	<10	<10	<10	<10
Cardiac ultrasound	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal

CRP, C-reactive protein; IVIG, intravenous immunoglobulins; mPDN, methylprednisolone; RT-PCR, real-time PCR; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

[9]Paediatric Hematology-Immunology and Rheumatology Department, Necker-Enfants-Malades University Hospital, AP-HP, Reference centre for Rheumatic, AutoImmune and Systemic diseases in children (RAISE), Paris, France

Correspondence to Dr Isabelle Melki, Laboratory of Neurogenetics and Neuroinflammation, Imagine Institute, Paris F-75019, France; isabelle.melki@aphp.fr

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Collaborators Great Paris Region (GPR) Kawa-COVID-19 consortium: Charlotte Borocco, M.D. (Department of Pediatric Rheumatology, Hôpital Bicêtre, AP-HP, Reference centre for Autoinflammatory diseases and amyloidosis (CEREMAIA), Le Kremlin-Bicêtre, France), Marion Caseris M.D. (General Paediatrics, Department of Infectious Disease and Internal Medicine, Robert Debré University Hospital, AP-HP, Paris, France, Reference centre for Rheumatic, AutoImmune and Systemic diseases in children (RAISE), Paris, France, Department of Microbiology, Robert Debré University Hospital, AP-HP, Paris, France), Romain Basmaci M.D., Ph.D. (Departments of General Paediatrics and Paediatric Emergency, Louis-Mourier Hospital, AP-HP, Colombes, France, Infection-Antimicrobials-Modelling-Evolution IAME, INSERM, UMR-1137, Université de Paris, 75018, Paris, France), Noémie Lachaume M.D. (Departments of General Paediatrics and Paediatric Emergency, Louis-Mourier Hospital, AP-HP, Colombes, France, Infection-Antimicrobials-Modelling-Evolution IAME, INSERM, UMR-1137, Université de Paris, 75018, Paris, France), Philippe Bensaid M.D. (Department of General Paediatrics, Victor Dupouy Hospital, Argenteuil, France), Samia Pichard M.D. (Department of General Paediatrics, Victor Dupouy Hospital, Argenteuil, France), Hanane Kouider M.D. (Department of General Paediatrics, René Dubos, Pontoise Hospital, France), Guillaume Morelle M.D. (Department of General Paediatrics Hôpital Bicêtre, AP-HP, Kremlin-Bicêtre, France), Irina Craiu M.D. (Paediatric emergency Department, Hôpital Bicêtre, AP-HP, Kremlin-Bicêtre, France), Corinne Pondarre M.D., Ph.D.(Sickle cell disease referal center, Centre hospitalier Intercommunal de Créteil, INSERM U955, Paris XII University), Arielle Maroni M.D. (Université de Paris, UFR de Médecine Paris Nord, 75010 Paris, France, Paediatric Intensive Care Unit, Robert Debré University Hospital, AP-HP, Paris, France), Mehdi Oualha M.D., Ph.D. (Paediatric Intensive Care Unit, Necker-Enfants-Malades University Hospital, AP-HP, Paris, France), Zahir Amoura M.D., M.Sc.(Sorbonne Université, Inserm UMR-S 1135, Department of Immunology and Infectious disease (CIMI-Paris), Pitié-Salpêtrière Hospital, AP-HP, Paris, France), Julien Haroche M.D., Ph.D. ((Sorbonne Université, Inserm UMR-S 1135, Department of Immunology and Infectious disease (CIMI-Paris), Pitié-Salpêtrière Hospital, AP-HP, Paris, France)), Juliette Chommeloux M.D. (Medical Intensive Care Unit, Institut de Cardiologie, AP-HP, Sorbonne University, Pitié-Salpêtrière Hospital, Paris, France), Fanny Bajolle M.D., Ph.D.(Cardiopaediatric Unit, M3-C Necker-Enfants-Malades University Hospital, AP-HP, Paris, France), Stéphane Bonacorsi M.D., Ph.D. (Université de Paris, UFR de Médecine Paris Nord, 75010 Paris, France, Department of Microbiology, Robert Debré University Hospital, AP-HP, Paris, France, Infection-Antimicrobials-Modelling-Evolution IAME, INSERM, UMR-1137, Université de Paris, 75018, Paris, France), Guislaine Carcelain M.D., Ph.D. (Department of Immunology, Robert Debré University Hospital, AP-HP, Paris, France), Isabelle Kone-Paut M.D. (Department of Pediatric Rheumatology, Hôpital Bicêtre, AP-HP, Reference centre for Autoinflammatory diseases and amyloidosis (CEREMAIA), Le Kremlin-Bicêtre, France, Université de Paris Sud Saclay), Brigitte Bader-Meunier M.D. (Paediatric Hematology-Immunology and Rheumatology Department, Necker-Enfants-Malades University Hospital, AP-HP, Paris, France, Reference centre for Rheumatic, AutoImmune and Systemic diseases in children (RAISE), Paris, France, Laboratory of Immunogenetics of paediatric autoimmune diseases, INSERM UMR 1163, Paris, France), Caroline Galeotti M.D., Ph.D. (Department of Pediatric Rheumatology, Hôpital Bicêtre, AP-HP, Reference centre for Autoinflammatory diseases and amyloidosis (CEREMAIA), Le Kremlin-Bicêtre, France, Laboratory of Immunogenetics of paediatric autoimmune diseases, INSERM UMR 1163, Paris, France).

Contributors MP, NO, AF, UM and IM designed the study. MP, NO, JL, CBen, CBey, AD and IM collected clinical data. MP, NO, AF and IM analysed data. MP, CB, CG and IM wrote the paper. JL, CBen, CBey, AD, UM, AF and IM supervised the study. All authors have read final approval of the version published.

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ORCID iD
Isabelle Melki http://orcid.org/0000-0002-8057-333X

REFERENCES

1 Pino R, Izurieta A, Rios-Barnes M, *et al*. Correspondence on 'Paediatric multisystem inflammatory syndrome temporally associated with SARS-CoV-2 mimicking Kawasaki disease (Kawa COVID-19): a multicentre cohort' by Pouletty *et al*. *Ann Rheum Dis* 2022;81:e158.
2 Pouletty M, Borocco C, Ouldali N, *et al*. Paediatric multisystem inflammatory syndrome temporally associated with SARS-CoV-2 mimicking Kawasaki disease (Kawa-COVID-19): a multicentre cohort. *Ann Rheum Dis* 2020;79:999–1006.
3 Whittaker E, Bamford A, Kenny J, *et al*. Clinical characteristics of 58 children with a pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2. *JAMA* 2020. doi:10.1001/jama.2020.10369. [Epub ahead of print: 08 Jun 2020].
4 Verdoni L, Mazza A, Gervasoni A, *et al*. An outbreak of severe Kawasaki-like disease at the Italian epicentre of the SARS-CoV-2 epidemic: an observational cohort study. *Lancet* 2020;395:1771–8.
5 Ouldali N, Pouletty M, Mariani P, *et al*. Emergence of Kawasaki disease related to SARS-CoV-2 infection in an epicentre of the French COVID-19 epidemic: a time-series analysis. *Lancet Child Adolesc Health* 2020.
6 Toubiana J, Poirault C, Corsia A, *et al*. Kawasaki-like multisystem inflammatory syndrome in children during the covid-19 pandemic in Paris, France: prospective observational study. *BMJ* 2020;369:m2094.
7 Feldstein LR, Rose EB, Horwitz SM, *et al*. Multisystem inflammatory syndrome in U.S. children and adolescents. *N Engl J Med* 2020;383:334–46.
8 Centers for Disease Control and Prevention. Centers for disease control and prevention. emergency preparedness and response: multisystem inflammatory syndrome in children (MIS-C) associated with coronavirus disease 2019 (COVID-19), 2020. Available: https://emergency.cdc.gov/han/2020/han00432.asp
9 European Centre for Disease Prevention and Control. Rapid risk assessment: paediatric inflammatory multisystem syndrome and SARS -CoV-2 infection in children, 2020. Available: https://www.ecdc.europa.eu/en/publications-data/paediatric-inflammatory-multisystem-syndrome-and-sars-cov-2-rapid-risk-assessment
10 McCrindle BW, Rowley AH, Newburger JW, *et al*. Diagnosis, treatment, and long-term management of Kawasaki disease: a scientific statement for health professionals from the American heart association. *Circulation* 2017;135:e927-e999.
11 Dufort EM, Koumans EH, Chow EJ, *et al*. Multisystem inflammatory syndrome in children in New York state. *N Engl J Med* 2020;383:347–58.
12 Wallace CA, French JW, Kahn SJ, *et al*. Initial intravenous gammaglobulin treatment failure in Kawasaki disease. *Pediatrics* 2000;105:E78.

Hydroxychloroquine ineffective for COVID-19 prophylaxis in lupus and rheumatoid arthritis

The viewpoint of Graef *et al* resonates more each day.[1] In a pandemic where the cries for certainty were met with a flow of mixed early study results, they admonish *festina lente ('make haste slowly')*! Since Graef, there have been many studies of hydroxychloroquine (HCQ) for treating COVID-19. These include a randomised controlled trial of 150 mild-to-moderate patients and three large observational studies, all inpatient studies that failed to show benefit of HCQ treatment for COVID-19.[2–5] Now a new inpatient study, with >80% administered HCQ within 24 hours, finds HCQ associated with substantial mortality reduction.[6] *Festina lente* indeed! A look at HCQ as prophylaxis, where its long half-life can be leveraged, may help.[7]

Bozzalla Cassione and colleagues described a northern Italian cohort of 165 patients with systemic lupus erythematosus (SLE).[8] HCQ users had 50% greater risk of COVID-19 (7.9% vs 5.3%; 95% CI for the difference −9.9% to 9.7%), but were limited by just 12 patients with COVID-19 and possible bias due to concomitant immunosuppressive therapy. A Belgian study of 225 patients with SLE found 7.9% of HCQ users and 8.2% of non-HCQ users had COVID-19 (95% CI for the difference −6.7% to 9.5%), and another Italian study of 914 rheumatologic patients found no preventive benefit for HCQ (0.89% vs 0.62%; 95% CI for the difference −0.84% to 4.28%).[9 10] These studies also had few cases (18 and 6) and possible confounding of immunosuppressive therapy. These three studies convincingly prove that HCQ users get COVID-19. However, they all lacked the sample size for meaningful CIs and could not rule out a strong preventive effect for HCQ. We employed a different methodology that accesses a larger population and expands the cohort to include both patients with SLE and rheumatoid arthritis (RA). This substantially increased the sample size despite only including patients on immunosuppressive therapy to minimise patient heterogeneity in sequestering behaviour and prioritisation for virus testing. If HCQ is effective prophylaxis, then the proportion of patients with SLE/RA on immunosuppressants using HCQ should be less for COVID-19 cases than for the general population.

We queried the commonly used TriNetX Research Network, a federated health research network that aggregates electronic health records from 36 US healthcare organisations (HCOs). Queries return population counts ≥10 patients. We included patients ≥18 years old with SLE or RA and a prescription for an immunosuppressant, diagnosed with COVID-19 since 20 January 2020. An outpatient encounter during the prior year was required to increase sensitivity of diagnoses and prescriptions. We then determined the proportion prescribed HCQ in the prior year. SLE/RA diagnoses and prescriptions were within the year preceding index diagnosis. With 90-day prescriptions and three refills common, many patients get one prescription per year, so only one prescription was required for HCQ or immunosuppressants.

We considered two control groups for the year prior to the COVID-19 study period[1]: patients diagnosed with influenza/pneumonia/other lower respiratory infection (I/P/LRI), as a group with similar symptoms, and[2] everyone with an outpatient visit (OP). Diagnoses were based on ICD-10 codes and prescriptions were identified using the Veterans Affairs Drug Classification System. Data were accessed on 13 July 2020.

A total of 159 patients with COVID-19 met criteria, 22.0% SLE and 80.5% RA (four diagnosed with both) (table 1). Also, 18.9% were hospitalised on day of diagnosis. This compared with 2609 I/P/LRI (22.5% hospitalised) and 32 599 OP. The proportion taking HCQ was similar for COVID-19 and I/P/LRI (34.6% vs 31.4%; CI for difference −4.4% to 10.8%; Fisher's exact test p=0.4290) and OP (34.6% vs 32.7%; CI for difference −5.5% to 9.4%; Fisher's exact test p=0.6115). Hypothesis that HCQ provides 25% protection was rejected versus I/P/LRI (p=0.0098) and OP (p=0.0252). To check if HCQ users only used HCQ for treating COVID-19 symptoms prior to diagnosis, we reran the analysis excluding HCQ prescriptions ≤14 days before COVID-19 diagnosis. This eliminated two HCQ users, as expected for refills in a 14-day period. We reran the analysis for patients under 65. Proportions using HCQ were even more similar: 37.2%, 37.0% and 36.9% for COVID-19, I/P/LRI and OP, respectively.

HCQ was not associated with COVID-19 prevention. A strength of this study is all patients were on an immunosuppressant, with similar high-risk status for COVID-19 regardless of HCQ use. A limitation is that a few patients might be misclassified as non-HCQ users if they had an immunosuppressant prescription in the HCO but filled their HCQ prescription(s) outside that HCO. While we only had data for prescriptions written, prescriptions filled and medications taken reflect real world adherence.

Our results suggest HCQ lacks *in vivo* activity against SARS-CoV-2, which might help explain seemingly contradictory treatment studies. Without antiviral activity, the success or failure of HCQ in treatment is likely due to immunomodulation, anti-inflammatory and anti-thrombotic effects, which may be more

Table 1 Proportion of patients taking hydroxychloroquine: COVID-19 vs two control groups

	COVID-19	Influenza/ pneumonia/LRI	CI for difference (COVID-19 vs I/P/LRI)	Any outpatient visit	CI for difference (COVID-19 vs OP)
Age 18 and over					
No of patients, N	16 869	198 114		3 970 695	
Lupus or RA on an immunosuppressant, N	159	2609		32 599	
Hydroxychloroquine, % (N)	34.6% (55)	31.4% (819)	(−4.4% to 10.8%) p=0.4290	32.7% (10 645)	(−5.5% to 9.4%) p=0.6115
Age 18–64					
No of patients, N	13 327	128 280		2 715 365	
Lupus or RA on an immunosuppressant, N	121	1477		20 256	
Hydroxychloroquine, % (N)	37.2% (45)	37.0% (547)	(−8.8% to 9.1%) p=1.0000	36.9% (7473)	(−8.3% to 8.9%) p=1.0000

I/P/LRI, influenza/pneumonia/other lower respiratory infection; LRI, lower respiratory infection; OP, outpatient; RA, rheumatoid arthritis.

beneficial earlier. Further, more severely ill patients may be especially vulnerable to HCQ's cardiotoxicity. Or maybe it's too soon to make conclusions. *Festina lente!*

Mendel E Singer ,[1] **David C Kaelber**,[1,2] **Maria J Antonelli**[3]

[1]Population and Quantitative Health Sciences, Case Western Reserve University School of Medicine, Cleveland, Ohio, USA
[2]Internal Medicine and Pediatrics, The MetroHealth System, Cleveland, Ohio, USA
[3]Internal Medicine and Rheumatology, Case Western Reserve University at The MetroHealth System, Cleveland, Ohio, USA

Correspondence to Prof Mendel E Singer, Population and Quantitative Health Sciences, Case Western Reserve University School of Medicine, Cleveland, OH 44106, USA; mendel@case.edu

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ORCID iD
Mendel E Singer http://orcid.org/0000-0002-3331-3340

REFERENCES

1 Graef ER, Liew JW, Putman MS, *et al*. *Festina lente*: hydroxychloroquine, COVID-19 and the role of the rheumatologist. *Ann Rheum Dis* 2020;79:734–6.
2 Tang W, Cao Z, Han M, *et al*. Hydroxychloroquine in patients mainly with mild to moderate COVID-19: an open-label, randomized, controlled trial. *BMJ* 2020:369.
3 Geleris J, Sun Y, Platt J, *et al*. Observational study of hydroxychloroquine in hospitalized patients with Covid-19. *N Engl J Med* 2020;382:2411–8.
4 Rosenberg ES, Dufort EM, Udo T, *et al*. Association of treatment with hydroxychloroquine or azithromycin with in-hospital mortality in patients with COVID-19 in New York state. *JAMA* 2020. doi:10.1001/jama.2020.8630. [Epub ahead of print: 11 May 2020].
5 Magagnoli J, Narendran S, Pereira F, *et al*. Outcomes of hydroxychloroquine usage in United States veterans hospitalized with Covid-19. *medRxiv* 2020. doi:10.1016/j.medj.2020.06.001. [Epub ahead of print: 21 Apr 2020].
6 Arshad S, Kilgore P, Chaudhry ZS, *et al*. Treatment with hydroxychloroquine, azithromycin, and combination in patients hospitalized with COVID-19. *Int J Infect Dis* 2020;97:396–403.
7 FDA approved drug products: hydroxychloroquine oral tablets. Available: https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/009768Orig1s051lbl.pdf [Accessed 8 Jul 2020].
8 Bozzalla Cassione E, Zanframundo G, Biglia A, *et al*. COVID-19 infection in a northern-Italian cohort of systemic lupus erythematosus assessed by telemedicine. *Ann Rheum Dis* 2020;79:1382–3.
9 Gendebien Z, von Frenckell C, Ribbens C, *et al*. Systematic analysis of COVID-19 infection and symptoms in a systemic lupus erythematosus population: correlation with disease characteristics, hydroxychloroquine use and immunosuppressive treatments. *Ann Rheum Dis* 2021;80:e94.
10 Favalli EG, De Lucia O, Biggioggero M, *et al*. Role of antimalarials in COVID-19: observational data from a cohort of rheumatic patients. *Ann Rheum Dis* 2021;80:e75.

Response to: 'Hydroxychloroquine ineffective for COVID-19 prophylaxis in lupus and rheumatoid arthritis' by Singer *et al*

We thank Singer *et al* for their correspondence[1] about our article related to hydroxychloroquine (HCQ) use, COVID-19 and rheumatology.[2] The authors present an interesting analysis using electronic health records from 36 US healthcare organisations, including patients with systemic lupus erythematosus (SLE) or rheumatoid arthritis (RA). They found no association of HCQ use versus non-use with COVID-19, influenza/pneumonia/other lower respiratory infections and any outpatient visit, suggesting that baseline use of antimalarials such as HCQ does not prevent COVID-19.

These results suggesting no prophylactic benefit for antimalarials complement findings from the physician-based registry of the COVID-19 Global Rheumatology Alliance (GRA). Among 80 patients with SLE and COVID-19 in the GRA registry, the rates of hospitalisation and requirement of supplemental oxygen were similar in those who were using antimalarials prior to the onset of COVID-19 and those who were not.[3] In the entire registry, which included 600 patients with systemic rheumatic disease (RMD) at that time, antimalarials were not associated with lower odds of hospitalisation after adjustment for age, sex, smoking, underlying RMD, comorbidities, use of glucocorticoids, non-steroidal anti-inflammatory drugs, or conventional, biological and targeted synthetic disease-modifying antirheumatic drugs (DMARDs) in monotherapy or in combination (excluding antimalarials).[4] Their results are also consistent with a recent trial showing that HCQ did not reduce COVID-19 risk compared with placebo when given prophylactically.[5] This lack of efficacy of HCQ prophlyaxis for COVID-19 in humans—despite encouraging results from in vitro studies—could be related to differences in dosing as well as viral replication mechanisms in vitro versus in vivo.[6] Moreover, we previously outlined a pharmacokinetic rationale why HCQ, at doses prescribed for the treatment of RMD, is unlikely to result in meaningful blood levels to inhibit viral replication.[3]

Patients with systemic RMD had similar rates of COVID-19 compared with the general population according to several reports.[7–9] In contrast, patients with RMD had a higher prevalence of PCR-confirmed COVID-19 compared with the reference population (0.76% vs 0.58%, OR=1.32, 95% CI 1.15 to 1.52) in a Spanish study.[10] However, that study only identified patients presenting to emergency departments, and patients with a milder disease were not included.[10]

Additionally, RMDs have been associated with a slightly increased risk of mortality due to COVID-19 in a large analysis of primary care records of more than 17 million adults.[11] That study found that a composite variable of RA/SLE/psoriasis was associated with an increased risk of death (HR=1.19, 95% CI 1.11 to 1.27) compared with absence of these diseases.[11]

These studies suggest that patients with RMDs may have a moderately increased risk of mortality due to COVID-19 and that antimalarials neither prevent severe acute respiratory syndrome coronavirus 2 infection nor reduce its severity. Whether the modest increase in COVID-19 mortality is due to the underlying RMD, associated with specific immunosuppressant use, or related to unmeasured risk factors (eg, accelerated cardiovascular disease or pulmonary damage from disease manifestations) currently remains uncertain. Further studies identifying disease-specific and DMARD-specific risks are needed to define the best approach for the prevention and management of COVID-19 in patients with systemic RMDs. In this regard, the real-world data provided by Singer and colleagues help provide a clearer picture of the long-lasting HCQ debate.

Manuel Francisco Ugarte-Gil ,[1] **Maximilian F Konig** ,[2] **Peter Korsten** ,[3] **Francis Berenbaum** ,[4] **Alfred Hyoungju Kim** ,[5] **Jeffrey A Sparks** [6]

[1]Department of Rheumatology, Universidad Cientifica del Sur, Lima, Peru
[2]Division of Rheumatology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA
[3]Department of Nephrology and Rheumatology, University Medical Center Göttingen, Gottingen, Germany
[4]Rheumatology, Sorbonne Université, Paris, France
[5]Medicine/Rheumatology, Washington University in Saint Louis School of Medicine, St. Louis, Missouri, USA
[6]Department of Medicine, Division of Rheumatology, Inflammation, and Immunity, Brigham and Women's Hospital, Boston, Massachusetts, USA

Correspondence to Dr Jeffrey A Sparks, 1Department of Medicine, Division of Rheumatology, Inflammation, and Immunity, Brigham and Women's Hospital, Boston, MA 02115, USA; jsparks@bwh.harvard.eduDr Alfred Hyoungju Kim; akim@wustl.edu

Handling editor Josef S Smolen

Twitter Manuel Francisco Ugarte-Gil @mugartegil, Maximilian F Konig @MaxKonigMD, Peter Korsten @pekor002, Francis Berenbaum @larhumato, Alfred Hyoungju Kim @alhkim and Jeffrey A Sparks @jeffsparks

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ORCID iDs
Manuel Francisco Ugarte-Gil http://orcid.org/0000-0003-1728-1999
Maximilian F Konig http://orcid.org/0000-0001-5045-5255
Peter Korsten http://orcid.org/0000-0001-6065-5680
Francis Berenbaum http://orcid.org/0000-0001-8252-7815
Alfred Hyoungju Kim http://orcid.org/0000-0003-4074-0516
Jeffrey A Sparks http://orcid.org/0000-0002-5556-4618

REFERENCES

1 Singer M, Kaelber D, Antonelli M. Hydroxychloroquine ineffective for COVID-19 prophylaxis in lupus and rheumatoid arthritis. *Ann Rheum Dis* 2022;81:e161.
2 Graef ER, Liew JW, Putman MS, *et al*. *Festina lente*: hydroxychloroquine, COVID-19 and the role of the rheumatologist. *Ann Rheum Dis* 2020;79:734–6.
3 Konig MF, Kim AH, Scheetz MH, *et al*. Baseline use of hydroxychloroquine in systemic lupus erythematosus does not preclude SARS-CoV-2 infection and severe COVID-19. *Ann Rheum Dis* 2020;79:1386–8.
4 Gianfrancesco M, Hyrich KL, Al-Adely S, *et al*. Characteristics associated with hospitalisation for COVID-19 in people with rheumatic disease: data from the COVID-19 global rheumatology alliance physician-reported registry. *Ann Rheum Dis* 2020;79:859–66.
5 Boulware DR, Pullen MF, Bangdiwala AS, *et al*. A randomized trial of hydroxychloroquine as postexposure prophylaxis for Covid-19. *N Engl J Med* 2020. doi:10.1056/NEJMoa2016638. [Epub ahead of print: 03 Jun 2020].
6 Hoffmann M, Mösbauer K, Hofmann-Winkler H, *et al*. Chloroquine does not inhibit infection of human lung cells with SARS-CoV-2. *Nature* 2020.
7 Michelena X, Borrell H, López-Corbeto M, *et al*. Incidence of COVID-19 in a cohort of adult and paediatric patients with rheumatic diseases treated with targeted biologic and synthetic disease-modifying anti-rheumatic drugs. *Semin Arthritis Rheum* 2020;50:564–70.
8 Quartuccio L, Valent F, Pasut E, *et al*. Prevalence of COVID-19 among patients with chronic inflammatory rheumatic diseases treated with biologic agents or small molecules: a population-based study in the first two months of COVID-19 outbreak in Italy. *Joint Bone Spine* 2020. doi:10.1016/j.jbspin.2020.05.003. [Epub ahead of print: 20 May 2020].
9 Favalli EG, Monti S, Ingegnoli F, *et al*. Incidence of COVID-19 in patients with rheumatic diseases treated with targeted immunosuppressive drugs: what can we learn from observational data? *Arthritis Rheumatol* 2020. doi:10.1002/art.41388. [Epub ahead of print: 07 Jun 2020].
10 Pablos JL, Abasolo L, Alvaro-Gracia JM, *et al*. Prevalence of hospital PCR-confirmed COVID-19 cases in patients with chronic inflammatory and autoimmune rheumatic diseases. *Ann Rheum Dis* 2020;79:1170–3.
11 Williamson EJ, Walker AJ, Bhaskaran K, *et al*. OpenSAFELY: factors associated with COVID-19 death in 17 million patients. *Nature* 2020. doi:10.1038/s41586-020-2521-4. [Epub ahead of print: 08 Jul 2020].

Correspondence on '*Festina lente*: hydroxychloroquine, COVID-19 and the role of the rheumatologist' by Graef *et al*

We read with interest the study by Graef *et al*,[1] who mentioned about the treatment and safety of hydroxychloroquine (HCQ) for the current COVID-19 pandemic. They described that decades of research strongly support the well control of disease activity and survival benefit of HCQ use in rheumatic diseases, such as systemic lupus erythematosus and rheumatoid arthritis (RA). They also highlight that HCQ should be used with caution in patients with COVID-19, including the safety concern, especially when combined with administration of azithromycin because both of them are known corrected QT interval (QTc) prolongation agents.

During early outbreak, HCQ, combined with azithromycin, has been used as a treatment option for COVID-19.[2 3] Recently, an observational study with 1446 patients with COVID-19 reported that HCQ administration was not associated with a lower risk of intubation or death.[4] However, the reasons for mortality were not illustrated. Multiple confounding factors like histories of ischaemic heart disease, heart failure and cardiac arrhythmia were not well adjusted. The main functional site of HCQ in COVID-19 is the entry via ACE2 preceptor.[5 6] We believe that patient selection in early phases of COVID-19 infection would be more appropriate than well-established pneumonia or cytokines storm cases.

The risk of cardiac arrhythmias is an important safety issue. HCQ inhibits the 'funny' current of sinoatrial node and rapid component of the delayed rectifier potassium current, causing lengthening of the action potential and QTc prolongation, which results in potential life-threatening ventricular arrhythmias such as torsades de pointes.[7–10] Nevertheless, previous reports showed limited and inconsistent arrhythmia risk of HCQ treatment.[11 12]

We investigated a report to clarify whether HCQ increased the new onset of arrhythmia in patients with RA by using a large population-based dataset from the National Health Insurance Research Database in Taiwan from 1999 to 2013. We enrolled all people aged 20 years or more who were newly diagnosed with RA (International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM)=714.0)[13 14] with at least ≥3 outpatient clinic or once admission. Patients with previous arrhythmia history (ICD-9-CM=426–427) or usage of antiarrhythmic agents such as amiodarone, propafenone and dronedarone were excluded. The end point was set as the first cardiac arrhythmia or 1 year from the index date. To minimise the effect of confounding factors, we used propensity score matching (PSM) to obtain 1:1 ratio matched by age and gender, and comorbidities such as hypertension, hyperlipidaemia, chronic liver disease, chronic kidney disease, diabetes mellitus, chronic obstructive pulmonary disease, ischaemic heart disease, heart failure, stroke, history of β-blocker usage and antibiotic macrolide treatment. χ^2 test for categorical variables and independent t-test for continuous variables were used. The Kaplan-Meier method was applied to obtain the cumulative incidences of newly diagnosed arrhythmia and log-rank test to determine the significance. We used a Cox proportional hazard model to estimate crude HRs, adjusted HRs (aHRs) and 95% CIs between the two groups.

A total of 8564 patients with newly diagnosed RA were selected to participate in the study. We excluded 1559 patients who had arrhythmia before their RA diagnosis and those using antiarrhythmic agents. After PSM, 2111 patients were enrolled in both HCQ and non-HCQ groups, respectively (see online supplementary figure 1). Table 1 shows the baseline patient demographic and clinical characteristics. The mean age of the patients was 52.7 (SD 14.1) years in the HCQ group and 53.6 (SD 14.4) years in the non-HCQ group. The cumulative risk of arrhythmia was not significantly higher in the HCQ group than in the non-HCQ group (log-rank test, p=0.99) (figure 1).

The incidence of arrhythmia did not increase when a combination of HCQ with or without a macrolide antibiotic was taken (aHR 2.7, 95% CI 0.73 to 9.97, p=0.114). Age above or below 50 years, gender and β-blocker usage also did not increase the risk of arrhythmia in patients either using HCQ or not using HCQ (see online supplementary table 2). We found that the risk of arrhythmia for HCQ was not significantly different regardless of the daily dose of <400 mg (aHR 1.0, 95% CI 0.65 to 1.53) or ≥400 mg (aHR

Table 1 Demographic characteristics of patients in the HCQ and non-HCQ groups

	Before PSM					After PSM				
	HCQ (N=2112)		Non-HCQ (N=4834)			HCQ (N=2111)		Non-HCQ (N=2111)		
	n	%	n	%	P value	n	%	n	%	P value
Age (years)					<0.001					0.473
<50	872	41.3	1746	36.1		872	41.3	895	42.4	
≥50	1240	58.7	3088	63.9		1239	58.7	1216	57.6	
Mean±SD	52.8±14.1		55.8±14.8		<0.001	52.7±14.1		53.6±14.4		0.058
Gender					<0.001					0.514
Female	1607	76.1	3075	63.6		1606	76.1	1624	76.9	
Male	505	23.9	1759	36.4		505	23.9	487	23.1	
Hypertension	503	23.8	1528	31.6	<0.001	503	23.8	492	23.3	0.690
Hyperlipidaemia	318	15.1	726	15.0	0.967	318	15.1	314	14.9	0.863
Chronic liver disease	225	10.7	580	12.0	0.107	225	10.7	215	10.2	0.614
Chronic kidney disease	37	1.8	79	1.6	0.725	36	1.7	31	1.5	0.538
Diabetes mellitus	246	11.6	721	14.9	<0.001	246	11.7	257	12.2	0.601
COPD	132	6.3	404	8.4	0.002	132	6.3	89	4.2	0.003
Ischaemic heart disease	155	7.3	468	9.7	0.002	155	7.3	142	6.7	0.434
Stroke	86	4.1	299	6.2	<0.001	86	4.1	77	3.6	0.472
Heart failure	37	1.8	98	2.0	0.444	37	1.8	25	1.2	0.125
Macrolides	246	11.6	611	12.6	0.248	246	11.7	221	10.5	0.220
β blocker	368	17.4	1015	21.0	0.001	368	17.4	333	15.8	0.148

COPD, chronic obstructive pulmonary disease; HCQ, hydroxychloroquine; PSM, propensity score matching.

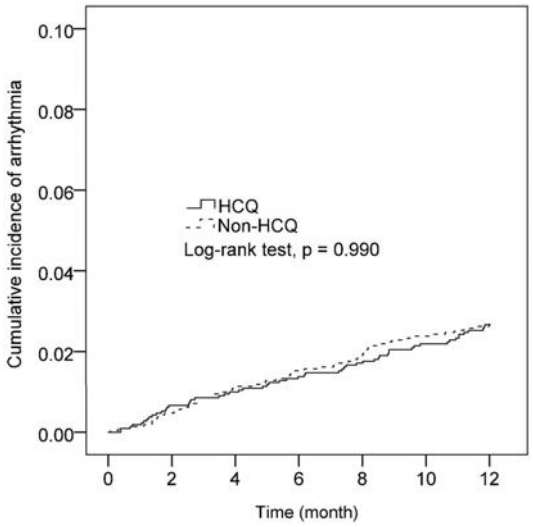

Figure 1 Cumulative incidence of cardiac arrhythmia between the HCQ group and the non-HCQ group (p value=0.99, log-rank test). HCQ, hydroxychloroquine.

0.85, 95% CI 0.52 to 1.4), and follow-up duration of <3 months (aHR 1.0, 95% CI 0.52 to 1.91) or ≥3 months (aHR 0.98, 95% CI 0.62 to 1.53) compared with non-HCQ usage (see online supplementary table 3).

This report may represent the first cohort study that used nationwide population-based data to assess the risk of arrhythmia with HCQ usage in patients with RA. The main results indicate that patients with RA using HCQ did not have a higher risk of cardiac arrhythmia. Larger daily HCQ dose, longer follow-up duration and combination therapy of HCQ with macrolides also did not increase any arrhythmia. Our result provides safety evidence of HCQ for rheumatic diseases. It may indirectly support the safety of HCQ therapy for other diseases such as COVID-19. Further prospective randomised controlled trial is required.

Chien Hsien Lo,[1,2] Yu-Hsun Wang,[3] Chin Feng Tsai,[1,2] Kuei Chuan Chan,[1,2] Li Ching Li,[4] Tse Hsien Lo,[4] Chun Hung Su ,[1,2] James Cheng-Chung Wei[5,6,7]

[1]Institute of Medicine, School of Medicine, Chung Shan Medical University, Taichung, Taiwan
[2]Division of Cardiology, Department of Internal Medicine, Chung Shan Medical University Hospital, Taichung, Taiwan
[3]Department of Medical Research, Chung Shan Medical University Hospital, Taichung, Taiwan
[4]Department of Internal Medicine, DaChien General Hospital, Miaoli, Taiwan
[5]Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan
[6]Division of Allergy, Immunology and Rheumatology, Chung Shan Medical University Hospital, Taichung, Taiwan
[7]Graduate Institute of Integrated Medicine, China Medical University, Taichung, Taiwan

Correspondence to Dr James Cheng-Chung Wei, Institute of Medicine, Chung Shan Medical University, Taichung 40201, Taiwan; wei3228@gmail.comDr Chun Hung Su; such197408@gmail.com

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ORCID iD
Chun Hung Su http://orcid.org/0000-0003-4003-008X

REFERENCES

1 Graef ER, Liew JW, Putman MS, *et al*. *Festina lente*: hydroxychloroquine, COVID-19 and the role of the rheumatologist. *Ann Rheum Dis* 2020;79:734–6.
2 Gautret P, Lagier J-C, Parola P, *et al*. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. *Int J Antimicrob Agents* 2020:105949.
3 Bhimraj A, Morgan R, Shumake A, *et al*. Infectious diseases Society of America guidelines on the treatment and management of patients with COVID-19. *Clin Infect Dis* 2020;4:ciaa478.
4 Geleris J, Sun Y, Platt J, *et al*. Observational study of hydroxychloroquine in hospitalized patients with Covid-19. *N Engl J Med* 2020;382:2411–8.
5 Klimke A, Hefner G, Will B, *et al*. Hydroxychloroquine as an aerosol might markedly reduce and even prevent severe clinical symptoms after SARS-CoV-2 infection. *Med Hypotheses* 2020;142:109783.
6 Picot S, Marty A, Bienvenu A-L, *et al*. Coalition: advocacy for prospective clinical trials to test the post-exposure potential of hydroxychloroquine against COVID-19. *One Health* 2020:100131.
7 Capel RA, Herring N, Kalla M, *et al*. Hydroxychloroquine reduces heart rate by modulating the hyperpolarization-activated current if: novel electrophysiological insights and therapeutic potential. *Heart Rhythm* 2015;12:2186–94.
8 Chugh SS, Reinier K, Singh T, *et al*. Determinants of prolonged QT interval and their contribution to sudden death risk in coronary artery disease: the Oregon sudden unexpected death study. *Circulation* 2009;119:663–70.
9 Morgan ND, Patel SV, Dvorkina O. Suspected hydroxychloroquine-associated QT-interval prolongation in a patient with systemic lupus erythematosus. *J Clin Rheumatol* 2013;19:286–8.
10 Simpson TF, Salazar JW, Vittinghoff E, *et al*. Association of QT-Prolonging medications with risk of autopsy-defined causes of sudden death. *JAMA Intern Med* 2020;180:698–9.
11 Yanturali S, Aksay E, Demir OF, *et al*. Massive hydroxychloroquine overdose. *Acta Anaesthesiol Scand* 2004;48:379–81.
12 Chen C-Y, Wang F-L, Lin C-C. Chronic hydroxychloroquine use associated with QT prolongation and refractory ventricular arrhythmia. *Clin Toxicol* 2006;44:173–5.
13 Chang Y-J, Lee Y-H, Leong P-Y, *et al*. Impact of rheumatoid arthritis on alopecia: a nationwide population-based cohort study in Taiwan. *Front Med* 2020;7:150.
14 Liu Y-T, Tsou H-K, Chiou J-Y, *et al*. Association of human papillomavirus infection with risk for rheumatoid arthritis: a population-based cohort study. *Ann Rheum Dis* 2019;78:1734–6.

Response to: 'Correspondence on '*Festina lente*: hydroxychloroquine, COVID-19and the role of the rheumatologist' by Graef *et al*' by Lo *et al*

We appreciate the interest of Lo *et al* in our opinion piece and thank them for the data presented in their letter.[1 2] Several reports of QT prolongation and torsades de pointes in patients with COVID-19 receiving antimalarials have been published.[3–5] These and other reports have indirectly raised questions regarding the arrhythmogenic potential of hydroxychloroquine (HCQ) when used to treat rheumatic disease.

Lo *et al* used the unique resource of the National Health Insurance Research Database of Taiwan. Through propensity scores, they matched patients who used HCQ and who did not use HCQ for the treatment of newly diagnosed rheumatoid arthritis (RA). Data about other disease-modifying antirheumatic drugs used by the patients was not reported. Therefore, it is unclear if the groups were similar in their RA disease severity, although they seemed similar related to comorbidities. The authors did not find differences in the cumulative risk of arrhythmia between the two groups after 1 year of follow-up. However, a prior meta-analysis on reported cardiotoxic events of antimalarials found that cardiac conduction abnormalities were the most common cardiac adverse events and were associated with higher cumulative doses (median cumulative HCQ dose of 1235 g, median treatment duration 8 years).[6]

The observations from Lo *et al* in the patients with RA are in alignment with the recent preliminary report of a trial regarding the effect of HCQ on hospitalised patients with COVID-19, which did not show any excess of arrhythmias in the HCQ arm after a 1600 mg load followed by 400 mg every 12 hours thereafter.[7] Of note, data regarding incidence of major cardiac arrhythmia was added after the trial launched and was therefore collected in approximately 40% of patients.

Similarly, a recently published of HCQ for COVID-19 showed a very low incidence of arrhythmias but did observe that up to 14.7% of those patients receiving HCQ had a QTc interval greater than 480 ms.[8] This is consistent with several observational studies described in our previous replies where clinically meaningful QTc prolongation occurred more frequently in hospitalised patients with COVID-19 treated with HCQ and azithromycin compared with either drug as monotherapy.[9–11] Risk of QTc prolongation and arrhythmias appear to be most prominent in patients requiring hospitalisation for COVID-19 though cardiac adverse events were not directly monitored in several recent randomised control trials in outpatient populations where HCQ was used for treatment or post exposure prophylaxis.[12–14]

It is important to consider several differences between the use of HCQ for the treatment of COVID-19 compared with its use in rheumatic diseases. HCQ treatment duration in COVID-19 has varied by protocol but lasts for several days. In contrast, patients with rheumatic diseases often are prescribed HCQ for years or even decades. This is relevant since HCQ has a long half-life and may take months to reach steady state concentrations.[15 16] Although a variety of dosing regimens for COVID-19 have been trialled, patients with COVID-19 tend to receive much higher daily doses than the current highest prescribed dose in rheumatology of 400 mg/day.

A recent study of the Veterans Affairs hospitals in the USA of patients using HCQ for prolonged periods of time, the majority of which had systemic lupus erythematosus (SLE), identified that close to 10% of the patients had a QTc of more than 470 ms.[17] Those with prolonged QTc had chronic kidney disease or pre-existent cardiac conditions such as congestive heart failure. Long-term HCQ users who had a prolonged QTc had greater mortality in the univariable analysis but not after adjustment for age, sex and comorbidities.

The COVID-19 pandemic has brought HCQ to the centre stage. As evidence expands, we are learning that HCQ is not an effective treatment for COVID-19, but the spotlight on this old drug has brought new concerns. The lack of association between HCQ use and incident arrhythmias in newly diagnosed the patients with RA in this study by Lo *et al* is reassuring. However, the prolonged QTc observed in other studies and in particular those with chronic kidney disease, a common comorbidity in SLE, and the potential interaction of HCQ with other QT prolonging agents are thought provoking, of clinical relevance and certainly further research is warranted.

Ali Duarte-García,[1] Elizabeth R Graef,[2] Jean W Liew,[3] Maximilian F Konig ,[4] Alfred Hyoungju Kim ,[5] Jeffrey A Sparks [6]

[1]Division of Rheumatology, Mayo Clinic, Rochester, Minnesota, USA
[2]COVID-19 Global Rheumatology Alliance, Boston, Massachusetts, USA
[3]Divison of Rheumatology, Boston University, Boston, Massachusetts, USA
[4]Division of Rheumatology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA
[5]Medicine/Rheumatology, Washington University in Saint Louis School of Medicine, Saint Louis, Missouri, USA
[6]Department of Medicine, Division of Rheumatology, Immunology and Allergy, Brigham and Women's Hospital, Boston, Massachusetts, USA

Correspondence to Dr Jeffrey A Sparks, Department of Medicine, Division of Rheumatology, Immunology and Allergy, Brigham and Women's Hospital, Boston, MA 02115, USA; jsparks@bwh.harvard.eduDr Alfred Hyoungju Kim; akim@wustl.edu

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Twitter Maximilian F Konig @MaxKonigMD, Alfred Hyoungju Kim @alhkim and Jeffrey A Sparks @jeffsparks

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ORCID iDs
Maximilian F Konig http://orcid.org/0000-0001-5045-5255
Alfred Hyoungju Kim http://orcid.org/0000-0003-4074-0516
Jeffrey A Sparks http://orcid.org/0000-0002-5556-4618

REFERENCES

1 Lo CH, Wang YH, Tsai CF, *et al*. 'Correspondence on "Festina lente: hydroxychloroquine, COVID-19 and the role of the rheumatologist" by Graef, et al. *Ann Rheum Dis* 2022;81:e163.
2 Graef ER, Liew JW, Putman MS, *et al*. *Festina lente*: hydroxychloroquine, COVID-19 and the role of the rheumatologist. *Ann Rheum Dis* 2020;79:734–6.
3 Szekely Y, Lichter Y, Shrkihe BA, *et al*. Chloroquine-Induced torsades de pointes in a patient with coronavirus disease 2019. *Heart Rhythm* 2020. doi:10.1016/j.hrthm.2020.04.046. [Epub ahead of print: 05 May 2020].
4 Jankelson L, Karam G, Becker ML, *et al*. Qt prolongation, torsades de pointes, and sudden death with short courses of chloroquine or hydroxychloroquine as used in COVID-19: a systematic review. *Heart Rhythm* 2020.
5 Chorin E, Wadhwani L, Magnani S, *et al*. Qt interval prolongation and torsade de pointes in patients with COVID-19 treated with hydroxychloroquine/azithromycin. *Heart Rhythm* 2020. doi:10.1016/j.hrthm.2020.05.014. [Epub ahead of print: 12 May 2020].
6 Chatre C, Roubille F, Vernhet H, *et al*. Cardiac complications attributed to chloroquine and hydroxychloroquine: a systematic review of the literature. *Drug Saf* 2018;41:919–31.
7 Horby P, Mafham M, Linsell L, *et al*. Effect of hydroxychloroquine in hospitalized patients with COVID-19: preliminary results from a multi-centre, randomized, controlled trial. *Infectious Diseases* 2020.
8 Cavalcanti AB, Zampieri FG, Rosa RG, *et al*. Hydroxychloroquine with or without azithromycin in mild-to-moderate Covid-19. *N Engl J Med* 2020. doi:10.1056/NEJMoa2019014. [Epub ahead of print: 23 Jul 2020].
9 Graef ER, Liew JW, Kim AH, *et al*. Response to: 'Antimalarial use and arrhythmias in COVID-19 and rheumatic patients: a matter of dose and inflammation?' by Erre *et al*. *Ann Rheum Dis* 2021;80:e30.
10 Mercuro NJ, Yen CF, Shim DJ, *et al*. Risk of QT interval prolongation associated with use of hydroxychloroquine with or without concomitant azithromycin among hospitalized patients testing positive for coronavirus disease 2019 (COVID-19). *JAMA Cardiol* 2020. doi:10.1001/jamacardio.2020.1834. [Epub ahead of print: 01 May 2020].
11 Rosenberg ES, Dufort EM, Udo T, *et al*. Association of treatment with hydroxychloroquine or azithromycin with in-hospital mortality in patients with COVID-19 in New York state. *JAMA* 2020;323:2493.
12 Ramireddy A, Chugh H, Reinier K, *et al*. Experience with hydroxychloroquine and azithromycin in the coronavirus disease 2019 pandemic: implications for QT interval monitoring. *J Am Heart Assoc* 2020;9:e017144.
13 Skipper CP, Pastick KA, Engen NW, *et al*. Hydroxychloroquine in nonhospitalized adults with early COVID-19: a randomized trial. *Ann Intern Med* 2020. doi:10.7326/M20-4207. [Epub ahead of print: 16 Jul 2020].
14 Boulware DR, Pullen MF, Bangdiwala AS, *et al*. A randomized trial of hydroxychloroquine as postexposure prophylaxis for Covid-19. *N Engl J Med Overseas Ed* 2020:NEJMoa2016638.
15 Yazdany J, Kim AHJ. Use of hydroxychloroquine and chloroquine during the COVID-19 pandemic: what every clinician should know. *Ann Intern Med* 2020;172:754–5.
16 Scheetz MH, Konig MF, Robinson PC, *et al*. A practical pharmacokinetics-informed approach to navigating hydroxychloroquine shortages in patients with rheumatic disease during the current COVID-19 pandemic 2020.
17 Hooks M, Bart B, Vardeny O, *et al*. Effects of hydroxychloroquine treatment on QT interval. *Heart Rhythm* 2020. doi:10.1016/j.hrthm.2020.06.029. [Epub ahead of print: 28 Jun 2020].

Correspondence on '2019 European League Against Rheumatism/American College of Rheumatology classification criteria for systemic lupus erythematosus' by Aringer *et al*

The 2019 European League against rheumatism/American College of Rheumatology classification criteria (EULAR/ACR 2019 criteria) for systemic lupus erythematosus (SLE) has introduced a new scoring system to classify SLE.[1] The EULAR/ACR 2019 criteria include positive antinuclear antibody at least once as obligatory entry criterion; followed by additive weighted criteria grouped in seven clinical and three immunological domains and weighted from 2 to 10. Patients fulfilling at least one clinical criterion and accumulating ≥10 points are classified. In validation cohort, a classification threshold score of ≥10 yielded a sensitivity similar to that of the Systemic Lupus International Collaborating Clinics (SLICC) 2012 criteria (96.1% vs 96.7%) and a specificity similar to that of the ACR 1997 criteria (93.4% vs 93.4%), demonstrating both excellent sensitivity and specificity. However, we have two concerns about its additive criteria and methodology.

First, some gastrointestinal injuries related to SLE, especially lupus enteritis, may be underestimated. Gastrointestinal symptoms are reported to occur in more than 50% of patients with SLE at some point in the course of their disease;[2] however, these symptoms are usually mild.[3] Although lupus enteritis manifestations are non-specific (eg, abdominal pain, nausea, vomiting, anorexia and diarrhoea) and have wide range from mild to life-threatening (perforation and fistulisation), it has relatively specific features ('double-halo' and 'comb sign') on contrast-enhanced CT.[4] The 'double-halo' (namely 'target sign') is a marker of abnormal bowel wall submucosal thickening, whereas the 'comb sign' correlates with mesenteric vessel prominence.[4] However, the described abnormalities can also be seen in patients with pancreatitis, mechanical bowel obstruction, peritonitis or inflammatory bowel disease.[5] Lupus enteritis mainly affects the small intestine; in rare circumstances, the colon and rectum can also be involved.[6–12] Because of lack of radiology and endoscopy studies on the newly onset SLE, the actual incidence rate of lupus enteritis remains unknown. Recently, we encountered a case of severe lupus enteritis with multiple rectal ulcers and fistulisation formation (figure 1). This is a male patient in his 30s who presented with severe diarrhoea, haematochezia and weight loss for 3 months. He had no dyspnoea, neuropsychiatric, musculoskeletal or mucocutaneous manifestations. Several days before admission, he had cough and low grade fever and this can be explained by mild community-acquired pneumonia and right-side pleural effusion confirmed by his chest CT. After admission, a transthoracic echocardiogram showed a slight pericardial effusion. Pleural or pericardial effusion can be explained by his hypoproteinemia, largely attributable to the protein-losing enteropathy caused by enteritis and rectal ulcers. Although the diagnosis of SLE was subsequently made according to his proteinuria (1.09 g/24 hours), hypocomplementemia (C3: 0.2 g/L, C4: 0.08 g/L) and SLE-specific antibody (anti-dsDNA antibody: >800 IU/mL), in terms of clinical domains in EULAR/ACR 2019 criteria, we felt lupus enteritis 'triumphing over' the seven orthodox clinical domains. Unfortunately, lupus enteritis has not yet been considered in ACR 1997 criteria, SLICC 2012 criteria or EULAR/ACR 2019. It is not even a candidate criteria in patients with early SLE,[13] some of which subsequently being refined and constitute the EULAR/ACR 2019 criteria.

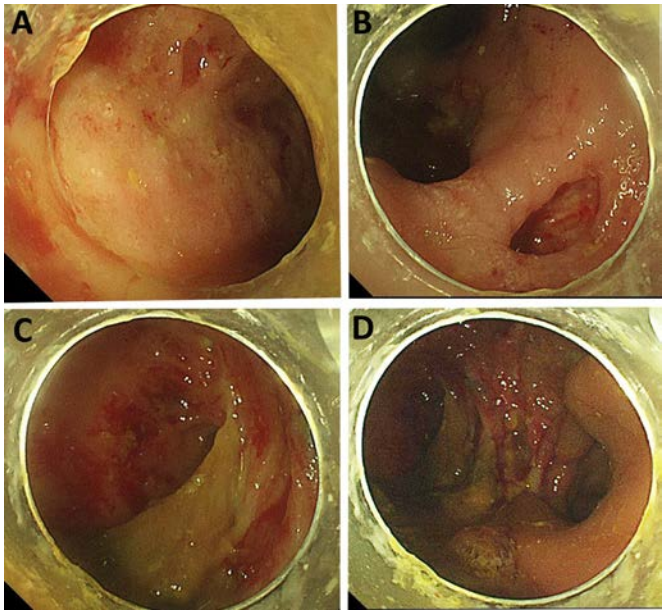

Figure 1 Endoscopic examination of the rectum. Multiple deep ulcers with mucosal friability, submucosal haemorrhage and purulent secretion (A–C) and fistulisation (D) were observed. These endoscopic findings are not exactly the same as ulcerative colitis and Crohn's disease.

Second, rheumatologists should be informed of exact probability of illness in patients with underlying SLE who are below the threshold (ie, total score <10) so as to provide better decision-making, evaluation and follow-up. It is preferable to use logistic regression and nomogram to predict the probability. In addition, when patients have signs or symptoms suggestive of but not diagnostic of SLE, their physician must decide whether to (1) treat empirically, (2) not treat or (3) perform further diagnostic testing before deciding between options 1 and 2. Under this circumstance, decision-making based on the threshold of 10 generated by the receiver operating characteristics analysis seems risky, especially when clinical and immunologic parameters are ambiguous. Rheumatologists should also be informed of the net benefit[14] from the patients when diagnosis is made and treatment is given at a threshold of 10. This net benefit comparison should be suggested to carry out among ACR 1997 criteria, SLICC 2012 criteria, and EULAR/ACR 2019 criteria.

Ran Cui ,[1] **Qian Wang**,[1] **Hua Zhang**,[1] **Shan Wu**,[2] **Xin-Jian Wan**,[2] **Sheng-Ming Dai**[1]

[1]Department of Rheumatology and Immunology, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China
[2]Department of Endoscopy, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China

Correspondence to Professor Sheng-Ming Dai, Department of Rheumatology and Immunology, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai 200233, China; shengmingdai@163.com

Contributors Concept and writing: RC and SMD; revising: SMD and XJW; acquisition of data: RC, QW, HZ, SW; analysis and interpretation of data: RC and SMD.

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ORCID iD
Ran Cui http://orcid.org/0000-0003-4923-5882

REFERENCES

1 Aringer M, Costenbader K, Daikh D, *et al*. 2019 European League against Rheumatism/American College of rheumatology classification criteria for systemic lupus erythematosus. *Ann Rheum Dis* 2019;78:1151–9.
2 Chng HH, Tan BE, Teh CL, *et al*. Major gastrointestinal manifestations in lupus patients in Asia: lupus enteritis, intestinal pseudo-obstruction, and protein-losing gastroenteropathy. *Lupus* 2010;19:1404–13.
3 Li Z, Xu D, Wang Z, *et al*. Gastrointestinal system involvement in systemic lupus erythematosus. *Lupus* 2017;26:1127–38.
4 Kröner PT, Tolaymat OA, Bowman AW, *et al*. Gastrointestinal manifestations of Rheumatological diseases. *Am J Gastroenterol* 2019;114:1441–54.
5 Janssens P, Arnaud L, Galicier L, *et al*. Lupus enteritis: from clinical findings to therapeutic management. *Orphanet J Rare Dis* 2013;8:67.
6 Yau AHL, Chu K, Yang HM, *et al*. Rectal ulcers induced by systemic lupus erythematosus. *BMJ Case Rep* 2014;2014:bcr2014205776.
7 Yuasa S, Suwa A, Hirakata M, *et al*. A case of systemic lupus erythematosus presenting with rectal ulcers as the initial clinical manifestation of disease. *Clin Exp Rheumatol* 2002;20:407–10.
8 Teramoto J, Takahashi Y, Katsuki S, *et al*. Systemic lupus erythematosus with a giant rectal ulcer and perforation. *Intern Med* 1999;38:643–9.
9 Chattopadhyay P, Abby Philips C, Dhua D, *et al*. Systemic lupus erythematosus presenting as ischaemic proctitis. *Lupus* 2011;20:653–5.
10 Mansoor AC, Jemshad A, Musambil M. Systemic lupus erythematosus presenting as rectal ulcer and longitudinal myelopathy. *Int J Rheum Dis* 2017;20:1818–20.
11 Kaieda S, Kobayashi T, Moroki M, *et al*. Successful treatment of rectal ulcers in a patient with systemic lupus erythematosus using corticosteroids and tacrolimus. *Mod Rheumatol* 2014;24:357–60.
12 Lee J-R, Paik C-N, Kim J-D, *et al*. Ischemic colitis associated with intestinal vasculitis: histological proof in systemic lupus erythematosus. *World J Gastroenterol* 2008;14:3591–3.
13 Tedeschi SK, Johnson SR, Boumpas D, *et al*. Developing and refining new candidate criteria for systemic lupus erythematosus classification: an international collaboration. *Arthritis Care Res* 2018;70:571–81.
14 Vickers AJ, Elkin EB. Decision curve analysis: a novel method for evaluating prediction models. *Med Decis Making* 2006;26:565–74.

Response to: "2019 European League Against Rheumatism/American College of Rheumatology classification criteria for systemic lupus erythematosus' by Aringer *et al*" by Cui *et al*

Dear Sir,

In their letter,[1] Dr Cui and colleagues include two interesting thoughts. Primarily, they argue, illustrated by one case, that gastrointestinal (GI) involvement may be more common in systemic lupus erythematosus (SLE) than usually thought. This thought is to some degree supported by the patient survey performed within the European League Against Rheumatism (EULAR)/ American College of Rheumatology (ACR) classification criteria project, in which we noted that more than 5% of the patients reported GI symptoms at the time of their SLE diagnosis,[2] even though this organ system was not included on the questionnaire.

However, no specific SLE GI pattern has been described so far, which would be a prerequisite of including a GI manifestation into SLE classification criteria. A large number of rare organ manifestations possible in SLE could not be included into the classification criteria, since this would be impracticable. Throughout the classification criteria development process, SLE experts opined for a comprehensive system balanced against computational ease in the clinic.[3 4] As in most instances, SLE affected multiple organs in the authors' patient, easily allowing for classification according to the EULAR/ACR 2019 SLE classification criteria.[5 6] On the other hand, in unusual situations of rare, isolated organ manifestations, failure to fulfil classification criteria should never prevent making a clinical diagnosis. While both try to correctly define whether a patient has SLE or not, diagnosis and classification are clearly distinct,[7] and we once again caution against using the EULAR/ACR 2019 classification criteria for making or even worse refuting a diagnosis of SLE. As result of a very stringent methodological process, lack of sufficient homogeneity of GI manifestations did not qualify as a classification domain, but this does not argue against their clinical occurrence.

The other idea Dr Cui *et al* express is direct translation of points received in the EULAR/ACR classification system to the probability that the patient has SLE. This has not yet been tried, but the line of thought is correct in that the system in fact provides for a measure of the probability a patient can be classified as having SLE.

Martin Aringer ,[1] **Karen H Costenbader,**[2] **Thomas Dörner** ,[3,4] **Sindhu R Johnson** [5,6]

[1]Division of Rheumatology, Department of Medicine III, University Medical Center and Faculty of Medicine TU Dresden, Dresden, Germany
[2]Division of Rheumatology, Inflammation and Immunity, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA
[3]Department of Rheumatology and Clinical Immunology, Charité – Universitätsmedizin Berlin, Corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, Berlin, Germany
[4]Department of Rheumatology and Clinical Immunology, Berlin Institute of Health, Berlin, Berlin, Germany
[5]Division of Rheumatology, Department of Medicine, Toronto Western Hospital, University Health Network, Mount Sinai Hospital, University of Toronto, Toronto Scleroderma Research Program, Toronto, Ontario, Canada
[6]University of Toronto Institute of Health Policy Management and Evaluation, Toronto, Ontario, Canada

Correspondence to Professor Martin Aringer, Division of Rheumatology, Department of Medicine III, University Medical Center and Faculty of Medicine TU Dresden, Dresden, Germany; martin.aringer@uniklinikum-dresden.de

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ORCID iDs
Martin Aringer http://orcid.org/0000-0003-4471-8375
Thomas Dörner http://orcid.org/0000-0002-6478-7725
Sindhu R Johnson http://orcid.org/0000-0003-0591-2976

REFERENCES

1 Cui R, Wang Q, Zhang H, *et al*. Correspondence on "2019 European League Against Rheumatism/ American College of Rheumatology classification criteria for systemic lupus erythematosus" by Aringer *et al*. *Ann Rheum Dis* 2022;81:e165.
2 Leuchten N, Milke B, Winkler-Rohlfing B, *et al*. Early symptoms of systemic lupus erythematosus (SLE) recalled by 339 SLE patients. *Lupus* 2018;27:1431–6.
3 Johnson SR, Khanna D, Daikh D, *et al*. Use of consensus methodology to determine candidate items for systemic lupus erythematosus classification criteria. *J Rheumatol* 2019;46:721–6.
4 Tedeschi SK, Johnson SR, Boumpas D, *et al*. Developing and refining new candidate criteria for systemic lupus erythematosus classification: an international collaboration. *Arthritis Care Res* 2018;70:571–81.
5 Aringer M, Costenbader K, Daikh D, *et al*. 2019 European League against Rheumatism/ American College of rheumatology classification criteria for systemic lupus erythematosus. *Ann Rheum Dis* 2019;78:1151–9.
6 Aringer M, Costenbader K, Daikh D, *et al*. 2019 European League against Rheumatism/ American College of rheumatology classification criteria for systemic lupus erythematosus. *Arthritis Rheumatol* 2019;71:1400–12.
7 Aringer M, Dörner T, Leuchten N, *et al*. Toward new criteria for systemic lupus erythematosus-a standpoint. *Lupus* 2016;25:805–11.

Women's journey in Mexican rheumatology. Comment on 'Gender gap in rheumatology: speaker representation at annual conferences' by Monga *et al*

We read with great interest the letter written by Monga *et al* addressing the narrowing of the gender gap in the annual meetings of the American College of Rheumatology, in which they reported an increase in female speakers from 42.8% in 2017 to 47.0% in 2018.[1] However, women under-representation goes beyond annual meetings as Adami *et al* found that of 366 guidelines and recommendations in Rheumatology published from 2004 to 2019, only 32% of first authors were women, a proportion which has been increasing in the last 15 years.[2] Their findings propelled us to evaluate women's participation as first authors of oral presentations in the annual meetings of the Mexican College of Rheumatology (MCR).

We evaluated the abstracts accepted as oral presentations of the MCR from 2011 to 2018; research using the first author's last name and affiliation was performed to identify authors and classify them as women or men. Presentations were classified as basic or clinical research, and according to the rheumatic disease evaluated: rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), osteoarthritis, idiopathic juvenile arthritis, vasculitis, myopathies, psoriatic arthritis, systemic sclerosis, spondyloarthropathies, antiphospholipid syndrome (APS), crystal arthropathies or miscellaneous. Authors who we were unable to identify as men or women were eliminated.

We found a total of 153 oral presentations; registries from 2015 were not found. Overall, 79 (51.6%) women were listed as first authors. The highest female participation was in 2011 (70.83%), and the lowest in 2017 (43.4%). Overall, women participation decreased by 20.8% from 2011 to 2018. The greatest fall in the percentage of women as first authors was from 2011 to 2012 (24.6%), and the highest increase from 2012 to 2013 (14.95%). Full results are shown in figure 1. Most oral presentations with a female first author were in clinical research (n=50, 63.3%), predominantly regarding SLE (n=13, 26%), RA (n=12, 24%), APS (n=6, 12%) and crystal arthropathies (n=4, 8%).

Women representation as first authors of the oral presentations of the MCR has followed a waxing and waning course. However, our findings are similar to those reported by Monga *et al* as women have steadily retained >40% of first authorship of the oral sessions.

In Mexico, up to 2017, a total of 379 (42.1%) rheumatologists were women.[3] This percentage could change soon as women currently represent 59.5% (n=75) of 126 rheumatology residents. Whether this female predominance in future rheumatologists will change female representation in the annual meetings of the MCR—and everywhere—is yet to be seen.

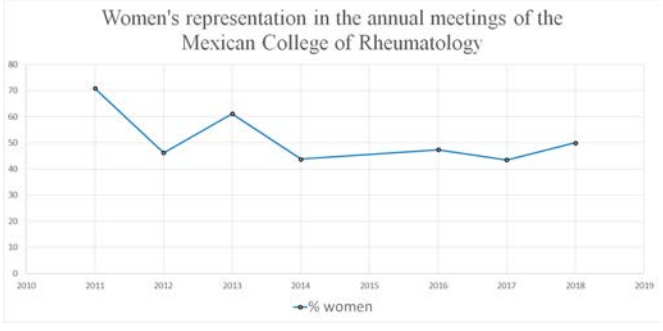

Figure 1 Women's representation through the years as first authors of oral presentations in the annual meetings of the Mexican College of Rheumatology.

Iris J Colunga-Pedraza [1], **Rosa I Arvizu-Rivera**,[2] **Griselda Serna-Peña** [1], **Ana Laura De-Leon-Ibarra**,[1] **Deshire Alpizar Rodriguez** [3], **Alejandra Pérez-Villar** [1], **Mayra Alejandra Reyes Soto** [1], **Dionicio Angel Galarza-Delgado** [1]

[1]Rheumatology, Hospital Universitario Dr Jose Eleuterio Gonzalez, Monterrey, Mexico
[2]Medicina Interna, Universidad Autonoma de Nuevo Leon, San Nicolas de los Garza, Nuevo Leon, Mexico
[3]Research Unit, Colegio Mexicano de Reumatología, Mexico, Mexico

Correspondence to Dr Iris J Colunga-Pedraza, Rheumatology, Hospital Universitario Dr Jose Eleuterio Gonzalez, Monterrey 64040, Mexico; iriscolunga@hotmail.com

Twitter Iris J Colunga-Pedraza @DraIrisColungaReuma and Griselda Serna-Peña @DraGrisSerna

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ORCID iDs
Iris J Colunga-Pedraza http://orcid.org/0000-0002-2786-5843
Griselda Serna-Peña http://orcid.org/0000-0002-7547-3975
Deshire Alpizar Rodriguez http://orcid.org/0000-0002-6930-0517
Alejandra Pérez-Villar http://orcid.org/0000-0002-0346-6469
Mayra Alejandra Reyes Soto http://orcid.org/0000-0001-9433-5976
Dionicio Angel Galarza-Delgado http://orcid.org/0000-0001-9714-2109

REFERENCES

1 Monga K, Liew J. Gender gap in rheumatology: SPEAKER representation at annual conferences. *Ann Rheum Dis* 2020. doi:10.1136/annrheumdis-2020-217673. [Epub ahead of print: 13 May 2020].
2 Adami G, Benini C, Vantaggiato E, *et al*. Gender disparity in authorship of guidelines and recommendations in rheumatology. *Ann Rheum Dis* 2020;79:1122–3.
3 Heinze-Martin G, Olmedo-Canchola VH, Bazán-Miranda G, *et al*. Los médicos especialistas en México. *Gac Med Mex* 2018;154:342–51.

Response to: 'Women's journey in Mexican rheumatology. Comment on 'Gender gap in rheumatology: speaker representation at annual conferences' by Monga *et al*' by Colunga-Pedraza *et al*

We read the correspondence by Colunga-Pedraza *et al* to our letter about the gender gap in the American College of Rheumatology (ACR) annual conference with great interest.[1 2] The authors provide data on the first authors of oral presentations in the Mexican College of Rheumatology (MCR) annual meetings from 2011 to 2018. Out of 153 oral presentations, 79 (51.6%) had women as first authors. While the overall percentage of oral presentations given by female authors surpassed the 42.1% reported as the total number of female rheumatologists in Mexico up to 2017, they found that there were fluctuations based on the year. If the data were available, an additionally valuable insight would be whether these fluctuations were correlated with the proportion of women among new rheumatologists entering the workforce in those years.

We have previously commented on the value of looking at both first and senior authors when responding to the work of Adami *et al*.[1 3] The representation of women among the first authors of original research in high impact general medical journals was significantly higher overall in 2014 compared with 1994, in a study by Filardo *et al*.[4] These authors also noted differences in gender representation that varied across journals, as well as plateauing or declining trends in some cases. An older study by Jagsi *et al* evaluated the percentage of both first and senior authors in high-impact medical journals[5] and found significantly increases over time for both. Looking at both authorship positions, whether it is for oral abstract presentations or publications, would provide some information on trends over time, as the last authorship position is usually reserved for a more senior researcher. It would also be helpful to note whether the selection process for these abstracts involves a double-blind peer review, as this tends to favour increased representation of female authors.[6 7]

In summary, Colunga-Pedraza *et al* present more data to support improved gender representation in rheumatology—but also the fact that we still have improvements left to make.

Kanika Monga,[1] **Jean Liew** [2]

[1]Rheumatology, University of Texas - McGovern Medical School, Houston, Texas, USA
[2]Medicine, Section of Rheumatology, Boston University, Boston, Massachusetts, USA

Correspondence to Dr Jean Liew, Medicine, Section of Rheumatology, Boston University, Boston, MA 02118, USA; liew.jw@gmail.com

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Twitter Jean Liew @rheum_cat

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ORCID iD
Jean Liew http://orcid.org/0000-0002-8104-2450

REFERENCES

1 Monga K, Liew J. Gender gap in rheumatology: SPEAKER representation at annual conferences. *Ann Rheum Dis* 2020. doi:10.1136/annrheumdis-2020-217673. [Epub ahead of print: 13 May 2020].
2 Colunga-Pedraza I, Arvizu-Rivera R, Serna-Pena G, *et al*. Women's journey in Mexican rheumatology. Comment on "Gender gap in rheumatology: speaker representation at annial conferences. *Ann Rheum Dis* 2022;81:e167.
3 Adami G, Benini C, Vantaggiato E, *et al*. Gender disparity in authorship of guidelines and recommendations in rheumatology. *Ann Rheum Dis* 2020;79:1122–3.
4 Filardo G, da Graca B, Sass DM, *et al*. Trends and comparison of female first authorship in high impact medical journals: observational study (1994-2014). *BMJ* 2016;352:i847.
5 Jagsi R, Guancial EA, Worobey CC, *et al*. The "gender gap" in authorship of academic medical literature--a 35-year perspective. *N Engl J Med* 2006;355:281–7.
6 Budden AE, Tregenza T, Aarssen LW, *et al*. Double-blind review favours increased representation of female authors. *Trends Ecol Evol* 2008;23:4–6.
7 Mulligan A, Hall L, Raphael E. Peer review in a changing world: an international study measuring the attitudes of researchers. *J Am Soc Inf Sci Tec* 2013;64:132–61.

Correspondence to 'Gender gap in rheumatology: speaker representation at annual conferences' by Monga and Liew—gender discrepancies at annual EULAR congresses: towards the gap narrowing

We read with interest the letter entitled 'Gender gap in rheumatology: speaker representation at annual conferences' by Kanika Monga and Jean Liew published in the *Annals of the Rheumatic Diseases*.[1]

The authors highlight the issue of a gender gap among speakers and moderators at the academic conferences investigating female representation at the American College of Rheumatology (ACR) in 2017 and 2018. Overall, the proportion of female speakers and moderators was 42.8%–47%and the gap was higher in the clinician sessions presentations compared with basic science ones (45.8% vs 40.5%). The highest proportion of female representation was detected in Alliance for Human Research Protection sessions (65.3%), and the lowest in Meet the Professors and workshop sessions (34.4% and 28.7%). Indeed, the gender gap was narrower as compared with other conferences.[2] Recently, European League Against Rheumatism (EULAR) established a task force on gender equity in rheumatology with the aim of developing a comprehensive intervention on gender equity.

We aimed at evaluating the gender gap in the EULAR meetings held in 2018 and 2019. Using the EULAR scientific programme, we determined the proportion of women for each session: invited speakers, abstract presenters selected by the congress scientific committee and moderators. We further categorised the prevalence according to the type of session: general session, people with arthritis and rheumatism (PARE) and health professionals in rheumatology (other than physicians, HRP).

Overall, 895 presentations were performed in 2018 (276 invited speakers and 619 selected abstract) requiring 462 moderations. Female prevalence was 44% among moderators, 31.5% among invited speakers and 52.1% among selected abstract. In 2019, there were 811 presentations (185 invited speakers and 626 selected abstract) and 422 moderations. Overall, the prevalence of female representation in 2019 among the moderators was significantly higher compared with that in 2018 (48.6% vs 44%, p=0.02).

In 2019, there was an increase in the female prevalence among the invited speakers compared with the previous year (43.2% vs 31.5%, p=0.03) while the selected abstracts were equally distributed between male and female speakers both in 2018 and 2019 (percentage of females=52.1%and 48.4%, respectively).

We also stratified female prevalence in 2018 and 2019 EULAR congress according to the type of session (figure 1): in 2018, the lowest female prevalence was recorded in the general scientific session (40.5% moderators, 30.5% invited speakers and 46.2% selected abstract) and the highest prevalence in PARE (63.9% moderators, 59.4% invited speakers and 71.7% selected abstract) and HRP sessions (77.8% moderators, 59.4% invited speakers and 69.3% selected abstract). Moreover, in the general sessions, the prevalence of female presenting invited speeches was significantly lower than that of selected abstracts presenters (p=0.003). The results recorded in the general scientific session of the 2019 EULAR congress showed 44.5% of female prevalence among the moderators, 31.7% among the invited speakers and 42.3% among the selected oral communications. Like in 2018, the highest prevalence of female representation was registered in the PARE and HRP sessions: moderators were present in 56.8% and 68%, respectively; invited speakers in 57.8% and 56.4%; selected abstracts in 73.8% and 64.2%.

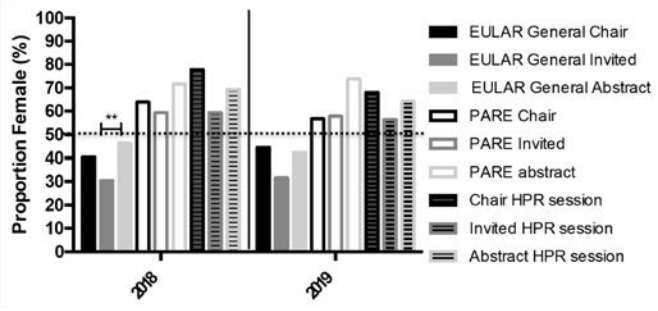

Figure 1 Prevalence of female presentations in 2018 and 2019 European League Against Rheumatism meetings. EULAR, european league against rheumatism; PARE, people with arthritis and rheumatism (PARE); HRP, health professionals in rheumatology.

These results are similar to that observed in ACR meetings by Monga K.[1] We also highlight that female gender in EULAR congresses is mostly represented in PARE and HRP sessions which account only for the 24.3%–28.3% of presentations and 19.5%–22.3% of moderations in 2018–2019. The women representation in PARE and HRP sessions reflects the gender bias characterising autoimmune diseases and the female predominance among health professionals; on the contrary, women are still under-represented in academic rheumatology.[3–5] The recent increase in proportion of females attending medical schools will, hopefully, further reduce the academic gender gap.

Paola Conigliaro ,[1] **Silvia Laura Bosello,**[2] **Cristina Iannuccelli,**[3] **Elisa Gremese** ,[2,4] **Francesca Romana Spinelli** ,[3] **Marta Vadacca,**[5] **Maria Sole Chimenti**[1]

[1]Department of "Medicina dei Sistemi", Rheumatology, Allergology and Clinical Immunology, Universita degli Studi di Roma Tor Vergata, Rome, Italy
[2]Division of Rheumatology, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy
[3]Department of "Scienze Cliniche, Internistiche, Anestesiologiche e Cardiovascolari" - Rheumatology Unit, Sapienza Università di Roma, Rome, Italy
[4]Institute of Rheumatology, Università Cattolica del Sacro Cuore, Rome, Italy
[5]Unit of Allergology, Clinical Immunology and Rheumatology, Università Campus Bio-Medico di Roma, Rome, Italy

Correspondence to Dr Francesca Romana Spinelli, Universita degli Studi di Roma La Sapienza, Roma 00161, Italy; francescaromana.spinelli@uniroma1.it

Contributors PC conceived the work and drafted the letter. All authors collected and analysed the data, revised the letter and approved the final version.

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ORCID iDs
Paola Conigliaro http://orcid.org/0000-0001-7905-8413
Elisa Gremese http://orcid.org/0000-0002-2248-1058
Francesca Romana Spinelli http://orcid.org/0000-0003-1969-2097

REFERENCES

1 Monga K, Liew J. Gender gap in rheumatology: speaker representation at annual conferences. *Ann Rheum Dis* 2020. doi:10.1136/annrheumdis-2020-217673. [Epub ahead of print: 13 May 2020].
2 Ruzycki SM, Fletcher S, Earp M, *et al*. Trends in the proportion of female speakers at medical conferences in the United States and in Canada, 2007 to 2017. *JAMA*.
3 Bosello SL, Chimenti MS, Conigliaro P, *et al*. Gender equality in rheumatology Reumatismo 2020;71:173–6.
4 Edmunds LD, Ovseiko PV, Shepperd S, *et al*. Why do women choose or reject careers in academic medicine? A narrative review of empirical evidence. *Lancet* 2016;388:2948–58.
5 Boniol M, McIsaac M, Xu L, *et al*. *Gender equity in the health workforce: analysis of 104 countries. Working paper 1*. Geneva: World Health Organization, 2019.

Response to: 'Correspondence to 'Gender gap in rheumatology: speaker representation at annual conferences' by Monga and Liew–gender discrepancies at annual EULAR congresses: towards the gap narrowing' by Conigliaro *et al*

We were pleased to read the correspondence by Conigliaro *et al* to our letter about the gender gap in the American College of Rheumatology (ACR) annual conference.[1 2]

The authors provide data on the gender gap in the European League Against Rheumatism (EULAR) meetings in 2018 and in 2019. Overall, the authors found that female speakers delivered 46% of presentations in 2018, and 44% in 2019—numbers that were similar to our findings for ACR meetings. The authors were able to break down the proportion of female speakers by invited speakers, moderators and selected abstracts. They found that these percentages were close to 50% for selected abstracts and moderators in 2019, a finding which is reassuring.

Interestingly, the lowest proportion of female speakers was recorded in the general scientific session during both years. We found there was a higher proportion of female speakers in the clinical than in the basic science presentations at ACR. If the data were available, it would be additionally informative to see what percentage of submitted abstracts by gender were selected for presentation. This would help identify whether the gender gap is driven by number of submissions vs the selection process; this knowledge could eventually help reduce the gap further.

Academic publications are used to disseminate scientific knowledge and are a way to measure research productivity. Publications can influence career prospects and visibility for authors. It would be also interesting to look at a country-stratified analysis of speaker gender using the EULAR data. Holman *et al* noted that countries, like Japan, Germany and Switzerland, which have higher per capita incomes, have fewer women authors representing the Science, Technology, Engineering, Mathematics and Medicine workforce.[3]

In summary, these data presented by Conigliaro *et al* are similar to ours and further support that a gender gap does exist among speakers during annual rheumatology meetings. Even though it has improved over the years, we must remain aware of its presence and continue to work towards equal representations. We are, thus, appreciative of the efforts of the EULAR Task Force on Gender Equity in Academic Rheumatology and look forward to their future outputs.[4]

Kanika Monga,[1] **Jean Liew** [2]

[1]Rheumatology, University of Texas McGovern Medical School, Houston, Texas, USA
[2]Medicine, Section of Rheumatology, Boston University School of Medicine, Boston, Massachusetts, USA

Correspondence to Dr Jean Liew, Medicine, Section of Rheumatology, Boston University School of Medicine, Boston, MA 02118, USA; liew.jw@gmail.com

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Twitter Jean Liew @rheum_cat

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ORCID iD
Jean Liew http://orcid.org/0000-0002-8104-2450

REFERENCES

1 Conigliaro P, Bosello S, Lannucelli C, *et al*. Correspondence to 'Gender gap in rheumatology: speaker representation at annualconferences' by Monga and Liew—gender discrepancies at annual EULARcongresses: towards the gap narrowing. *Ann Rheum Dis* 2022;81:e169.
2 Monga K, Liew J. Gender gap in rheumatology: SPEAKER representation at annual conferences. *Ann Rheum Dis* 2020. doi:10.1136/annrheumdis-2020-217673. [Epub ahead of print: 13 May 2020].
3 Holman L, Stuart-Fox D, Hauser CE. The gender gap in science: how long until women are equally represented? *PLoS Biol* 2018;16:e2004956.
4 Ovseiko PV, Gossec L, *et al*, on behalf of EULAR Task Force on Gender Equity in Academic Rheumatology. THU0580 EULAR Task force on gender equity in academic rheumatology: preliminary survey findings. *Ann Rheum Dis* 2020;79:531–2.

Case of postpartum axial spondyloarthritis

In a recent issue of the *Annals of Rheumatic Diseases*, Hoballah *et al* reported high false-positive results on sacroiliac MRI based on the Assessment of SpondyloArthritis international Society (ASAS) criteria in the early postpartum period.[1] Similarly, Renson *et al* reported a markedly high prevalence of postpartum sacroiliac bone marrow oedema on sacroiliac MRI and recommended waiting at least 6 months after delivery to perform sacroiliac MRI.[2] Both studies excluded patients with inflammatory bowel disease. We would like to share below a case of new onset axial spondyloarthritis in an early postpartum patient with ulcerative colitis.

A Japanese female patient in her 30s with ulcerative colitis in clinical remission without treatment was evaluated for a 2-month history of progressive right buttock pain which began 6 months after childbirth. Naproxen was ineffective in relieving the pain.

Physical examination showed tenderness in the right sacroiliac joint and was positive for Patrick's test and Gaenslen test. Laboratory analysis showed serum C reactive protein 1.7 mg/dL and was negative for human leucocyte antigen-B27. A plain radiograph of the sacroiliac joint was normal. MRI revealed bone marrow oedema without erosion or ankylosis in the right sacroiliac joint, thus satisfying the ASAS criteria for active sacroiliitis (figure 1A). The pain dramatically improved 4 days after adalimumab treatment was begun, and the MRI findings improved by week 8 (figure 1B).

In patients with inflammatory bowel disease, mechanical stress on the sacroiliac joint during labour may induce persistent inflammation leading to axial spondyloarthritis. Although the MRI findings are indistinguishable from non-specific bone marrow oedema, response to treatment can confirm the diagnosis of postpartum axial spondyloarthritis. In cases with a high index of suspicion, clinical diagnosis and prompt treatment can improve the patient's quality of life.

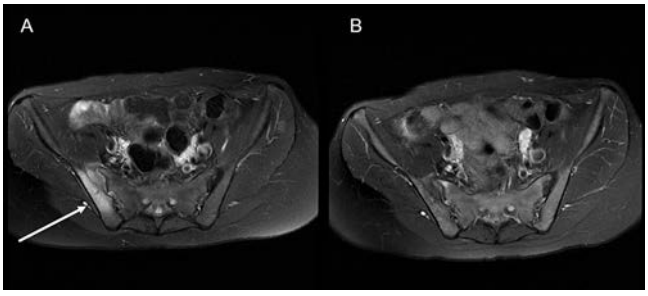

Figure 1 (A) bone marrow oedema in the right sacroiliac joint on short tau inversion recovery images at baseline; (B)improvement of bone marrow oedema after treatment.

Kazunori Furuhashi,[1] Nanase Honda,[2] Yuji Miyoshi,[2] Naoto Yokogawa [1,2]

[1]Department of Internal Medicine, Hino Municipal Hospital, Hino, Japan
[2]Department of Rheumatic Diseases, Tokyo Metropolitan Tama Medical Center, Fuchu, Japan

Correspondence to Dr Naoto Yokogawa, Department of Rheumatic Diseases, Tokyo Metropolitan Tama Medical Center, Fuchu 183-8524, Japan; yokogawan@aol.com

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ORCID iD
Naoto Yokogawa http://orcid.org/0000-0002-7814-443X

REFERENCES

1 Hoballah A, Lukas C, Leplat C, *et al*. Mri of sacroiliac joints for the diagnosis of axial spa: prevalence of inflammatory and structural lesions in nulliparous, early postpartum and late postpartum women. *Ann Rheum Dis* 2020;79:1063–9.
2 Renson T, Depicker A, De Craemer A-S, *et al*. High prevalence of spondyloarthritis-like MRI lesions in postpartum women: a prospective analysis in relation to maternal, child and birth characteristics. *Ann Rheum Dis* 2020;79:929–34.

Response to: 'Case of postpartum axial spondyloarthritis' by Furuhashi *et al*

We were pleased to read the correspondence of Furuhashi *et al*[1] who highlighted the importance of considering coexisting risk factors when diagnosing axial spondyloArthritis (ax-SpA) in early postpartum women. The authors correctly pointed out that patients with inflammatory bowel disease are more likely to develop ax-SpA due to common pathogenic mechanisms.[2] In fact, the presence of Crohn's disease or ulcerative colitis is part of the Assessment of SpondyloArthritis international Society criteria for the diagnosis of ax-SpA.[3]

In our study, on the prevalence of bone marrow oedema (BME) at the sacroiliac joint (SIJ) in postpartum women,[4] we excluded those with known risk factors for developing ax-SpA, such as family or patient history of inflammatory diseases. The exclusion of women with known risk factors for developing ax-SpA was deemed important to eliminate potential confounding factors, and hence ascertain whether observations of sacroiliitis were triggered by, or related to, pregnancy and childbirth as opposed to other aetiologies.

In our experience, women with inflammatory bowel disease who have low back pain within the first 6 months after delivery should be assessed using MRI to identify the causes of pain, which could include infections or degenerative diseases. However, we believe that BME at the SIJ observed in an MRI taken during the early postpartum period is insufficient to diagnose ax-SpA, since BME at the SIJ may be merely transient and could disappear over time. It is also important to note the limitations of MRI for the diagnosis of ax-SpA, due to limited sensitivity and specificity of detecting 'suggestive BME' lesions, and the role/necessity of conventional radiography for the standardisation and contextual evaluation.[5] While response to tumour necrosis factor inhibitors, such as adalimumab, can confirm diagnosis and relieve symptoms, immunosuppressive drugs are associated with a number of adverse events. We endeavour to improve the efficacy of clinical and imaging diagnostic tools to prescribe the most appropriate treatment and avoid such adverse events, especially in patients that are unlikely to benefit from immunosuppressive drugs.

Adel Hoballah,[1] Cédric Lukas,[2] Christophe Leplat,[1] Patrice Taourel,[1] Jean-Baptiste Pialat,[3] Nicolas Sans,[4] Sonia Ramos-Pascual ,[5] Mo Saffarini,[5] Catherine Cyteval[1]

[1]Osteoarticular Medical Imaging Section, Department of Medical Imaging, University Hospital Centre Montpellier, Montpellier, Languedoc-Roussillon, France
[2]Department of Rheumatology, University Hospital Centre Montpellier, Montpellier, Languedoc-Roussillon, France
[3]Department of Radiology, Groupement Hospitalier Edouard Herriot, Lyon, Rhône-Alpes, France
[4]Department of Radiology, Toulouse University Hospital, Paul Sabatier University, Toulouse, Occitanie, France
[5]ReSurg SA, Nyon, Vaud, Switzerland

Correspondence to Dr Sonia Ramos-Pascual, ReSurg SA, Nyon 1560, Vaud, Switzerland; journals@resurg.com

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ORCID iD
Sonia Ramos-Pascual http://orcid.org/0000-0002-0409-613X

REFERENCES

1 Furuhashi K, Honda N, Miyoshi Y, *et al*. Case of postpartum axial spondyloarthritis. *Ann Rheum Dis* 2022;81:e171.
2 Fragoulis GE, Liava C, Daoussis D, *et al*. Inflammatory bowel diseases and spondyloarthropathies: from pathogenesis to treatment. *World J Gastroenterol* 2019;25:2162–76.
3 Sieper J, Rudwaleit M, Baraliakos X, *et al*. The assessment of spondyloarthritis International Society (ASAS) Handbook: a guide to assess spondyloarthritis. *Ann Rheum Dis* 2009;68 Suppl 2:ii1–44.
4 Hoballah A, Lukas C, Leplat C, *et al*. Mri of sacroiliac joints for the diagnosis of axial spa: prevalence of inflammatory and structural lesions in nulliparous, early postpartum and late postpartum women. *Ann Rheum Dis* 2020;79:1063–9.
5 Lukas C, Cyteval C, Dougados M, *et al*. MRI for diagnosis of axial spondyloarthritis: major advance with critical limitations 'Not everything that glisters is gold (standard)'. *RMD Open* 2018;4:e000586.

Correspondence on 'EULAR recommendations for the management of psoriatic arthritis with pharmacological therapies: 2019 update'

We read with interest the recently published European League Against Rheumatism (EULAR) recommendations for the management of psoriatic arthritis (PsA) with pharmacological therapies[1] and the associated systematic literature research (SLR)[2]; we welcome the clarity that they offer for patient care.

We would like to bring three points to your readers' attention to correct and clarify the narrative supporting Recommendation 7 (Janus kinase (JAK) inhibitors).

Recommendation 7 states: "… Our SLR indicated tofacitinib may have similar efficacy as the TNFi adalimumab for joint involvement, but numerically lower efficacy in skin psoriasis.[1,15,72] … Safety signals exist for some infections, especially herpes zoster, as well as a recent signal for deep vein thrombosis especially with a high dose of tofacitinib which is not approved for PsA, but also the usual 5 mg twice daily dose particularly in those with cardiovascular risk factors and older patients.[15,72,73]"

Regarding the statement: "… as well as a recent signal for deep vein thrombosis especially with a high dose of tofacitinib which is not approved for PsA …":

We would like to respectfully correct this statement, as this signal was seen for pulmonary embolism (PE) rather than deep vein thrombosis (DVT); this was observed in the 10 mg twice daily dose of tofacitinib, which is not approved for PsA.

In February 2019, during a routine safety analysis of Study A3921133 (NCT02092467; database not locked, data not yet source-verified or subjected to standard quality-check procedures that occur at the time of database lock, therefore may be subject to change), an ongoing postauthorisation safety surveillance study for tofacitinib in patients with rheumatoid arthritis (RA) aged ≥50 years and with one or more cardiovascular risk factor, the independent tofacitinib Data Safety Monitoring Board reported a statistically increased incidence of PE events in patients receiving tofacitinib 10 mg twice daily versus tumour necrosis factor inhibitors (TNFi). The incidence of venous thromboembolism (VTE; PE or DVT) from this ad hoc safety analysis of Study A3921133 has been reported as an identified risk (adverse drug reaction) in the Summary of Product Characteristics (SmPC) for tofacitinib (table 1).[3]

Regarding the statement: "… but also the usual 5 mg twice daily dose particularly in those with cardiovascular risk factors and older patients.[15,72,73]":

The SLR publication states: "while no venous thromboembolic events or pulmonary embolisms were observed in patients with PsA treated with tofacitinib or filgotinib,[13,55] such events were seen in other indications when tofacitinib, baricitinib and upadacitinib were used, especially in an ongoing study on patients with RA with high cardiovascular risk (tofacitinib study A3921133); warnings in these regards were issued by regulators, especially with respect to patients with a high risk for venous thromboembolic events.[56,57]"[2]

There were no statistically significant differences in the incidence rates (IRs) for DVT among tofacitinib 5 mg twice daily (the licenced dose), tofacitinib 10 mg twice daily and TNFi (table 1). Additionally, the difference in IRs for PE between tofacitinib 5 mg twice daily and TNFi was not statistically significant. Based on the totality of available information, including, but not limited to, analyses of Study A3921133 data, VTE has been determined to be an important identified risk for tofacitinib treatment. Consequently, the tofacitinib SmPC was updated following the European Commission decision on 31 January 2020,[4] to include additional text in the 'Special warnings and precautions for use', 'Undesirable effects' and 'Pharmacodynamic properties' sections pertaining to VTE.[3] We believe that it is important to clarify for your readers that the SmPC updates related to VTE are relevant for the treatment of patients with any condition for which tofacitinib is indicated, including patients with RA or PsA.

Regarding the statement: "tofacitinib may have similar efficacy as the TNFi adalimumab for joint involvement, but numerically lower efficacy in skin psoriasis,[1,15,72]"

Consistent with the results of OPAL Broaden[5] and the SLR publication,[2] tofacitinib has been shown to have similar efficacy to adalimumab in joint and skin psoriasis.

Results from OPAL Broaden show that the proportion of patients achieving ≥75% improvement in the Psoriasis Area and Severity Index at month 3 (secondary endpoint) was 43% for tofacitinib 5 mg twice daily and 39% for adalimumab 40 mg every other week (the study was not designed to compare non-inferiority or superiority between tofacitinib and adalimumab).[5] These results are accurately reported in table 3 in the SLR publication, which also states: "tofacitinib was superior to placebo in csDMARD-IR patients and, although not formally tested, exhibited numerically similar results as adalimumab in OPAL Broaden".[2] To inform understanding of the available data from the tofacitinib clinical programme, we respectfully draw this inaccuracy in the recommendations to the attention of your readers.

Recognising the independence of the EULAR Taskforce from pharmaceutical companies, we seek to correct and clarify the information about JAK inhibitors, and specifically tofacitinib, within the EULAR recommendations, and to provide your readers with the correct information to inform patient care.

Table 1 IRs and HRs of PE and DVT for tofacitinib 5 and 10 mg twice daily versus TNFi (95% CI) from the ad hoc safety analysis of ongoing Study A3921133 (data cut-off: February 2019; database not locked; data not yet source-verified or subjected to standard quality-check procedures that would occur at the time of database lock and may therefore be subject to change)

Safety endpoint		Tofacitinib 5 mg twice daily	Tofacitinib 10 mg twice daily	TNFi
PE	IR (95% CI)	0.27 (0.12 to 0.52)	0.54 (0.32 to 0.87)	0.09 (0.02 to 0.26)
	HR versus TNFi (95% CI)	2.99 (0.81 to 11.06)	5.96 (1.75 to 20.33)	–
DVT	IR (95% CI)	0.30 (0.14 to 0.55)	0.38 (0.20 to 0.67)	0.18 (0.07 to 0.39)
	HR versus TNFi (95% CI)	1.66 (0.60 to 4.57)	2.13 (0.80 to 5.69)	–
Subgroup analysis in patients with VTE risk factors				
PE	HR versus TNFi (95% CI)	3.92 (0.83 to 18.48)	9.14 (2.11 to 39.56)	–

DVT, deep vein thrombosis; IR, incidence rate (unique patients with events per 100 patient-years); PE, pulmonary embolism; TNFi, tumour necrosis factor inhibitor; VTE, venous thromboembolism.

Lara Fallon,[1] Thomas V Jones[2]

[1]Inflammation & Immunology – Global Medical Affairs, Pfizer Inc, Kirkland, Quebec, Canada
[2]Worldwide Medical and Safety, Pfizer Inc, Collegeville, Pennsylvania, USA

Correspondence to Dr Lara Fallon, Inflammation & Immunology – Global Medical Affairs, Pfizer Inc, Kirkland, Quebec, Canada; lara.fallon@pfizer.com

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REFERENCES

1 Gossec L, Baraliakos X, Kerschbaumer A, *et al*. EULAR recommendations for the management of psoriatic arthritis with pharmacological therapies: 2019 update. *Ann Rheum Dis* 2020;79:700–12.
2 Kerschbaumer A, Smolen JS, Dougados M, *et al*. Pharmacological treatment of psoriatic arthritis: a systematic literature research for the 2019 update of the EULAR recommendations for the management of psoriatic arthritis. *Ann Rheum Dis* 2020;79:778–86.
3 European Medicines Agency. Xeljanz (tofacitinib): summary of product characteristics, 2020. Available: https://www.ema.europa.eu/en/documents/product-information/xeljanz-epar-product-information_en.pdf [Accessed 10 Jul 2020].
4 European Medicines Agency. EMA confirms Xeljanz to be used with caution in patients at high risk of blood clots, 2020. Available: https://www.ema.europa.eu/en/documents/referral/xeljanz-article-20-procedure-ema-confirms-xeljanz-be-used-caution-patients-high-risk-blood-clots_en.pdf [Accessed 10 Jul 2020].
5 Mease P, Hall S, FitzGerald O, *et al*. Tofacitinib or adalimumab versus placebo for psoriatic arthritis. *N Engl J Med* 2017;377:1537–50.

Response to: 'Correspondence on 'EULAR recommendations for the management of psoriatic arthritis with pharmacological therapies: 2019 update' by Fallon *et al*

We thank Fallon and Jones[1] for their correspondence on the European League Against Rheumatism (EULAR) recommendations for the management of psoriatic arthritis (PsA) and their comments on the clarity of the specific recommendation regarding the use of Janus kinase inhibitors (JAKi) in the management of PsA,[2] which was based on the associated systematic literature research (SLR).[3]

We appreciate the support of the authors to provide as much clarification to the wording of our recommendations as possible, since these are the currently most up-to-date literature for the current and future treatment of patients with PsA.

In their remarks, Fallon and Jones refer to recommendation 7 and especially the wording on the safety signals of tofacitinib related to events of venous thromboembolism (VTE, including pulmonary embolism (PE) and deep vein thrombosis (DVT)). Indeed, the conclusions of the available literature, so far, are as they describe: in an interim analysis of a study in patients with rheumatoid arthritis (RA) aged ≥50 years and with ≥1 cardiovascular risk factor, the incidence of PE events has been found to be statistically significantly increased in the group treated with tofacitinib 10 mg two times per day (a dose not approved in PsA or RA), when compared with tumour necrosis factor inhibitors (TNFi); however, while not statistically significantly different from the control arm, the data of the 5 mg arm still show a numerical increase in thromboembolic events and thus are right in between control and 10 mg arms. Similarly, the HR for DVT was 1.7 and 3-fold increased for PE compared with control, as Fallon and Jones show in their table. While no data on patients with PsA with cardiovascular risk factors exist, the task force felt that it was important to make the readers aware of these risks, even if primarily coming from RA. Indeed, the warning by the regulators also does not exempt PsA from the risks. Importantly, recommendation 7 is not only referring to the comparison of JAKi with TNFi but also to the use of JAKi in PsA in general.

Fallon and Jones also comment on the sentence comparing the efficacy of tofacitinib and adalimumab on skin psoriasis in the text accompanying recommendation 7. As they mention, the OPAL Broaden data were fully presented in the SLR.[2] However, EULAR recommendations are not solely based on evidence but include experts' opinion and the discussions among the experts are reflected in the text accompanying the recommendations, as is the case here. Of note, the text clearly said that tofacitinib 'may' have 'numerically lower efficacy in skin psoriasis' and not 'has lower efficacy', reflecting the various positions within the expert committee.

Finally, we fully agree with the remark that it is important for the readers to remember that the Summary of Product Characteristics updates related to VTE are relevant for the treatment of patients with any condition for which tofacitinib is indicated, including patients with RA or PsA.

Xenofon Baraliakos ,[1] **Laure Gossec** ,[2,3] **Iain McInnes**,[4] **Andreas Kerschbaumer** ,[5] **Maarten de Wit** ,[6] **Maxime Dougados**,[7] **Jette Primdahl** ,[8,9] **Désirée van der Heijde** ,[10,11] **Josef S Smolen**[5]

[1]Rheumazentrum Ruhrgebiet, Ruhr University Bochum, Herne, Nordrhein-Westfalen, Germany
[2]Institut Pierre Louis d'Epidémiologie et de Santé Publique, INSERM, Sorbonne Universite, Paris, France
[3]APHP, Rheumatology Department, Hopital Universitaire Pitie Salpetriere, Paris, France
[4]Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, UK
[5]Division of Rheumatology, Department of Medicine 3, Medical University of Vienna, Wien, Austria
[6]EULAR past Vice President representing People with Arthritis/Rheumatism in Europe (PARE), Zurich, Switzerland
[7]Department of Rheumatology - Hôpital Cochin. Assistance Publique, Hôpitaux de Paris INSERM (U1153): Clinical epidemiology and biostatistics, PRES Sorbonne Paris-Cité, Université de Paris, Paris, France
[8]Department of Regional Health Research, University of Southern Denmark, Odense, Denmark
[9]Danish Hospital for Rheumatic Diseases, University Hospital of Southern Denmark, Sønderborg, Denmark
[10]Rheumatology, Leiden University Medical Center, Leiden, The Netherlands
[11]Rheumatology, Diakonhjemmet Hospital, Oslo, Norway

Correspondence to Dr Xenofon Baraliakos, Rheumazentrum Ruhrgebiet, Herne 44649, Germany; baraliakos@me.com

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ORCID iDs
Xenofon Baraliakos http://orcid.org/0000-0002-9475-9362
Laure Gossec http://orcid.org/0000-0002-4528-310X
Andreas Kerschbaumer http://orcid.org/0000-0002-6685-8873
Maarten de Wit http://orcid.org/0000-0002-8428-6354
Jette Primdahl http://orcid.org/0000-0002-1049-4150
Désirée van der Heijde http://orcid.org/0000-0002-5781-158X

REFERENCES

1 Fallon L, Jones TV. Correspondence on 'EULAR recommendations for the management of psoriatic arthritis with pharmacological therapies: 2019 update'. *Ann Rheum Dis* 2022;81:e173.
2 Gossec L, Baraliakos X, Kerschbaumer A, *et al*. EULAR recommendations for the management of psoriatic arthritis with pharmacological therapies: 2019 update. *Ann Rheum Dis* 2020;79:700–12.
3 Kerschbaumer A, Smolen JS, Dougados M, *et al*. Pharmacological treatment of psoriatic arthritis: a systematic literature research for the 2019 update of the EULAR recommendations for the management of psoriatic arthritis. *Ann Rheum Dis* 2020;79:778–86.

Diagnostic value of ultrasound halo count and Halo Score in giant cell arteritis: a retrospective study from routine care

We read with great interest the paper published by van der Geest *et al*[1] on 'Novel ultrasonographic Halo Score for giant cell arteritis (GCA): assessment of diagnostic accuracy and association with ocular ischaemia'. The authors aimed to quantify the extent of vascular inflammation by ultrasound (US) in patients with GCA and developed two novel US scoring systems, the halo count and Halo Score, including the assessment of the three temporal artery (TA) segments and axillary arteries. First, we would like to congratulate them for the novelty of their work that opens up new perspectives in the use of US in the assessment of GCA. According to recent EULAR recommendations, US is recommended as the first imaging modality in patients with suspected predominantly cranial GCA.[2] The halo sign is the most relevant US finding in GCA and is defined as a homogeneous, hypoechoic wall thickening, well delineated towards the luminal side, visible in two perpendicular planes, most commonly concentric in transverse scan.[3] The halo count and Halo Score constitute the first quantitative tools to assess the extent of vascular inflammation by US in GCA.[1] According to their findings, a high volume of vascular inflammation on US might strongly support the diagnosis of GCA, is linked to systemic markers of inflammation and identifies patients at risk for ocular ischaemia. On the other hand, a modified Halo Score has been recently proposed by Chattopadhyay *et al*[4] including the assessment of three vascular territories (bilateral temporal, subclavian and axillary arteries) instead of two, as the original Halo Score may underestimate the burden of the inflammation in large-vessel GCA and Takayasu arteritis.

We aim to assess the diagnostic value of both scoring systems and its association with systemic inflammation in patients with GCA seen in routine care. This was a retrospective observational study including patients suspected of having GCA over a 9-month period. All patients underwent bilateral US examination of the three TA segments (common superficial TA, its parietal and frontal branches) and extracranial (carotid, subclavian and axillary) arteries as part of a diagnostic fast track pathway (FTP)[5] where US is undertaken within 24 hours. The extent of vascular inflammation was quantified according to the halo count (number of TA segments and axillary arteries with a halo) ranging from 0 to 8 and the Halo Score (a composite index that incorporates both the number of halos and the maximum halo thickness in each region) ranging from 0 to 48.[1] TA biopsy was performed according to the treating clinician criteria. The gold standard for GCA was the clinical diagnosis after 6 months of follow-up. Validity was analysed by receiver operating characteristic (ROC) curves and correlations were determined by Spearman's rank correlation coefficient (rho).

Fifty-eight patients were evaluated in the FTP (mean age 74.7 years, 65.5% females). Clinical and US variables of patients with and without GCA are shown in table 1. A clinical diagnosis of GCA was established in 15 (25.9%) patients. Only 4.7% patients without GCA versus 86.7% with GCA had positive US findings according to the ultrasonographer criteria (sensitivity (Sens) 86.7%, specificity (Spec) 95.3%, positive likelihood ratio (LR+) 18.4 and negative likelihood ratio (LR−) 0.14). Halo count and Halo Score showed similar diagnostic accuracy for a clinical diagnosis of GCA (area under the ROC

Table 1 Clinical, laboratory and ultrasound findings of patients included in the fast track pathway

	Total, n=58	Patients with GCA, n=15	Patients without GCA, n=43	P value
Age, mean (SD)	74.7 (10.9)	76.5 (10.2)	74 (11.3)	0.431
Sex, no. of female	38 (65.5%)	8 (53.3%)	30 (69.8%)	0.249
Baseline use of steroids, no. of patients	28 (49.1%)	6 (40%)	22 (52.4%)	0.410
TA biopsy positive n=11, no. of patients	3 (27.3%)	3 (37.5%)	0 (0%)	0.491
TA biopsy length (mm) n=11, mean (SD)	5.5 (3.1)	5.9 (3.6)	4.7 (1.5)	0.6
^{18}F-FDG-PET/CT positive n=10, no. of patients	5 (50%)	4 (66.7%)	1 (25%)	0.197
Fulfilling 1990 GCA criteria, no. of patients	13 (22.4%)	5 (33.3%)	8 (18.6%)	0.239
Headache, no. of patients	30 (51.7%)	11 (73.3%)	19 (44.2%)	0.052
Scalp tenderness, no. of patients	4 (6.9%)	2 (13.3%)	2 (4.7%)	0.273
Jaw claudication, no. of patients	10 (17.2%)	7 (46.7%)	3 (7%)	**0.002**
Visual symptoms, no. of patients	10 (17.2%)	5 (33.3%)	5 (11.6%)	0.055
Fever, no. of patients	7 (12.1%)	2 (13.3%)	5 (11.6%)	1
Polymyalgia, no. of patients	27 (46.6%)	10 (66.7%)	17 (39.5%)	0.07
Ocular ischaemia, no. of patients	3 (5.2%)	1 (6.7%)	2 (4.7%)	1
Abnormal TA clinical examination, no. of patients	4 (6.9%)	2 (13.3%)	2 (4.7%)	0.273
CRP (mg/dL), mean (SD)	4.5 (6.7)	9.3 (8.8)	2.7 (4.6)	**0.001**
ESR (mm/h), mean (SD)	51.2 (33.7)	65.7 (33.2)	46.1 (33.1)	0.075
Haemoglobin (g/dL), mean (SD)	12.6 (1.7)	11.9 (1.6)	12.9 (1.6)	0.05
Platelets 10^9/L, mean (SD)	266.8 (96)	307.5 (104.1)	252.2 (89.7)	0.081
Positive US findings, no. of patients	15 (25.9%)	13 (86.7%)	2 (4.7%)	**<0.001**
TA positive US findings, no. of patients	11 (19%)	10 (66.7%)	1 (2.3%)	**<0.001**
Axillary positive US findings, no. of patients	8 (13.8%)	7 (46.7%)	1 (2.3%)	**<0.001**
TA+axillary positive US findings, no. of patients	4 (7%)	4 (26.7%)	0 (0%)	**0.003**
Halo sign positive, no. of patients	15 (25.9%)	13 (86.7%)	2 (4.7%)	**<0.001**
Compression sign positive, no. of patients	8 (13.8%)	7 (46.7%)	1 (2.3%)	**<0.001**
Halo count, mean (SD)	0.7 (1.4)	2.5 (1.9)	0.04 (0.2)	**<0.001**
Halo Score, mean (SD)	4.5 (8.7)	15.8 (9.9)	0.5 (2.7)	**<0.001**

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; 18F-FDG-PET/CT, Fluorine-18 Fluorodeoxyglucose Positron Emission Tomography/Computed Tomography; GCA, giant cell arteritis; TA, temporal artery; US, ultrasound.

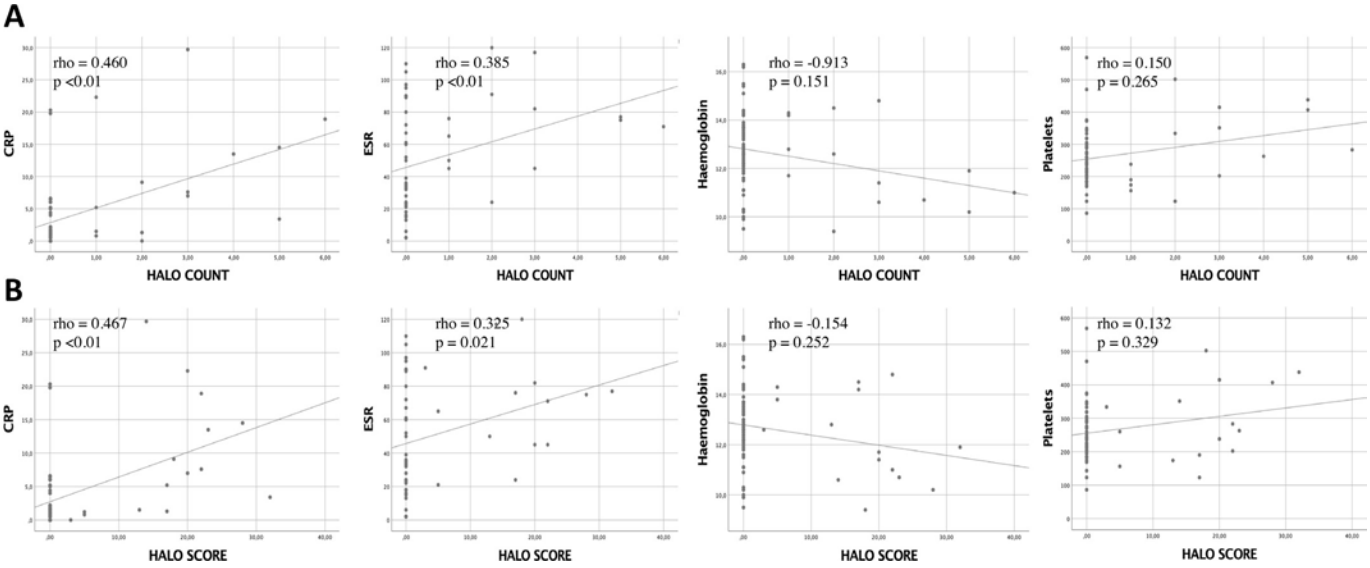

Figure 1 Correlations between halo count (A) and Halo Score (B) with markers of systemic inflammation (CRP, ESR, haemoglobin and platelets). CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

curve of 0.892 and 0.921, respectively). The optimal cut-off point for halo count was ≥1 (Sens 80%, Spec 95.3, LR+ 17.02, LR− 0.21) and for Halo Score ≥2 (Sens 86.7%, Spec 95.3%, LR+18.4, LR− 0.14). Statistically moderate positive correlations were found between halo count and Halo Score and ESR (rho 0.385 and 0.325, $p<0.05$) and C-reactive protein (CRP) (rho 0.460 and 0.467, $p<0.01$), but not with haemoglobin and platelet count ($p>0.05$) (figure 1).

To our knowledge, this is the first study to assess the diagnostic value of the halo count and Halo Score in a routine clinical setting, after its first description by van der Geest *et al*.[1] They first demonstrated that the Halo Score correlated positively with CRP levels and platelet counts and negatively with haemoglobin levels, but they found no correlation with ESR. Our findings confirm the link between both scoring systems with systemic inflammation in GCA, both with CRP and ESR, and show a good diagnostic accuracy in a clinical setting. In summary, the extent of vascular inflammation by US halo count and Halo Score can help to support the diagnosis of GCA in routine care as they correlate with laboratory markers of systemic inflammation. In the future, they may also have a role in monitoring disease activity. Although both scoring systems needs further validation, they can be easily implemented in FTP of patients with GCA.

Juan Molina Collada, Julia Martínez-Barrio, Belén Serrano-Benavente, Isabel Castrejón, Liz Rocío Caballero Motta, Laura Trives Folguera, José María Álvaro-Gracia

Rheumatology, Hospital General Universitario Gregorio Marañón, Madrid, Spain

Correspondence to Juan Molina Collada, Rheumatology, Hospital General Universitario Gregorio Marañón, Madrid 28007, Spain; molinacolladajuan@gmail.com

Twitter Juan Molina Collada @jmolinacollada

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Contributors All authors made substantial contributions to the conception and design of this study. Study design was performed by JMC and IC. Subject recruitment and US examination were performed by JMC. JMC and LRCM collected the epidemiological and clinical data. JMC and IC performed the statistical analysis. JMC, JM-B, BS-B, IC, LRCM, LTF and JMA-G drafted the manuscript. All coauthors revised the final manuscript.

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ORCID iDs
Juan Molina Collada http://orcid.org/0000-0001-5191-7802
José María Álvaro-Gracia http://orcid.org/0000-0002-0343-3747

REFERENCES

1 van der Geest KSM, Borg F, Kayani A, *et al*. Novel ultrasonographic halo score for giant cell arteritis: assessment of diagnostic accuracy and association with ocular ischaemia. *Ann Rheum Dis* 2020;79:393–9.
2 Dejaco C, Ramiro S, Duftner C, *et al*. EULAR recommendations for the use of imaging in large vessel vasculitis in clinical practice. *Ann Rheum Dis* 2018;77:636–43.
3 Bruyn GA, Iagnocco A, Naredo E, *et al*. OMERACT definitions for ultrasonographic pathologies and elementary lesions of rheumatic disorders 15 years on. *J Rheumatol* 2019;46:1388–93.
4 Chattopadhyay A, Ghosh A. 'Halo Score': missing large-vessel giant cell arteritis- do we need a 'modified Halo Score'? *Ann Rheum Dis* 2022;81:e118.
5 Diamantopoulos AP, Haugeberg G, Lindland A, *et al*. The fast-track ultrasound clinic for early diagnosis of giant cell arteritis significantly reduces permanent visual impairment: towards a more effective strategy to improve clinical outcome in giant cell arteritis? *Rheumatology* 2016;55:66–70.

Response to: 'Diagnostic value of ultrasound halo count and Halo Score in giant cell arteritis: a retrospective study from routine care' by Molina Collada *et al*

We would like to thank Molina Collada *et al*[1] for their interest in our paper on the ultrasonographic Halo Score in giant cell arteritis (GCA).[2] We welcome their effort to validate our findings.

The authors have performed a retrospective analysis of the Southend Halo Score and halo count in a GCA fast-track clinic. The authors report an excellent diagnostic accuracy of the Halo Score/halo count for a clinical diagnosis of GCA. The authors also observed a positive correlation between the Halo Score/halo count and systemic inflammation, that is, C reactive protein levels and the erythrocyte sedimentation rate (ESR). The correlation with ESR may reflect measurement by Westergren or a similar accurate method.

Thus, the study by Molina Collada *et al* is indeed the first to validate the feasibility and diagnostic performance of the Southend Halo Score in routine clinical care. Their findings confirm that the Halo Score may help to estimate the burden of inflammation in GCA. As previously stated,[2 3] we agree with the authors that the Halo Score requires further validation. The utility of the Halo Score for the diagnosis, prognosis and monitoring of GCA disease activity is currently under investigation in prospective, multicentre studies (Halo Score for Giant Cell Arteritis (HAS-GCA) National Institute for Health Research Portfolio study #264 294 and ClinicalTrials. gov, NCT03765788). The practicality of the Southend Halo Score and halo count, as recently discussed,[4] could be an important advantage in this context. There is a need for an intrarater and inter-rater reliability exercise to validate these quantitative assessments, and this is planned for the eighth International Ultrasound Workshop on GCA, large-vessel vasculitis and polymyalgia rheumatica at Southend in March 2021.

Kornelis SM van der Geest [1,2] **Bhaskar Dasgupta** [2]

[1]Rheumatology and Clinical Immunology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands
[2]Rheumatology, Southend University Hospital NHS Foundation Trust, Westcliff-on-Sea, UK

Correspondence to Dr Bhaskar Dasgupta, Rheumatology, Southend University Hospital NHS Foundation Trust, Westcliff-on-Sea SS0 0RY, UK; bhaskar.dasgupta@southend.nhs.uk

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Twitter Bhaskar Dasgupta @profbdasgupta

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ORCID iDs
Kornelis SM van der Geest http://orcid.org/0000-0003-2798-6765
Bhaskar Dasgupta http://orcid.org/0000-0002-5523-6534

REFERENCES

1 Molina Collada J, Martínez-Barrio J, Serrano-Benavente B, *et al*. Diagnostic value of ultrasound halo count and halo score in giant cell arteritis: a retrospective study from routine care. *Ann Rheum Dis* 2022;81:e175.
2 van der Geest KSM, Borg F, Kayani A, *et al*. Novel ultrasonographic halo score for giant cell arteritis: assessment of diagnostic accuracy and association with ocular ischaemia. *Ann Rheum Dis* 2020;79:393–9.
3 van der Geest KSM, Borg F, Kayani A, *et al*. Response to: 'Diagnostic accuracy of novel ultrasonographic halo score for giant cell arteritis: methodological issues' by Ghajari and Sabour. *Ann Rheum Dis* 2022;81:e106.
4 van der Geest KSM, Dasgupta B. Response to: "Halo Score': missing large vessel giant cell arteritis- do we need a modified 'Halo Score?" by Chattopadhyay and Ghosh. *Ann Rheum Dis* 2022;81:e119.

Exaggerated neutrophil extracellular trap formation in Kawasaki disease: a key phenomenon behind the outbreak in western countries?

We read with great interest the article by Pouletty *et al* recently published in your journal.[1] The authors described a series of 16 cases with paediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2 (PIMS-TS) in the Paris area. While the affected children exhibited, in a complete or incomplete form, clinical features of Kawasaki disease (KD), they also presented several features distinct from KD, such as an older age at onset and a higher frequency of myocarditis and/or pericarditis, and of resistance to first treatment with intravenous immunoglobulin (IVIG). Clusters of similar cases have been identified in the USA and other European countries since April 2020.[2 3] However, it is still a matter of debate whether PIMS-TS and KD share aetiology and/or pathophysiology, or represent two distinct clinical entities. Herein, we explore the cause behind its outbreak in relation to neutrophil extracellular traps (NETs), a novel killing mechanism of neutrophils.

KD is a multisystem vasculitis that primarily affects coronary arteries of young children, especially in Japan. Although

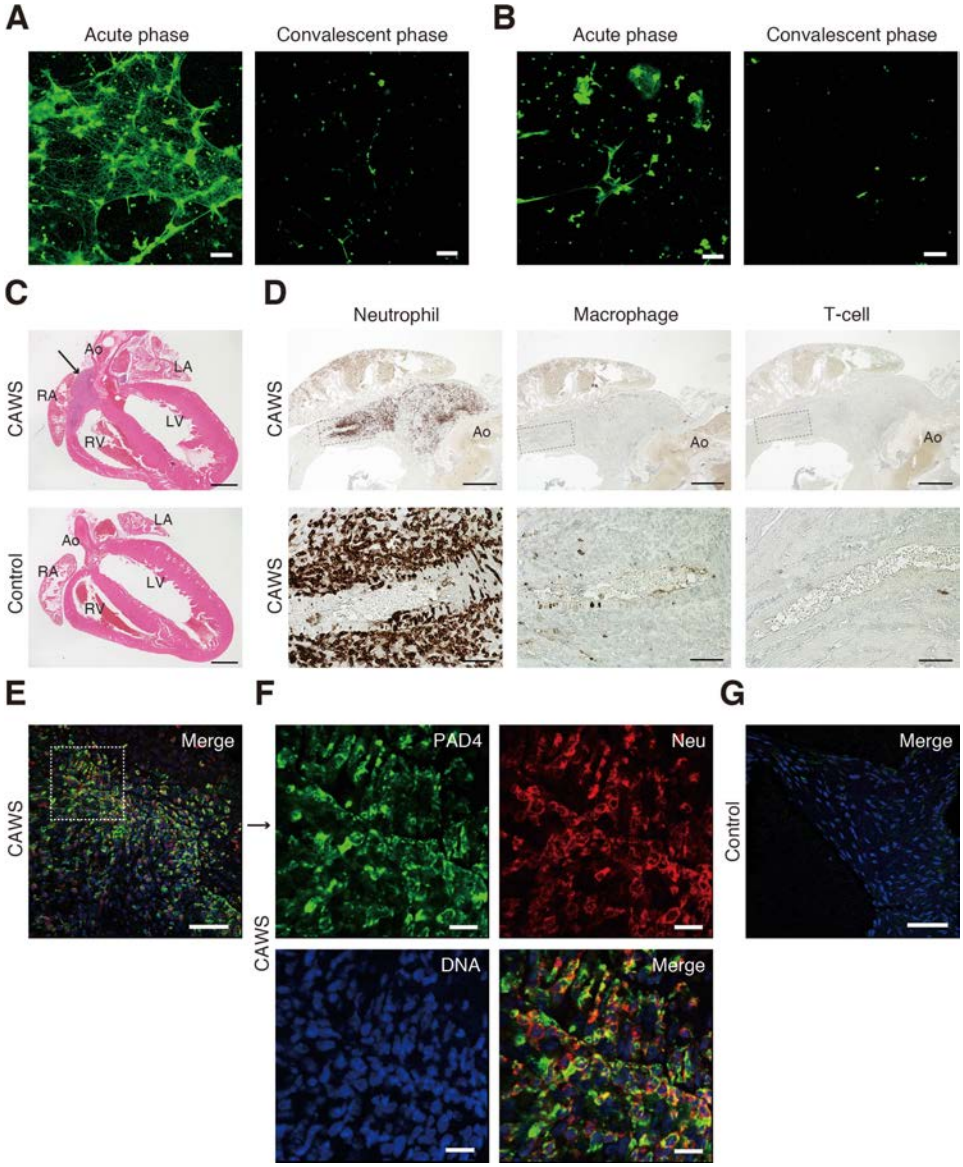

Figure 1 Exaggerated NET formation in patients with KD and model mice. (A, B) The effect of human KD serum on NET formation. Human neutrophils were incubated with the serum from KD cases with serious (A) or mild (B) illness at acute (left panel) or convalescent (right panel) phase for 3 hours. NET formation was visualised using laser scanning fluorescence confocal microscopy. Representative micrographs are shown. Scale bars represent 100 µm. (C) H&E staining of longitudinal sections of hearts from CAWS-treated (upper panel) and control (lower panel) mice. Ao, aorta; LA, left atrium; LV, left ventricle; RA, right atrium; RV, right ventricle. Scale bars represent 1 mm. Arrow indicates panvasculitis at the aortic roots of CAWS-treated mice. (D) Immunoperoxidase staining with anti-Ly-6B.2 (neutrophil) (left panels), anti-F4/80 (macrophage) (middle panels) and CD3 (T-cell) (right panels) in CAWS-treated mice. The lower panels represent high-power views of the boxed areas in the corresponding upper panels. Ao, aorta. Scale bars represent 500 µm (upper panels) and 50 µm (lower panels). (E–G) Immunofluorescence staining with anti-PAD4 (green) and anti-Ly-6B.2 (neutrophil) (red) in CAWS-treated (E, F) and control (G) mice. Nuclei were counterstained with DAPI (blue). Photographs in (F) are high-power views of the boxed areas in (E). Scale bars represent 50 µm (E, G) and 10 µm (F).

the aetiology of KD remains unclear, it may be triggered by infectious agents, leading to exaggerated activation of immune systems in genetically susceptible children. We first investigated whether KD patients' serum stimulates NET formation in human neutrophils. NETs are extracellular structures primarily composed of DNA fibres, histones, and antimicrobial granule proteins such as neutrophil elastase and myeloperoxidase. Although NETs can fight diseases, excessive NET formation is associated with the pathogenesis of autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus, as well as atherosclerosis and thrombosis. The study was approved by the Institutional Review Board at Kyoto University Hospital, and written informed consent was obtained from their parents. Nine cases were analysed at the acute phase before IVIG treatment and convalescent phase 5–10 days after treatment. Serious cases were determined by the risk score proposed by Kobayashi *et al*,[4] and given corticosteroids in addition to IVIG. In all cases, healthy neutrophils stimulated with KD sera in the acute phase produced NETs. Serum from serious cases (figure 1A) stimulated formation of spider-like NETs to a greater extent than milder cases (figure 1B). Serum in the convalescent phase failed to simulate NET formation (figure 1A and B). NETs were not induced by serum from patients with infectious diseases such as upper respiratory infection or gastroenteritis (data not shown).

The role of NETs in the pathogenesis of KD was explored using a mouse model of vasculitis that mimics that of human KD, elicited by *Candida albicans* water-soluble fraction (CAWS). In CAWS-treated mice, a large number of neutrophils infiltrated the aortic root and coronary artery, leading to severe panvasculitis, consistent with KD autopsy findings (figure 1C and D).[5] However, macrophages and/or T cells were scarcely detected (figure 1D). Next, we examined whether infiltrative neutrophils expressed peptidylarginine deiminase 4 (PAD4). NET formation requires conversion of histone arginine residues to citrulline residues by PAD4, an effect that promotes chromatin decondensation. Immunohistochemistry analysis revealed most neutrophils at the aortic root and coronary artery in CAWS-treated mice strongly expressed PAD4 (figure 1E and F), whereas PAD4 expression was not detected in control mice (figure 1G). Results suggest that infiltrative neutrophils in KD-like mice are primed to produce NETs, resulting in severe vasculitis.

SARS-CoV-2 accesses the endothelium via ACE2 binding, which can lead to endothelial injuries, vascular inflammation, and cardiovascular symptoms, such as hypertension and thromboembolism.[6] Zuo *et al* reported high levels of NETs in patients with COVID-19.[7] NET formation induces endothelial injury and vice versa. Excessive NET formation is associated with pathogenesis of thrombosis. Therefore, NETs may play a crucial role in development of cardiovascular defects in patients with COVID-19.[8] This study demonstrated that NET formation is significantly increased in patients with KD, irrespective of pathogen, and mice model. Taken together, we speculate that KD and Kawasaki-like PIMS-TS share the pathophysiology, thus SARS-CoV-2 infection may trigger the development of Kawasaki-like symptoms at least in part via exaggerated NET formation even in relatively non-susceptible children in western countries. Kawasaki-like PIMS-TS may represent a more severe form of KD. Findings may provide insight into development of therapeutic strategies to treat SARS-CoV-2-induced Kawasaki-like PIMS-TS.

Kouhei Yamashita,[1] Akifumi Takaori-Kondo,[1] Kiyomi Mizugishi [1,2]

[1]Department of Hamatology, Kyoto University Hospital, Kyoto, Japan
[2]Department of Pediatrics, Yaizu City Hospital, Yaizu, Shizuoka, Japan

Correspondence to Dr Kiyomi Mizugishi, Department of Hematology, Kyoto University Hospital, Kyoto 606-8507, Japan; kiyomim@kuhp.kyoto-u.ac.jp

Contributors KY performed the experiments, analysed the data and interpreted the data. AT-K interpreted the data and critically revised the manuscript. KM designed the study, performed the experiments, analysed the data, interpreted the data and wrote the manuscript. All authors reviewed the results and approved the final version of the manuscript.

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ORCID iD
Kiyomi Mizugishi http://orcid.org/0000-0001-7558-4352

REFERENCES

1 Pouletty M, Borocco C, Ouldali N, *et al*. Paediatric multisystem inflammatory syndrome temporally associated with SARS-CoV-2 mimicking Kawasaki disease (Kawa-COVID-19): a multicentre cohort. *Ann Rheum Dis* 2020;79:999–1006.
2 Verdoni L, Mazza A, Gervasoni A, *et al*. An outbreak of severe Kawasaki-like disease at the Italian epicentre of the SARS-CoV-2 epidemic: an observational cohort study. *Lancet* 2020;395:1771–8.
3 Feldstein LR, Rose EB, Horwitz SM, *et al*. Multisystem inflammatory syndrome in U.S. children and adolescents. *N Engl J Med* 2020. [Epub ahead of print: 29 Jun 2020].
4 Kobayashi T, Inoue Y, Takeuchi K, *et al*. Prediction of intravenous immunoglobulin unresponsiveness in patients with Kawasaki disease. *Circulation* 2006;113:2606–12.
5 Noval Rivas M, Arditi M. Kawasaki disease: pathophysiology and insights from mouse models. *Nat Rev Rheumatol* 2020;16:391–405.
6 Liu PP, Blet A, Smyth D, *et al*. The science underlying COVID-19: implications for the cardiovascular system. *Circulation* 2020;142:68–78.
7 Zuo Y, Yalavarthi S, Shi H, *et al*. Neutrophil extracellular traps in COVID-19. *JCI Insight* 2020;5:138999.
8 Barnes BJ, Adrover JM, Baxter-Stoltzfus A, *et al*. Targeting potential drivers of COVID-19: neutrophil extracellular traps. *J Exp Med* 2020;217:e20200652.

Response to: 'Exaggerated neutrophil extracellular trap formation in Kawasaki disease: a key phenomenon behind the outbreak in western countries?' by Yamashita *et al*

We read with interest the correspondence from Mizugishi *et al*.[1] The pathophysiology of Kawasaki disease (KD) and more recently Kawasaki-like paediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2 (PIMS-TS) or Kawa-COVID-19 remains largely unknown, even if the infectious trigger by SARS-CoV-2 in the prior weeks seems to be a key feature.[2] Furthermore, it is still uncertain whether Kawasaki-like PIMS-TS can be considered as the same entity as KD or if it should be individualised as a novel distinct condition, as it may have been suggested with several significant clinical and biological differences between classical KD and Kawa-COVID-19.[3]

Mizugishi *et al*[1] speculate that KD and Kawa-COVID-19 share a common pathophysiology through excessive neutrophil extracellular trap (NET) formation. Similar to Yoshida *et al*,[4] Mizugishi *et al*[1] showed increased NET formation in KD patients sera. Through a KD mouse model, the authors describe that severe vasculitis (in the aorta and coronary arteries) was associated with infiltrative neutrophils. Those neutrophils were primed to produce NETs through the PAD4 pathway and seemed to produce a specific type of NET formation (enriched in citrullinated histones). Excessive NET formation was also described in sera of adult COVID-19 patients with endothelial injuries. Kawa-COVID-19 could represent a severe form of KD triggered by an exaggerated NET formation induced by SARS-CoV-2.

NETs are an important first-line defence mechanism against bacterial, viral, fungal and parasitic infections, but they have been also suspected to play a role in autoimmune diseases such as systemic lupus erythematosus (SLE) or antineutrophil cytoplasmic antibodies-associated vasculitis (AAV) for example.[5] Van Dam *et al*[5] showed that NET formation is involved in the pathophysiology of two clinically and pathologically distinct forms of glomerulonephritis in AAV and SLE. The triggers and pathways leading to excessive NET formation in these renal autoimmune diseases are fundamentally different. Therefore, the elucidation of the disease-specific triggers of NET formation and the pathways that are involved is essential to understand the role of NETs and decipher their role in these different pathologies. Moreover, other pathways may be involved in SARS-CoV-2 postinflammatory diseases.

KD seasonality and peaks after viral outbreaks strongly suggest that KD is triggered by an infectious agent.[6 7] It has been shown that, compared with healthy control individuals, patients with KD have an altered Vβ T-cell repertoire (increased frequencies of circulating Vβ2+ and Vβ8.1+ T cells), leading to the hypothesis that a superantigen toxin might have a role in triggering KD.[8–11] The recent increase in KD-like patients after the SARS-CoV-2 outbreak (+497% increase (95% CI: 72 to 1082))[6] corroborates the hypothesis of a viral trigger in KD. Finally, Cheng *et al*[12] have recently found that SARS-CoV-2 encoded a superantigen motif near its S1/S2 cleavage site witch interacts with both the TCR and CD28,[13] resulting in massive production of proinflammatory cytokines including IFNγ, TNFα and IL-2 from T cells, as well as IL-1.[14] This cytokine storm leads to multiorgan tissue damage similar to what is now observed in PIMS-TS. Mice models and human tissue analysis have largely helped to better understand KD pathophysiology. In recent studies, it has been shown that neutrophilic infiltrations of the vessels were responsible for necrotising arthritis as well as other inflammatory mechanisms linked to the innate immune response and more broadly to the cytokine storm.[8]

As shown in our cohort,[3] the onset of the disease occurred 2–4 weeks after acute SARS-CoV-2 infection or exposure and the majority of patients presented no or low nasal SARS-CoV-2 viral loads (Ct >35 in 86%) and positive IgG antibodies, suggesting a postinfectious process. These results are in contrast to severe adult COVID-19 patients, where high viral loads were reported.[15] Thus, NET formation in these patients might be constitutionally different and linked to various pathways.

In conclusion, excessive NET formation seems to be an interesting lead to study and may help to better understand KD and more recently PIMS-TS. However, to better assess the link between KD and Kawa-COVID-19 and the potential role of SARS-CoV-2, NET formation response should be tested with serum from Kawa-COVID-19 patients and compared with classical KD patients and paediatric patients with mild SARS-CoV-2 infection, as a control group. To go further, this comparison should be stretched to severe versus non-severe Kawa-COVID-19 patients, as compared in our cohort, similar to the work of Mizugichi *et al*[1] in classical KD. Finally, larger studies seem necessary and should focus on NET formation molecules as well as morphology, kinetics, specific triggers, pathways and associated immune responses.

Marie Pouletty,[1,2] Glory Dingulu,[1] Naim Ouldali,[1,2,3] Olivier Corseri,[1] Camille Ducrocq,[1,2] Ulrich Meinzer,[1,2,4,5] Albert Faye,[1,2,3] Caroline Galeotti,[6] Isabelle Melki ,[1,7,8] Great Paris Region (GPR) Kawa-COVID-19 consortium

[1]General Paediatrics, Department of Infectious Disease and Internal Medicine, Reference centre for Rheumatic, AutoImmune and Systemic diseases in children (RAISE), Robert Debré University Hospital, AP-HP, Paris, France
[2]Université de Paris, UFR de Médecine Paris Nord, 75010 Paris, France
[3]INSERM UMR 1123, ECEVE, Paris, France
[4]Center for Research on Inflammation, UMR1149, INSERM, Paris, France
[5]Biology and Genetics of Bacterial Cell Wall Unit, Pasteur Institute, Paris, France
[6]Department of Pediatric Rheumatology, Reference centre for Autoinflammatory diseases and amyloidosis (CEREMAIA), Hôpital Bicêtre, AP-HP, Le Kremlin-Bicêtre, France
[7]Paediatric Hematology-Immunology and Rheumatology Department, Reference centre for Rheumatic, AutoImmune and Systemic diseases in children (RAISE), Paris, France, Necker-Enfants-Malades University Hospital, AP-HP, Paris, France
[8]Laboratory of Neurogenetics and Neuroinflammation, Imagine Institute, Paris, France

Correspondence to Isabelle Melki, General Paediatrics, Infectious Diseases and Internal Medicine, Hopital Universitaire Robert Debre, Paris, F-75019, France; isabelle.melki@aphp.fr

Handling editor Josef S Smolen

Collaborators Great Paris Region (GPR) Kawa-COVID-19 consortium: Charlotte Borocco, MD(Department of Pediatric Rheumatology, Hôpital Bicêtre, AP-HP, Reference centre for Autoinflammatory diseases and amyloidosis (CEREMAIA), Le Kremlin-Bicêtre, France, Department of Immunology, Robert Debré University Hospital, AP-HP, Paris, France), Marion Caseris MD (General Paediatrics, Department of Infectious Disease and Internal Medicine, Robert Debré University Hospital, AP-HP, Paris, France, Reference centre for Rheumatic, AutoImmune and Systemic diseases in children (RAISE), Paris, France, Department of Microbiology, Robert Debré University Hospital, AP-HP, Paris, France), Johanna Lokmer MD (Cardiopaediatric Unit, Robert Debré University Hospital, AP-HP, Paris, France), Cherine Benzouid MD (Cardiopaediatric Unit, Robert Debré University Hospital, AP-HP, Paris, France), Constance Beyler MD (Cardiopaediatric Unit, Robert Debré University Hospital, AP-HP, Paris, France), Romain Basmaci MD, PhD (Departments of General Paediatrics and Paediatric Emergency, Louis-Mourier Hospital, AP-HP, Colombes, France, Infection-Antimicrobials-Modelling-Evolution IAME, INSERM, UMR-1137, Université de Paris, 75018, Paris, France), Noémie Lachaume MD (Departments of General Paediatrics and Paediatric Emergency, Louis-Mourier Hospital, AP-HP, Colombes, France, Infection-Antimicrobials-Modelling-Evolution IAME, INSERM, UMR-1137, Université de Paris, 75018, Paris, France), Philippe Bensaid MD (Department of General Paediatrics, Victor Dupouy Hospital, Argenteuil, France), Samia Pichard MD (Department of General Paediatrics, Victor Dupouy Hospital, Argenteuil, France),

Hanane Kouider MD (Department of General Paediatrics, René Dubos, Pontoise Hospital, France), Guillaume Morelle MD (Department of General Paediatrics Hôpital Bicêtre, AP-HP, Kremlin-Bicêtre, France), Irina Craiu MD (Paediatric emergency Department, Hôpital Bicêtre, AP-HP, Kremlin-Bicêtre, France), Corinne Pondarre MD, PhD (Sickle cell disease referal center, Centre hospitalier Intercommunal de Créteil, INSERM U955, Paris XII University), Arielle Maroni MD (Université de Paris, UFR de Médecine Paris Nord, 75010 Paris, France, Paediatric Intensive Care Unit, Robert Debré University Hospital, AP-HP, Paris, France), Anna Deho, MD (Paediatric Intensive Care Unit, Robert Debré University Hospital, AP-HP, Paris, France), Mehdi Oualha MD, PhD (Paediatric Intensive Care Unit, Necker-Enfants-Malades University Hospital, AP-HP, Paris, France), Zahir Amoura MD, MSc (Sorbonne Université, Inserm UMR-S 1135, Department of Immunology and Infectious disease (CIMI-Paris), Pitié-Salpêtrière Hospital, AP-HP, Paris, France), Julien Haroche MD, PhD (Sorbonne Université, Inserm UMR-S 1135, Department of Immunology and Infectious disease (CIMI-Paris), Pitié-Salpêtrière Hospital, AP-HP, Paris, France), Juliette Chommeloux MD (Medical Intensive Care Unit, Institut de Cardiologie, AP-HP, Sorbonne University, Pitié-Salpêtrière Hospital, Paris, France), Fanny Bajolle MD, PhD (Cardiopaediatric Unit, M3-C Necker-Enfants-Malades University Hospital, AP-HP, Paris, France), Stéphane Bonacorsi MD, PhD (Université de Paris, UFR de Médecine Paris Nord, 75010 Paris, France, Department of Microbiology, Robert Debré University Hospital, AP-HP, Paris, France, Infection-Antimicrobials-Modelling-Evolution IAME, INSERM, UMR-1137, Université de Paris, 75018, Paris, France), Guislaine Carcelain MD, PhD (Department of Immunology, Robert Debré University Hospital, AP-HP, Paris, France), Isabelle Kone-Paut MD (Department of Pediatric Rheumatology, Hôpital Bicêtre, AP-HP, Reference centre for Autoinflammatory diseases and amyloidosis (CEREMAIA), Le Kremlin-Bicêtre, France, Department of Immunology, Robert Debré University Hospital, AP-HP, Paris, France), Brigitte Bader-Meunier MD (Paediatric Hematology-Immunology and Rheumatology Department, Necker-Enfants-Malades University Hospital, AP-HP, Paris, France, Reference centre for Rheumatic, AutoImmune and Systemic diseases in children (RAISE), Paris, France, Laboratory of Neurogenetics and Neuroinflammation, Imagine Institute, Paris, France).

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ORCID iD
Isabelle Melki http://orcid.org/0000-0002-8057-333X

REFERENCES

1 Yamashita K, Takaori-Kondo A, Mizugishi K. Exaggerated neutrophil extracellular trap formation in Kawasaki disease: a key phenomenon behind the outbreak in Western countries? *Ann Rheum Dis* 2022;81:e177.
2 Feldstein LR, Rose EB, Horwitz SM, *et al*. Multisystem inflammatory syndrome in U.S. children and adolescents. *N Engl J Med* 2020;383:334–46.
3 Pouletty M, Borocco C, Ouldali N, *et al*. Paediatric multisystem inflammatory syndrome temporally associated with SARS-CoV-2 mimicking Kawasaki disease (Kawa-COVID-19): a multicentre cohort. *Ann Rheum Dis* 2020;79:999–1006.
4 Yoshida Y, Takeshita S, Kawamura Y, *et al*. Enhanced formation of neutrophil extracellular traps in Kawasaki disease. *Pediatr Res* 2020;87:998–1004.
5 van Dam LS, Rabelink TJ, van Kooten C, *et al*. Clinical implications of excessive neutrophil extracellular trap formation in renal autoimmune diseases. *Kidney Int Rep* 2019;4:196–211.
6 Ouldali N, Pouletty M, Mariani P, *et al*. Emergence of Kawasaki disease related to SARS-CoV-2 infection in an epicentre of the French COVID-19 epidemic: a time-series analysis. *Lancet Child Adolesc Health* 2020.
7 Turnier JL, Anderson MS, Heizer HR, *et al*. Concurrent respiratory viruses and Kawasaki disease. *Pediatrics* 2015;136:e609–14.
8 Noval Rivas M, Arditi M. Kawasaki disease: pathophysiology and insights from mouse models. *Nat Rev Rheumatol* 2020;16:391–405.
9 Abe J, Kotzin BL, Jujo K, *et al*. Selective expansion of T cells expressing T-cell receptor variable regions V beta 2 and V beta 8 in Kawasaki disease. *Proc Natl Acad Sci U S A* 1992;89:4066–70.
10 Abe J, Kotzin BL, Meissner C, *et al*. Characterization of T cell repertoire changes in acute Kawasaki disease. *J Exp Med* 1993;177:791–6.
11 Curtis N, Zheng R, Lamb JR, *et al*. Evidence for a superantigen mediated process in Kawasaki disease. *Arch Dis Child* 1995;72:308–11.
12 Cheng MH, Zhang S, Porritt RA, *et al*. An insertion unique to SARS-CoV-2 exhibits superantigenic character strengthened by recent mutations. *bioRxiv* 2020. doi:10.1101/2020.05.21.109272. [Epub ahead of print: 21 May 2020].
13 Arad G, Levy R, Nasie I, *et al*. Binding of superantigen toxins into the CD28 homodimer interface is essential for induction of cytokine genes that mediate lethal shock. *PLoS Biol* 2011;9:e1001149.
14 Krakauer T. Staphylococcal superantigens: pyrogenic toxins induce toxic shock. *Toxins* 2019;11:178.
15 Zheng S, Fan J, Yu F, *et al*. Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang Province, China, January-March 2020: retrospective cohort study. *BMJ* 2020;369:m1443.

2019 American College of Rheumatology/ European League Against Rheumatism classification criteria for IgG4-related disease by Wallace *et al*

We read with interest the original article by Wallace *et al* proposing the new classification for IgG4-related disease (IgG4-RD).[1] So far, the comprehensive diagnostic criteria for IgG4-RD (the comprehensive criteria) have been widely used,[2] but recently, the 2019 American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) classification criteria for IgG4-RD have been developed and validated (the 2019 ACR/EULAR criteria). To determine the agreement rate between the comprehensive and the 2019 ACR/EULAR criteria, we applied the 2019 ACR/EULAR criteria to 40 patients with definite IgG4-RD based on the comprehensive criteria and retrospectively reviewed their medical records. Based on the inclusion criteria of the 2019 ACR/EULAR criteria, total points of ≥20 indicated the classification of IgG4-RD.

The mean age of the patients was 60.2 years, and 29 patients (72.5%) were men. With respect to the immunostaining items, no points were assigned to five patients for lymph node biopsy, although they exhibited both IgG4+/IgG+ cells ratio of >40% and IgG4+ cells/high power field (HPF) of >10 on immunostaining. Finally, 1 of 40 patients (2.5%) with definite IgG4-RD was not reclassified as having IgG4-RD according to the 2019 ACR/EULAR criteria. The 2019 ACR/EULAR criteria do not approve the results of immunostaining if the disease involves the lymph nodes. We described five patients who received no points with respect to the immunostaining items in the 2019 ACR/EULAR criteria in table 1.

Patient 1 exhibited neither information on typical histopathological features of IgG4-RD nor definite evidence of IgG-RD involvement in the chest, pancreas and biliary tree, kidney or retroperitoneum. Thus, patient 1 obtained only 4 points and could not be reclassified as having IgG4-RD despite the increased concentration of serum IgG4 (4 points). Patient 2 exhibited dense lymphocytic infiltrate and storiform fibrosis in the biopsy samples (13 points) and the highest serum IgG4 concentration range (11 points). Patients 3 and 4 exhibited no information on typical histopathological features of IgG4-RD. However, they had the highest serum IgG4 concentration range and definite evidence of IgG-RD involvement in the chest and kidney on a CT scan. Patient 5 exhibited only dense lymphocytic infiltrate in the biopsy sample (4 points) and the highest serum IgG4 concentration range (11 points). In addition, this patient showed abnormalities in one set of glands (6 points) and in the renal pelvic soft tissue (8 points) on a CT scan.

In this study, we elucidated that 97.5% of the patients with definite IgG4-RD were also reclassified as having IgG4-RD according to the 2019 ACR/EULAR criteria in Korea. We also revealed that the biopsy result of the lymph nodes was a crucial negative factor for the classification of IgG4-RD despite the highest serum IgG4 concentration range. Patient 1 had experienced recurrent lymph node enlargement for 3 years. This patient underwent lymph node biopsy five times to exclude malignancies. During this period, the serum IgG4 concentration had consistently increased, and the last two biopsy samples showed markedly increased infiltrating IgG4+ cells counts. Therefore, this patient was diagnosed with IgG4-related lymphadenopathy[3 4] and received glucocorticoids and mycophenolate mofetil.[5] We believe that this paper will be of interest to the readership of the journal because of its immediate clinical impact to patients with suspected IgG4-related disease, as well as our recommendations to physicians attempting to diagnose them. To the best of our knowledge, this is the first pilot study investigating the agreement between the comprehensive criteria and the 2019 ACR/EULAR criteria for IgG4-RD.

Byung-Woo Yoo,[1] Jason Jungsik Song,[1,2] Yong-Beom Park,[1,2] Sang-Won Lee [1,2]

[1]Division of Rheumatology, Department of Internal Medicine, Yonsei University College of Medicine, Seoul, South Korea
[2]Institute for Immunology and Immunological Diseases, Yonsei University College of Medicine, Seoul, South Korea

Correspondence to Professor Sang-Won Lee, Internal Medicine, Yonsei University College of Medicine, Seoul 03722, Korea (the Republic of); SANGWONLEE@yuhs.ac

Contributors B-WY and S-WL designed the report and wrote the paper; B-WY, JJS and S-WL participated in the data acquisition and interpretation; B-WY, Y-BP and S-WL drafted and revised the manuscript; S-WL designed the concept and approved the final paper. All authors have taken care to ensure the integrity of this work, and the final manuscript has been seen and approved by all authors.

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Patient consent for publication Not required.

Ethics approval This study was approved by the institutional review board of Severance Hospital (4-2019-0695), which waived the requirement for obtaining written informed consent from the patients as this was a retrospective study.

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ORCID iD
Sang-Won Lee http://orcid.org/0000-0002-8038-3341

REFERENCES

1 Wallace ZS, Naden RP, Chari S, *et al*. The 2019 American College of Rheumatology/ European League against rheumatism classification criteria for IgG4-related disease. *Ann Rheum Dis* 2020;79:77–87.
2 Umehara H, Okazaki K, Masaki Y, *et al*. Comprehensive diagnostic criteria for IgG4-related disease (IgG4-RD), 2011. *Mod Rheumatol* 2012;22:21–30.
3 Wick MR, O'Malley DP. Lymphadenopathy associated with IgG4-related disease: Diagnosis & differential diagnosis. *Semin Diagn Pathol* 2018;35:61–6.
4 Cheuk W, Chan JKC. Lymphadenopathy of IgG4-related disease: an underdiagnosed and overdiagnosed entity. *Semin Diagn Pathol* 2012;29:226–34.
5 Kamisawa T, Zen Y, Pillai S, *et al*. Igg4-Related disease. *Lancet* 2015;385:1460–71.

Table 1 Patients who underwent biopsy on lymph node

Patients	Biopsy sites	Histology points	Immunostaining points	Serum IgG4 points	Image points	Total inclusion points	Images of organ involved
1	Lymph node	0	0	4	0	4	Only lymph node
2	Lymph node	13	0	11	0	24	Only lymph node
3	Lymph node	0	0	11	14	25	Septal thickening and renal cortex low-density area
4	Lymph node	0	0	11	18	29	Paravertebral lesion and renal pelvis soft tissue
5	Lymph node	4	0	11	14	29	One set of gland and renal pelvis soft tissue

Glucosamine and mortality: a note of caution

I read with interest the paper by Li *et al*[1] reporting the association of regular glucosamine use with lower mortality. The authors report significantly lower all-cause mortality HR 0.85 (95% CI 0.82 to 0.89), cardiovascular mortality HR 0.82 (95% CI 0.74 to 0.90), cancer mortality HR 0.94 (95% CI 0.88 to 0.99), respiratory mortality HR 0.73 (95% CI 0.66 to 0.81) and digestive mortality HR 0.74 (95% CI 0.62 to 0.90). The magnitude of the reported reduction in mortality is striking, as is the consistency across major disease categories. The results reported by the authors are consistent with other prior epidemiological studies looking at glucosamine and mortality.[2–4]

The biological plausibility for glucosamine having such pronounced causative effects on mortality, particularly across the entire spectrum of disease, is somewhat tenuous. The authors suggest inhibition of NF-kB(nuclear factor-kappa B) thereby reducing inflammation and glucosamine triggering a mimic response of low carbohydrate diet in animal models as potential explanations.[1] While there is validity to these hypotheses it is difficult to envisage the translation of these pathways with such marked improvements in mortality.

We have seen such beneficial associations with mortality reported for other supplements previously, perhaps most notably with vitamin D. Later detailed analyses have revealed that these associations appear to be due to other factors including unmeasured confounders and reverse causation. In the current work the authors have attempted to correct for important potential confounding factors including deprivation, lifestyle behaviours and non-steroidal anti-inflammatory drug use. However, despite such efforts it is likely that residual unmeasured confounders remain when using observational data such as this. Whether these unmeasured factors are important determinants of outcomes may be difficult to ascertain. As an illustration of this point, we recently reported an analysis of broadband Internet access as a predictor of emergency medical admission rates.[5 6] We chose broadband access for this purpose as an example of a clear non-causative association, yet despite controlling for other measures of deprivation, it remained a significant predictor of admission rates, almost certainly as a surrogate of other socioeconomic factors. Similarly, I suggest it is more likely that the association between mortality and glucosamine use reflects unmeasured underlying healthcare behaviours or other confounders. For example, those taking regular glucosamine supplements may be more likely to engage in a multitude of other beneficial health-related behaviours which have a cumulative effect on decreasing mortality.

Randomised controlled trials (RCTs) represent the ideal setting to clarify these issues. To my knowledge, previous RCTs of glucosamine have not suggested any evidence of a beneficial effect on mortality. One could argue that any such RCT would need to be so large and of such long duration as to be unfeasible, however, given the magnitude of benefit of glucosamine suggested by the current report, this is not the case and a RCT to assess this degree of benefit should be manageable.

In conclusion, while it would be gratifying to believe that glucosamine could be a panacea for a longer life, the current data are more likely to reflect regular glucosamine use as a surrogate marker for other unmeasured factors.

Richard Conway

Correspondence to Dr Richard Conway, Rheumatology, Saint James's Hospital, Dublin, Ireland; drrichardconway@gmail.com

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ORCID iD
Richard Conway http://orcid.org/0000-0003-2538-3362

REFERENCES

1 Li Z-H, Gao X, Chung VC, *et al*. Associations of regular glucosamine use with all-cause and cause-specific mortality: a large prospective cohort study. *Ann Rheum Dis* 2020;79:829–36.
2 Bell GA, Kantor ED, Lampe JW, *et al*. Use of glucosamine and chondroitin in relation to mortality. *Eur J Epidemiol* 2012;27:593–603.
3 Brasky TM, Lampe JW, Slatore CG, *et al*. Use of glucosamine and chondroitin and lung cancer risk in the vitamins and lifestyle (vital) cohort. *Cancer Causes Control* 2011;22:1333–42.
4 Ma H, Li X, Sun D, *et al*. Association of habitual glucosamine use with risk of cardiovascular disease: prospective study in UK Biobank. *BMJ* 2019;365:l1628.
5 Conway R, Cournane S, Byrne D, *et al*. Broadband access, housing and CAR ownership as deprivation predictors of the emergency medical admission. *Eur J Intern Med* 2016;35:e25–6.
6 Conway R, Cournane S, Byrne D, *et al*. Interaction of broadband access and education levels in determining the rate of emergency medical admissions. *Ir Med J* 2017;110:636.

Response to: 'Glucosamine and mortality: a note of caution' by Conway

We appreciate the comments by Conway[1] on our manuscript which investigated the association of regular glucosamine use with all-cause and cause-specific mortality.[2]

First, we agree that the underlying mechanisms, including inhibition of nuclear factor-κB thereby reducing inflammation and glucosamine triggering a mimic response of low carbohydrate diet, might partially explain the association between glucosamine use and mortality. Future studies are needed to better understand underlying pharmacological roles of glucosamine on health outcomes.

Second, the association of mortality with glucosamine use might be confused by unmeasured underlying lifestyle-related factors or other confounders. Nevertheless, in our analyses, we had carefully adjusted for several important confounders, including sociodemographic factors, lifestyle behaviours, health status, drug use and other supplements use. In total, 27 confounders were included in our fully adjusted models, and the adjustment for confounding was sufficient.

Third, randomised controlled trial (RCT) is indeed the ideal study design to clarify these issues. However, most previous RCTs, investigating the association between glucosamine use and health outcomes, mainly focused on the therapy in treating patients with osteoarthritis or other chronic diseases,[3–5] who might have relatively poorer prognoses than healthy people in primary prevention trials. Moreover, several previous large-scale cohort studies found the similar magnitude of benefit of glucosamine on mortality,[6–8] which are generally consistent with our study. Therefore, in the absence of sufficient sample size and sufficient trail duration, it might be difficult for previous RCTs to find such magnitude of benefit of glucosamine. Further studies are necessary to better clarify the association between glucosamine use and mortality.

Zhi-Hao Li, Qing-Mei Huang, Wen-Fang Zhong, Xi-Ru Zhang, Chen Mao

Department of Epidemiology, School of Public Health, Southern Medical University, Guangzhou, Guangdong, China

Correspondence to Professor Chen Mao, Department of Epidemiology, School of Public Health, Southern Medical University, Guangzhou, Guangdong 510515, China; maochen9@smu.edu.cn

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ORCID iD
Chen Mao http://orcid.org/0000-0002-6537-6215

REFERENCES

1 Conway. Glucosamine and mortality, a note of caution. *Ann Rheum Dis* 2022;81:e180.
2 Li Z-H, Gao X, Chung VC, *et al*. Associations of regular glucosamine use with all-cause and cause-specific mortality: a large prospective cohort study. *Ann Rheum Dis* 2020;79:829–36.
3 Towheed TE, Maxwell L, Anastassiades TP, *et al*. Glucosamine therapy for treating osteoarthritis. *Cochrane Database Syst Rev* 2005;51:CD002946.
4 Towheed TE, Anastassiades TP. Glucosamine and chondroitin for treating symptoms of osteoarthritis: evidence is widely touted but incomplete. *JAMA* 2000;283:1483–4.
5 Runhaar J, Rozendaal RM, van Middelkoop M, *et al*. Subgroup analyses of the effectiveness of oral glucosamine for knee and hip osteoarthritis: a systematic review and individual patient data meta-analysis from the oa trial bank. *Ann Rheum Dis* 2017;76:1862–9.
6 Bell GA, Kantor ED, Lampe JW, *et al*. Use of glucosamine and chondroitin in relation to mortality. *Eur J Epidemiol* 2012;27:593–603.
7 Ma H, Li X, Sun D, *et al*. Association of habitual glucosamine use with risk of cardiovascular disease: prospective study in UK Biobank. *BMJ* 2019;365:l1628.
8 Pocobelli G, Kristal AR, Patterson RE, *et al*. Total mortality risk in relation to use of less-common dietary supplements. *Am J Clin Nutr* 2010;91:1791–800.

Correspondence to 'Associations of regular glucosamine use with all-cause and cause-specific mortality: a large prospective cohort study' by Li *et al*

With great interest, we have read the recent article from Li *et al*, which addressed the link between regular glucosamine use and all-cause and cause-specific mortality in a large prospective cohort from the UK biobank.[1] These authors are dedicated to providing valuable insights and comprehensive analysis for HRs associated with glucosamine use, 0.85 (95% CI 0.82 to 0.89) of all-cause mortality, 0.82 (95% CI 0.74 to 0.90) of cardiovascular disease mortality, 0.94 (95% CI 0.88 to 0.99) of cancer mortality, 0.73 (95% CI 0.66 to 0.81) of respiratory mortality and 0.74 (95% CI 0.62 to 0.90) of digestive mortality. However, some methodological issues of these findings must be considered.

First, the definition of regular glucosamine use should be described more detailed. It is important to present factors such as dosage, frequency and treatment adherence, which may make confounded dose–response effects. Optimal dosage, which builds a basis for exhibiting the effectiveness of drugs at various levels of dosage, can also provide a better approach to assess the protective effect of glucosamine. For example, Simon *et al* used high/low dose, date, number of pills, defined daily dose, cumulative dose and duration to evaluate the association of aspirin with hepatocellular carcinoma and liver-related mortality.[2] Second, we observed that some residual confounders would have to be strongly related to HRs of mortality, like stress, air pollution and nutrition status. To the best of our knowledge, negative controls can be a method of detecting uncontrolled confounding. They are irrelevant factors designed for finding spurious causal inference and generally expected to give a result of no association after an analysis. When it turns out different, the main association may be biased by the same procedures which brought about the failure of negative-control experiment.[3–5] Therefore, we recommend negative-control outcome (ie, glucosamine and accidental trauma) and negative-control exposure (ie, dental care participation and mortality) as two kinds of negative control design to improve causal inference of this study. Third, the habits of glucosamine use are likely to have inference with HRs of mortality. Glucosamine users often take glucosamine as a nutritional supplement. They regard it as one of the methods to keep a healthy lifestyle, thus be more physically active as well as attentive to keep their bodies in a robust condition. Therefore, the protective effect of glucosamine in mortality might be overestimated.

Besides, glucosamine is also used for treatments in patients with the history of osteoarthritis who may have relatively poor prognoses.[1] Therefore, the protective effect of glucosamine in mortality might be underestimated. These choices cause doubts of confounding by indication that tends to happen when the clinical indication for selecting a particular medicine is also linked to the outcome of interest.[6]

As mentioned above, the bias of (1) confounded dose–response effect, (2) residual confounders and (3) confounding by indication should be taken into consideration to make this study more convincing based on the adequate database.

Hann-Ziong Yueh [1], **Chih-Jung Yeh**,[2] **James Cheng-Chung Wei** [3,4,5]

[1]Department of Medical Education, Chung Shan Medical University Hospital, Taichung, Taiwan
[2]Department of Public Health, Chung Shan Medical University, Taichung, Taiwan
[3]Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan
[4]Department of Allergy, Immunology & Rheumatology, Chung Shan Medical University Hospital, Taichung, Taiwan
[5]Graduate Institute of Integrated Medicine, China Medical University, Taichung, Taiwan

Correspondence to Dr James Cheng-Chung Wei, Institute of Medicine, Chung Shan Medical University, Taichung 40201, Taiwan; jccwei@gmail.com

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ORCID iDs
Hann-Ziong Yueh http://orcid.org/0000-0002-6776-0982
James Cheng-Chung Wei http://orcid.org/0000-0003-0310-2769

REFERENCES

1 Li Z-H, Gao X, Chung VC, *et al*. Associations of regular glucosamine use with all-cause and cause-specific mortality: a large prospective cohort study. *Ann Rheum Dis* 2020;79:829–36.
2 Simon TG, Duberg A-S, Aleman S, *et al*. Association of aspirin with hepatocellular carcinoma and liver-related mortality. *N Engl J Med* 2020;382:1018–28.
3 Lousdal ML, Lash TL, Flanders WD, *et al*. Negative controls to detect uncontrolled confounding in observational studies of mammographic screening comparing participants and non-participants. *Int J Epidemiol* 2020. doi:10.1093/ije/dyaa029. [Epub ahead of print: 25 Mar 2020].
4 Lipsitch M, Tchetgen Tchetgen E, Cohen T. Negative controls: a tool for detecting confounding and bias in observational studies. *Epidemiology* 2010;21:383–8.
5 Hamer M, Bauman A, Bell JA, *et al*. Examining associations between physical activity and cardiovascular mortality using negative control outcomes. *Int J Epidemiol* 2019;48:1161–6.
6 Kyriacou DN, Lewis RJ. Confounding by indication in clinical research. *JAMA* 2016;316:1818–9.

Response to: 'Correspondence to 'Associations of regular glucosamine use with all-cause and cause-specific mortality: a large prospective cohort study' by Li *et al*' by Yueh *et al*

As suggested by Yueh *et al*,[1] it is important to present factors such as dosage, frequency and treatment adherence, which may make confounded dose–response effects. However, as explained at length in the limitation section,[2] the UK Biobank did not gather detailed information on the dosage, forms or duration of glucosamine use to perform further analyses on the dose relationship of glucosamine with the mortality, like Simon *et al*.[3] And the data collection on dietary supplements intake was not conducted in clinical settings in order to promote more truthful reporting. Further studies are necessary to better clarify the dose-relationship of glucosamine use with the mortality.

Yueh *et al*[1] mentioned that some residual confounders would have to be strongly related to HRs of mortality, such as stress, air pollution, and nutrition status. According to the published literature,[4–6] a total of 27 confounders, the most important risk factors for mortality, were included in our fully adjusted models, among which depression status reflects stress, as well as Townsend Deprivation Index, body mass index and the use of various drugs reflect nutritional status to some extent. Actually, we also evaluated the impact of air pollution, including nitrogen oxides and particulate matter, on the relationship between glucosamine and mortality, and found that the results did not vary substantially. Due to the hard limit on word count of the manuscript required by the journal, the content for the impact of air pollution on results was not included in the manuscript.

Obviously, with the current observational study design, the possibility of residual confounding due to imprecise measurements or unknown factors cannot be excluded for all findings in our study, despite our careful adjustment of all measured confounders.[2] Negative controls may be an effective tool for detecting confounding and bias in observational studies.[7] Future studies with negative control design are necessary to improve causal inference, as the Yueh *et al* suggested.[1]

In regard to the question of the bias of confounding by healthy lifestyle, we acknowledged that regular glucosamine use may be a marker for a healthy lifestyle, as the results of our study showed, compared with non-users, glucosamine users were more likely to be current non-smokers and more physically active.[2] However, it is difficult to disentangle the effects of a healthy lifestyle from the use of glucosamine in an observational study, although the potential confounders were carefully adjusted in our analyses. We also clarified this issue in the limitations of the manuscript.[4]

Finally, glucosamine is also used to treat patients with arthritis, which might lead to underestimation of the protective effect of glucosamine due to the poor prognoses of arthritic patients. Therefore, we have included arthritis in the fully adjusted models in our study. In addition, we conducted additional subgroup analysis to assess potential modification effect by modelling the cross-product term of arthritis (yes or no) with glucosamine use in the fully adjusted model, and found that the associations of glucosamine use with all-cause and cause-specific mortality were not significantly modified by arthritis.

Zhi-Hao Li, Wen-Fang Zhong, Qing-Mei Huang, Xi-Ru Zhang, Chen Mao

Department of Epidemiology, School of Public Health, Southern Medical University, Guangzhou, Guangdong, China

Correspondence to Professor Chen Mao, School of Public Health, Southern Medical University, Guangzhou 510515, China; maochen9@smu.edu.cn

Contributors Z-HL, W-FZ, Q-MH, X-RZ and CM wrote the manustrcipt.

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ORCID iD
Chen Mao http://orcid.org/0000-0002-6537-6215

REFERENCES

1 Yueh H-Z, Yeh C-J, JC-C W. Correspondence to 'Associations of regular glucosamine use with all-cause and cause-specific mortality: a large prospective cohort study' by Li et al. *Ann Rheum Dis* 2022;81:e182.
2 Li Z-H, Gao X, Chung VC, *et al*. Associations of regular glucosamine use with all-cause and cause-specific mortality: a large prospective cohort study. *Ann Rheum Dis* 2020;79:829–36.
3 Simon TG, Duberg A-S, Aleman S, *et al*. Association of aspirin with hepatocellular carcinoma and Liver-Related mortality. *N Engl J Med* 2020;382:1018–28.
4 Kantor ED, Lampe JW, Navarro SL, *et al*. Associations between glucosamine and chondroitin supplement use and biomarkers of systemic inflammation. *J Altern Complement Med* 2014;20:479–85.
5 Ma H, Li X, Sun D, *et al*. Association of habitual glucosamine use with risk of cardiovascular disease: prospective study in UK Biobank. *BMJ* 2019;365:l1628.
6 Bell GA, Kantor ED, Lampe JW, *et al*. Use of glucosamine and chondroitin in relation to mortality. *Eur J Epidemiol* 2012;27:593–603.
7 Lipsitch M, Tchetgen Tchetgen E, Cohen T. Negative controls: a tool for detecting confounding and bias in observational studies. *Epidemiology* 2010;21:383–8.

Persistence of rT-PCR-SARS-CoV-2 infection and delayed serological response, as a possible effect of rituximab according to the hypothesis of Schulze-Koops *et al*

The large study of 600 cases from 40 countries of the Covid-Global Rheumatology Alliance has shown that the use of conventional disease-modifying antirheumatic drug, alone or in combination with biologics/Janus Kinase inhibitors, and tumour necrosis factor inhibitor was associated with a reduced odds of hospitalisation.[1] Schulze-Koops *et al* described two fatal outcomes in patients with rheumatoid arthritis treated with rituximab and focuses on careful vigilance on immunosuppression in the treatment of immune-mediated rheumatic diseases.[2] To reinforce this observation, we report the case of a female patient aged about 60 years old, with a history of polymyositis and Sjögren's syndrome. Her history was significant for previous cancer: thyroid carcinoma in 1995, phylloid tumour of the right breast in 2010 and carcinoid of the annexes in 2011. In January 2020, the patient had problem of fatigue, myalgia, together with rise in creatinekinase (CK) and Aldolase with positive anti-Mi-2 antibody, while on stable maintenance treatment with methotrexate 12.5 mg/weekly and prednisone 10 mg/day, rituximab 1 g 2 weeks apart was employed since 2017 with further cycles on the basis of B lymphocyte count (about 39 weeks between each cycle, accounting for four cycles in total). The last rituximab cycle was administered on February 2020. On 4 April, the patient showed fever 38.5°C, cough, dyspnoea, with the Horovitz Index of 108 and an X-ray demonstrating bilateral basal pulmonary thickenings with Echo Score of 38/42. The nose-pharyngeal swab rT-PCR test for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was found positive. She was admitted to the Covid-Unit Emergency Department, and soon transferred in intensive care unit for assisted ventilation and for subsequent endotracheal invasive ventilation. Table 1 shows clinical, laboratory parameters and treatments from hospitalisation to home discharge and in the subsequent follow-up. Laboratory data of the patient show an absence of replication in rT-PCR after treatment with remdesivir consisting of a loading dose of 200 mg intravenously on day 1, plus 100 mg daily for the following 9 days and 3 U of convalescent plasma. The treatments used also included tocilizumab (two 800 mg cumulative dose infusions), three-cycle intravenous immunoglobulins (0.4 g/kg day for 5 consecutive days) and dexamethasone 6 mg one time a day (table 1). During the hospitalisation and also 1-month follow-up after discharge, we did not detect anti-SARS-CoV-2 IgM and IgG production above the cut-off (iFlash1800 CLIA analyzer for anti-SARS CoV-2 antibodies IgM and IgG with a cut-off value of 10.0 AU/mL for both IgM and IgG antibodies). Furthermore, we did not observe B lymphocyte recovery in such subpopulations ($CD27^+$ naive, $CD27^+$ memory, $CD38^+$, $CD20^+$, $CD19^+$) evaluated by flow cytometry (FACS CANTO II, BD Biosciences). B-lymphocytes play a key role through the B cell receptor in the early stages of innate immunity, along with the natural killer (NK) cells. B cells are important in the viral antigen processing mechanism, and also $CD19^+IgG^+$ memory elements are crucial to build up an immunological memory as well as in the production of antibodies with IgM (5–15 day) and IgG (10–21 day) isotypes, also in the response to a reinfection.[3] Thus, rituximab may be hazardous in the present pandemic scenario, since it alarmingly inhibits the humoral response to SARS-CoV-2 infection and might contribute to possible secondary worsening.[4] Differently from Schulze-Kopp's cases, who underwent a fixed retreatment

Table 1 Clinic and laboratory data before treatment with rituximab, during hospitalisation, and in the follow-up

	January 28	April 4	April 10	April 17	April 24	May 1	May 8	May 15	May 25	June 6	July 2
RT-PCR		+	+	+	+	+	+	+	+	–	–
IgM-SARS-CoV-2 AU/mL		0.2	0.3	0.2	0.2	0.32	0.23	0.26	0.25	0.32	0.1
IgG-SARS-CoV-2 AU/mL		2.3	2.5	0.7	0.6	0.41	0.21	0.34	9.6	11.6	0.3
CD3+ cells/µL	2452	1802	2387	1238	1341	1156	858	784	778	1356	2078
CD4+ cell/µL	1315	1223	1677	856	736	578	416	389	403	758	905
CD8+ cell/µL	689	531	625	212	578	411	302	305	285	378	1107
CD56+ cell/µL	337	137	129	78	134	98	65	53	89	215	433
CD19+ cell/µL	26	1	3	2	0.2	0.4	0.3	0.6	0.1	0.6	1
CD20+ cell/µL	20	1	2	1	0.5	0.7	0.8	0.4	0.4	0.5	0.1
CD27+ naive cell/µL	22	0.1	0.6	0.6	0.1	0.6	0.4	0.7	0.6	0.3	1
CD27+ memory cell/µL	12	1.3	0.4	0.4	0.4	0.5	0.5	0.7	0.4	0.5	0.1
CD38+ cell/µL	9	0.4	0.5	0.7	0.6	0.3	0.1	0.6	0.6	2.3	1.4
IgG mg/dL	643	689	673	613	518	568	1856	1789	2134	789	637
IgA mg/dL	67	56	79	69	87	73	77	89	78	81	97
IgM mg/dL	71	68	62	56	53	51	89	91	98	101	78
Horovitz Index		108	96	48	51	49	46	56	170	280	300
Echo Score		38	40	38	36	32	38	36	28	14	8
Antimalarials		+	+	+	+	+	+	–	–	–	+
Antiviral treatments		LOP-RIT	LOP-RIT	LOP-RIT					REM	REM	
Biological agent			TOC			TOC	IVIG	IVIG	IVIG-CP	CP	
Other treatments	PDN MTX	DEX HEP AZI	DEX HEP AZI	DEX HEP AZI	DEX HEP	DEX HEP	DEX HEP MER LIN	DEX HEP MER LIN	DEX HEP	PDN HEP	PDN HEP

Horovitz Index (SpO_2/FiO_2 ratio).
-, absent; +, present; CP, convalescent plasma; DEX, dexamethasone; HEP, heparin; IVIG, intravenous immunoglobulin; LOP-RIT, lopinavir/ritonavir; MER-LIN, meropenem/linezolid; MTX, methotrexate; PDN, prednisone; REM, remdesivir; TOC, tocilizumab.

rituximab schedule, our patient undergoing a retreatment regimen at B cell recovery showed a good outcome. Notably, IgG levels before the last rituximab infusion were low, but over the dangerous threshold of 400 mg/dL. Therefore, it might be argued that rituximab retreatment regimen at B cell recovery rather than fixed retreatment schedule might be safer. Moreover, dexamethasone may have contributed to the favourable outcome of our patient.[5] According to European League Against Rheumatism recommendations,[6] patients undergoing cyclophosphamide or rituximab-mediated immunosuppression should represent the population of patients with rheumatic disease at highest risk of COVID-19 infection and its most severe consequences at this time; therefore, administration of these treatments should be based on a careful risk–benefit ratio. If needed, retreatment with rituximab should be based on B cell recovery or clinical (or laboratory) relapse, according to the disease, rather than with a fixed time schedule. Finally, a deeper analysis of the effect of each biological agent on COVID-19 infection is warranted.

Maurizio Benucci ,[1] **Luca Quartuccio**,[2] **Francesca Li Gobbi**,[3] **Arianna Damiani**,[4] **Valentina Grossi**,[5] **Maria Infantino** ,[6] **Mariangela Manfredi**[7]

[1]Rheumatology Unit, Ospedale San Giovanni di Dio, Firenze, Toscana, Italy
[2]Internal Medicie and Rheumatology Unit University of Udine, Udine, Italy
[3]Rheumatology Unit, Ospedale San Giovanni di Dio Hospital, Florence, Italy
[4]Rheumatology Unit Department Experimental Medicine University of Florence, Florence, Italy
[5]Maria Infantino Mariangela Manfredi Laboratory of Immunology and Allergology SGiovanni di Dio Hospital, Florence, Italy
[6]Laboratory of Immunology and Allergology, Ospedale San Giovanni di Dio, Firenze, Italy
[7]Ospedale San Giovanni di Dio, Firenze, Italy

Correspondence to Dr Maurizio Benucci, Rheumatology Unit, Ospedale San Giovanni di Dio, Firenze, Toscana, Italy; maubenucci@tiscali.it

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ORCID iDs
Maurizio Benucci http://orcid.org/0000-0001-8180-6660
Maria Infantino http://orcid.org/0000-0002-6200-4467

REFERENCES

1 Gianfrancesco M, Hyrich KL, Al-Adely S, *et al*. Characteristics associated with hospitalisation for COVID-19 in people with rheumatic disease: data from the COVID-19 global rheumatology alliance physician-reported registry. *Ann Rheum Dis* 2020;79:859–66.
2 Schulze-Koops H, Krueger K, Vallbracht I, *et al*. Increased risk for severe COVID-19 in patients with inflammatory rheumatic diseases treated with rituximab. *Ann Rheum Dis* 2021;80:e67.
3 Vabret N, Britton GJ, Gruber C, *et al*. Immunology of COVID-19: current state of the science. *Immunity* 2020;52:910–41.
4 Avouac J, Airó P, Carlier N, *et al*. Severe COVID-19-associated pneumonia in 3 patients with systemic sclerosis treated with rituximab. *Ann Rheum Dis* 2021;80:e37.
5 Johnson RM, Vinetz JM. Dexamethasone in the management of covid -19. *BMJ* 2020;370:m2648.
6 Landewé RB, Machado PM, Kroon F, *et al*. EULAR provisional recommendations for the management of rheumatic and musculoskeletal diseases in the context of SARS-CoV-2. *Ann Rheum Dis* 2020;79:851–8.

Treatment of patients with inflammatory rheumatic diseases with rituximab should be carefully considered during the SARS-CoV-2/COVID-19 pandemic. Response to: 'Persistence of rT-PCR-SARS-CoV-2 infection and delayed serological response, as a possible effect of rituximab according to the hypothesis of Schulze-Koops et al' by Benucci *et al*

We thank Dr Benucci *et al* for their comments[1] on our report on fatalities of patients with inflammatory rheumatic diseases (IRDs) treated with rituximab (RTX) during the SARS-CoV-2/COVID-19 pandemic.[2] The authors present a case of COVID-19 in a patient with myositis treated with RTX, who required assisted ventilation and eventually recovered after intensive care including invasive ventilation and medication with remdesivir, dexamethason and tocilizumab. While emphasising the potential of RTX to lead to severe courses of COVID-19, a particularly interesting aspect of the report is the complete absence of antibodies to SARS-CoV-2 even up to 4 weeks after discharge of the patient. The authors therefore conclude that RTX may be hazardous in the present pandemic as it may inhibit the humoral response to SARS-CoV-2 and contribute to secondary worsening of COVID-19.

The case of Dr Benucci reinforces our recommendation for caution and careful vigilance when considering treating patients with IRD with RTX in times of SARS-CoV-2. We had illustrated our concerns on two patients with RTX-treated rheumatoid arthritis who developed fatal COVID-19 and we had hypothesised that persistent B cell depletion and comedication with glucocorticoids may have resulted in severe combined cellular and humoral immunodeficiency. This assumption was based on the well-known association of RTX treatment with an increased risk for the development of viral infections, such as JC virus, hepatitis B virus or cytomegalovirus[3] and the aggressive course of COVID-19 in patients with common variable immunodeficiency.[4] Supporting our hypothesis is a recent publication on persistent SARS-CoV-2 viraemia in two rituximab-treated patients with severe COVID-19 pneumonia until death without any sign of viral clearance.[5] It is intriguing to speculate that a defect in viral clearance may underlie also the unusual course of COVID-19 in patients with IRD treated with RTX that was recently published: Three patients with systemic sclerosis routinely treated with RTX who were affected by COVID-19 and also a patient with granulomatosis with polyangiitis treated with RTX developed atypical late clinical worsening to severe pneumonia.[6 7] Whether these patients and the patients initially reported by us[2] also had a defect in viral clearance or even developed viraemia,[5] a rather unusual situation in viral respiratory diseases, and, if so, whether decreased viral clearance contributed to delayed clinical worsening in the reported clinical cases is unknown. These cases, however, highlight the possibility that rituximab is associated with a specific risk in SARS-CoV-2 infections and in the outcome of COVID-19. Current data from the National Registry for patients with IRD infected with SARS-CoV-2 in Germany support the contention of such a risk as in this registry, 11 out of 18 patients (61.1%) treated with RTX required hospitalisation, with 9 of the 18 patients (50%) required ventilation, whereas only 28 out of 95 patients (28.6%) treated with biological disease modifying anti-rheumatic drugs (bDMARDs) needed hospital care and only 12 (12.2%) required ventilation (Hasseli *et al*, submitted for publication, 2020). While further data on the risk of RTX during the SARS-CoV-2/COVID-19 pandemic and its precise mechanisms are urgently required, physicians should be aware of the potential of RTX-associated severe courses of the infection and remain to be extremely vigilant and cautious when considering RTX treatment.

Hendrik Schulze-Koops ,[1] **Klaus Krueger,**[2] **Inka Vallbracht Vallbracht,**[3] **Rebecca Hasseli** ,[4] **Alla Skapenko**[1]

[1]Division of Rheumatology and Clinical Immunology, Department of Medicine IV, Ludwig-Maximilians-Universitat Munchen, Munchen, Germany
[2]Praxiszentrum St. Bonifatius Muenchen, Muenchen, Germany
[3]Department of Rheumatology, Clinical Immunology and Osteology, Munich Clinic Bogenhausen, Munich, Germany
[4]Department of Rheumatology, Kerckhoff-Klinik GmbH, Bad Nauheim, Germany

Correspondence to Professor Hendrik Schulze-Koops, Division of Rheumatology and Clinical Immunology, Internal Medicine IV, Ludwig-Maximilians-Universitat Munchen, Munchen 80336, Germany; hendrik.schulze-koops@med.uni-muenchen.de

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ORCID iDs
Hendrik Schulze-Koops http://orcid.org/0000-0002-1681-491X
Rebecca Hasseli http://orcid.org/0000-0002-2982-8253

REFERENCES

1 Benucci M, Li Gobbi F, Damiani A, *et al*. Presistence of rT-PCR-SARS-CoV-2 infection and delayed serological resoinse, as a possible effect of rituximab according to the hypothesis of Schulze-Koops, et al. *Ann Rheum Dis* 2022;81:e184.
2 Schulze-Koops H, Krueger K, Vallbracht I, *et al*. Increased risk for severe COVID-19 in patients with inflammatory rheumatic diseases treated with rituximab. *Ann Rheum Dis* 2021;80:e67.
3 Tony H-P, Burmester G, Schulze-Koops H, *et al*. Safety and clinical outcomes of rituximab therapy in patients with different autoimmune diseases: experience from a national registry (GRAID). *Arthritis Res Ther* 2011;13:R75.

4 Quinti I, Lougaris V, Milito C, *et al*. A possible role for B cells in COVID-19? lesson from patients with agammaglobulinemia. *J Allergy Clin Immunol* 2020;S0091-6749(20)30557-1.
5 Tepasse P-R, Hafezi W, Lutz M, *et al*. Persisting SARS-CoV-2 viraemia after rituximab therapy: two cases with fatal outcome and a review of the literature. *Br J Haematol* 2020;190:185–8.
6 Avouac J, Airó P, Carlier N, *et al*. Severe COVID-19-associated pneumonia in 3 patients with systemic sclerosis treated with rituximab. *Ann Rheum Dis* 2021;80:e37.
7 Guilpain P, Le Bihan C, Foulongne V, *et al*. Rituximab for granulomatosis with polyangiitis in the pandemic of covid-19: lessons from a case with severe pneumonia. *Ann Rheum Dis* 2021;80:e10.

Terminology and definition of 'antinuclear antibodies': history and current debate

Recently, a series of letters has been published in the *Annals of the Rheumatic Diseases*[1–10] in response to an article by Pisetsky *et al* on the variability of indirect immunofluorescence (IIF) assays for testing for antinuclear antibodies (ANAs) in systemic lupus erythematodes (SLE).[11] This discussion has focused on the merits and shortcomings of different assays used in the detection of ANA. Only one of the contributions briefly addresses one of the key questions: 'What is an ANA?'.[3] Because the term ANA itself has been judged anachronistic and misleading by many,[12] with efforts to replace it under way,[13 14] this is not a trivial question to answer. Since the terminology around ANA has developed historically, it is useful to explore the contexts in which these terms originated in order to understand their original definition and the changes therein over time.

From the late 1940s onwards research into the serology of SLE gradually led to the discovery that autoantibodies against components of the cells' nucleus could be found in sera of SLE patients.[13–21] Because the exact target antigens remained largely unknown at first (except for dsDNA and histone) Holman *et al* coined the term 'antinuclear antibody' or ANA for them.[22 23] With the advent of IIF testing on HEp-2-cells as standard method for the detection of ANA, it was possible to detect autoantibodies against a range of different target antigens; including antigens of the cytoplasm, the nuclear envelope and the mitotic spindle apparatus. Since these autoantibodies were detected in the context of ANA diagnostics, the term ANA was soon applied to them as well.[13] This led to a gradual expansion of what was commonly understood to constitute an ANA, without any official consensus on the definition of the term itself, causing many to view it as anachronistic and misleading.

Several proposals have been made on how to improve the situation, perhaps most prominently by the International Consensus on ANA Patterns (ICAP) group, who have suggested the name 'anticellular antibodies'[12 13] in order to broaden the definition to include cytoplasmic and mitotic antigens as well. There have, however, been other proposals as well, such as 'antibodies to intracellular antigens'.[14] Since the widely shared opinion is that the term 'ANA' is too well known and too embedded in guidelines, classifications and even legislation to be replaced abruptly, other possible solutions include adding a clarifying subtitle to the term ANA (eg, 'ANA—antibodies to intracellular autoantigens'[14] or 'ANA is actually a test that detects autoantibodies to cellular antigens—thus encompassing the whole cellular anatomy and all cellular structures'[12]) when reporting ANA results, possibly leading to a transitional period after which the term ANA might be replaced altogether.[12 14 24]

Ultimately, the debate about terminology should be a secondary one. First, a consensus should be reached on which autoantibodies are to be described by the new terminology. The US National Library of Medicine in its Medical Subject Headings descriptor 2020 currently defines ANA as 'Autoantibodies directed against various nuclear antigens including DNA, RNA, histones, acidic nuclear proteins, or complexes of these molecular elements. Antinuclear antibodies are found in systemic autoimmune diseases including systemic lupus erythematosus, Sjogren's syndrome, scleroderma, polymyositis, and mixed connective tissue disease.'.[25] This definition, as well as others currently used, appears at the same time to be too narrow in its exclusion of autoantibodies against non-nuclear antigens, as well as too vague, not specifying whether any antibody against a nuclear antigen qualifies as an ANA or what, if any, connection there has to be between an ANA and one of the systemic autoimmune diseases, an inexhaustive list of which is given in the definition. Good examples for this are the autoantibodies against DFS70. Since their antigen is nuclear, they are considered to be ANA even in the stricter sense, but evidence has accumulated that the detection of anti-DFS70-autoantibodies, especially when isolated, rather serves to exclude the possibility of a systemic autoimmune disease.[26] Therefore, their inclusion in the definition of the term ANA may be counterproductive for any classification or guideline that lists the general presence of ANA as diagnostic criterion for systemic autoimmune diseases.

Therefore, in order for any future nomenclature that is to replace the term ANA to be precise the following questions concerning its definition should be considered.

Considering a definition based on the target antigens: this is the definition with the most historical precedence, the term ANA itself being defined by the nuclear nature of the corresponding antigens. This is probably why proposed new terms such as 'anticellular antibodies' and 'antibodies to intracellular antigens' also focus on the properties of the target antigens. But is it prudent to go down the same road again when the antigens in question are so diverse? Are not the proposed terms, in their attempt to be more inclusive, too vague? What makes an antibody 'anticellular'? Are antibodies against antigens like GAD65 or Hu to be included in the definition of antibodies 'to intracellular antigens'?

Considering a definition based on detection method: since it is the current diagnostic gold standard, should only autoantibodies detectable via IIF using HEp-2-cells be considered, thus including antibodies against cytoplasmic and mitotic antigens, but excluding autoantibodies against antigens such as MDA5[27] and HMGCR,[28] which are tested for via immunoblotting?

Considering a definition based on disease association: should the definition include specifications about possible disease associations, thereby excluding autoantibodies against DFS70? If yes, which diseases are to be included and which to be excluded? What about anti-Ro52, which has no specific disease?[29] Should all autoantibodies be included that are associated with a certain ANA-associated systemic autoimmune disease, even if some are currently not considered to be ANA (eg, autoantibodies against the angiotensin II type 1 receptor and the endothlin-1 type A receptor which are associated with systemic sclerosis[30])?

The replacement of the term ANA and of the network of related and similarly outdated terms by a clearly defined and widely agreed terminology is still some way off. We hope to have added to the debate some helpful impulses towards a more appropriate nomenclature of autoantibodies.

Robert Daniel Heinrich Markewitz , **Klaus-Peter Wandinger**

Institute of Clinical Chemistry, University Hospital Schleswig Holstein, Lübeck, Germany

Correspondence to Dr Robert Daniel Heinrich Markewitz, Institut für Klinische Chemie, Universitätsklinikum Schleswig-Holstein - Campus Lübeck, Lubeck 23538, Germany; robert.markewitz@uksh.de

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ORCID iD
Robert Daniel Heinrich Markewitz http://orcid.org/0000-0003-1676-1085

REFERENCES

1 Bizzaro N. Can solid-phase assays replace immunofluorescence for ANA screening? *Ann Rheum Dis* 2020;79:e32.
2 Infantino M, Manfredi M, Soda P, *et al*. ANA testing in 'real life'. *Ann Rheum Dis* 2020;79:e3.
3 Mahler M. Lack of standardisation of ANA and implications for drug development and precision medicine. *Ann Rheum Dis* 2019;78:e33.
4 Meroni PL, Chan EK, Damoiseaux J, *et al*. Unending story of the indirect immunofluorescence assay on HEp-2 cells: old problems and new solutions? *Ann Rheum Dis* 2019;78:e46.
5 Pacheco Y, Monsalve DM, Acosta-Ampudia Y, *et al*. Antinuclear autoantibodies: discordance among four different assays. *Ann Rheum Dis* 2020;79:e6.
6 Pregnolato F, Borghi MO, Meroni PL. Forum Interdisciplinare per La Ricerca sulle Malattie Autoimmuni (firma) Study Group. pitfalls of antinuclear antibody detection in systemic lupus erythematosus: the positive experience of a national multicentre study. *Ann Rheum Dis* 2019;78:e50.
7 Van Hoovels L, Schouwers S, Van den Bremt S, *et al*. Variation in antinuclear antibody detection by automated indirect immunofluorescence analysis. *Ann Rheum Dis* 2019;78:e48.
8 Willems P, De Langhe E, Westhovens R, *et al*. Antinuclear antibody as entry criterion for classification of systemic lupus erythematosus: pitfalls and opportunities. *Ann Rheum Dis* 2019;78:e76.
9 Bossuyt X, Claessens J, De Langhe E, *et al*. Antinuclear antibodies by indirect immunofluorescence and solid phase assays. *Ann Rheum Dis* 2020;79:e65.
10 Pisetsky DS, Spencer DM, Rovin B, *et al*. Role of ANA testing in the classification of patients with systemic lupus erythematosus. *Ann Rheum Dis* 2021;80:e124.
11 Pisetsky DS, Spencer DM, Lipsky PE, *et al*. Assay variation in the detection of antinuclear antibodies in the sera of patients with established SLE. *Ann Rheum Dis* 2018;77:911–3.
12 Damoiseaux J, von Mühlen CA, Garcia-De La Torre I, *et al*. International consensus on ANA patterns (ICAP): the bumpy road towards a consensus on reporting ANA results. *Auto Immun Highlights* 2016;7:1.
13 Agmon-Levin N, Damoiseaux J, Kallenberg C, *et al*. International recommendations for the assessment of autoantibodies to cellular antigens referred to as anti-nuclear antibodies. *Ann Rheum Dis* 2014;73:17–23.
14 Tozzoli R, Villalta D, Bizzaro N. Challenges in the standardization of autoantibody testing: a comprehensive review. *Clin Rev Allergy Immunol* 2017;53:68–77.
15 Hargraves MM, Richmond H, Morton R. Presentation of two bone marrow elements; the tart cell and the L.E. cell. *Proc Staff Meet Mayo Clin* 1948;23:25–8.
16 Haserick JR, Lewis LA, Bortz DW. Blood factor in acute disseminated lupus erythematosus 1. Determination of gamma globulin as specific plasma fraction. *Am J Med Sci* 1950;219:660–3.
17 Haserick JR, Bortz DW. Normal bone marrow inclusion phenomena induced by lupus erythematosus plasma. *J Invest Dermatol* 1949;13:47–9.
18 Miescher P, Fauconnet M. L'absorption du facteur «L.E.» par des noyaux cellulaires isolés. *Experientia* 1954;10:252–4.
19 Miescher P, Strässle R. New serological methods for the detection of the L. E. factor. *Vox Sang* 1957;2:283–7.
20 Holman HR, Kunkel HG. Affinity between the lupus erythematosus serum factor and cell nuclei and nucleoprotein. *Science* 1957;126:162–3.
21 Seligmann M, Milgrom F. Mise en evidence dans Le serum de malades atteints de lupus erythemateux dissemine diune substance determinant une reaction de precipitation avec liacide desoxyribonucleique. *Comptes Rendus Hebd Seances Acad Sci* 1957;245:1472–5.
22 Holman H, Kunkel H. Antinuclear antibodies and their detection in systemic lupus erythematosus (SLE). *Bull Rheum Dis* 1959;10:197–8.
23 Holman H, Robbins W. Antinuclear antibodies in systemic lupus erythematosus. *Arthritis Rheum* 1959;2:468–71.
24 Hernández-Flórez D, Valor L. Neither the anti-nuclear antibodies nor the anti-extractable nuclear antigens are what they used to be. A future change of Nomenclature. *Reumatol Clin* 2018;14:317–9.
25 MeSH Browser. Available: https://meshb.nlm.nih.gov/record/ui?name=Antinuclear%20antibodies [Accessed 13 Jan 2020].
26 Mahler M, Andrade LE, Casiano CA, *et al*. Anti-DFS70 antibodies: an update on our current understanding and their clinical usefulness. *Expert Rev Clin Immunol* 2019;15:241–50.
27 Hoa S, Troyanov Y, Fritzler MJ, *et al*. Describing and expanding the clinical phenotype of anti-MDA5-associated rapidly progressive interstitial lung disease: case series of nine Canadian patients and literature review. *Scand J Rheumatol* 2018;47:210–24.
28 Christopher-Stine L, Casciola-Rosen LA, Hong G, *et al*. A novel autoantibody recognizing 200-kd and 100-kd proteins is associated with an immune-mediated necrotizing myopathy. *Arthritis Rheum* 2010;62:2757–66.
29 Murng SHK, Thomas M. Clinical associations of the positive anti Ro52 without Ro60 autoantibodies: undifferentiated connective tissue diseases. *J Clin Pathol* 2018;71:12–19.
30 Riemekasten G, Philippe A, Näther M, *et al*. Involvement of functional autoantibodies against vascular receptors in systemic sclerosis. *Ann Rheum Dis* 2011;70:530–6.
31 Ceppellini R, Polli E, Celada F. A DNA-Reacting factor in serum of a patient with lupus erythematosus Diffusus. *Exp Biol Med* 1957;96:572–4.
32 Hiepe F, Dörner T, Burmester G-R. Editorial overview: antinuclear antibody- and extractable nuclear antigen-related diseases. *Int Arch Allergy Immunol* 2000;123:5–9.

Correction: *Cliniclal benefit of 1-year certolizumab pegol(CZP) add-on therapy to methotrexate treatment in patients with early rheumatoid arthritiswas observed following CZP discontinuation: 2-year results of the C-OPERA study, a phase III randomised trial*

Atsumi T, Tanaka Y, Yamamoto K, *et al*. Clinical benefit of 1-year certolizumab pegol (CZP) add-on therapy to methotrexate treatment in patients with early rheumatoid arthritis was observed following CZP discontinuation: 2-year results of the C-OPERA study, a phase III randomised trial. *Ann Rheum Dis* 2017;76:1348–56. doi:10.1136/annrheumdis-2016-210246.

There are some minor errors in the numbers report in table 1. The correct values are:

Table 1 Baseline demographics and patient characteristics

	CZP+MTX®MTX			PBO+MTX®MTX		
	Total patients	Patients entering PT period		Total patients	Patients entering PT period	
	n=159	n=108		n=157	n=71	
	DB baseline(Week 0)	DB baseline(Week 0)	PT baseline(Week 52)	DB baseline(Week 0)	DB baseline(Week 0)	PT baseline(Week 52)
Age (years)	49.4±10.6	48.8±11.2	–	49.0±10.3	48.6±10.8	–
Female, n (%)	129 (81.1)	85 (78.7)	–	127 (80.9)	58 (81.7)	–
Weight (kg)	57.4±11.3	57.0±11.5	–	57.4±10.6	57.4±10.3	–
BMI (kg/m^2)	22.4±3.9	22.2±3.7	–	22.5±3.7	22.4±3.7	–
RA duration (months)*	4.0±2.9	4.4±3.1	–	4.3±2.8	4.4±3.1	–
Anti-CCP antibody positive, n (%)	159 (100.0)	108 (100.0)	–	157 (100.0)	71 (100.0)	–
RF positive, n (%)	153 (96.2)	104 (96.3)	–	146 (93.0)	68 (95.8)	–
Bone erosion (judged by physician), n (%)	79 (49.7)	51 (47.2)	–	80 (51.0)	34 (47.9)	–
TJC (/28 joints)	8.4±6.1	7.5±5.8	0.5±1.1	8.9±6.5	7.3±6.1	0.6±1.6
SJC (/28 joints)	8.3±5.3	7.6±4.6	0.3±0.7	8.4±5.3	7.0±4.2	0.4±1.4
ESR (mm/h)	38.4±25.3	36.3±23.7	12.8±9.9	43.7±28.2	36.5±22.2	15.5±14.3
CRP (mg/dl)	1.29±1.82	1.12±1.51	0.06±0.13	1.52±1.91	1.03±1.39	0.17±0.37
MMP-3 (ng/ml)	130.4±135.4	125.3±135.4	47.7±25.7	185.4±214.9	167.3±204.3	52.5±31.1
DAS28(ESR)	5.4±1.1	5.2±1.1	1.9±0.8	5.5±1.2	5.1±1.0	2.2±0.7
SDAI	28.7±12.5	27.0±11.2	2.4±2.6	30.0±13.6	24.6±11.3	2.7±3.1
HAQ-DI score	1.01±0.64	1.04±0.63	0.14±0.26	1.05±0.69	0.79±0.57	0.07±0.14
mTSS	4.1±7.4	3.8±7.4	3.7±7.4	5.5±15.0	3.2±6.2	3.4±6.3
Erosion score	1.9±4.0	1.6±3.9	1.6±3.7	2.5±7.8	1.6±3.3	1.8±3.2
Joint space narrowing score	2.1±4.6	2.2±4.8	2.2±4.8	2.9±8.3	1.5±4.0	1.6±4.1
Average weekly MTX dose (mg/week)†	11.4±3.1	11.3±3.2	10.9±4.1	11.5±2.8	11.5±3.1	11.1±3.7

Values are mean ±SD unless otherwise indicated. Data in DB baseline columns represent average during weeks 0–104, whereas data in PT baseline columns represent average during weeks 52–104.
*Time from onset of persistent arthritic symptoms.
†MTX dose was initiated at 8 mg/week and escalated to the maximum tolerated dose (up to 16 mg/week) by week 8.
BMI, body mass index; CCP, cyclic citrullinated peptide; CRP, C reactive protein; CZP, certolizumab pego; DB, double blind; ESR, erythrocyte sedimentation rate; HAQ-DI, Health Assessment Questionnaire Disability Index; MMP-3, matrix metalloproteinase-3; mTSS, modified total Sharp score; MTX, methotrexate; PBO, placebo; PT, post treatment; RA, rheumatoid arthritis; RF, rheumatoid factor; SJC, swollen joint count; TJC, tender joint count.