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ISSN 0003-4967 (print); 1468-2060 (online)

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BMJ Journals, BMA House, Tavistock Square
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E: **ard@bmj.com**

Production Editor
Teresa Jobson
E: **production.ard@bmj.com**

EULAR
EULAR Executive Secretariat
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E: **eular@eular.org**
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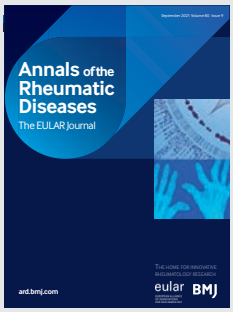

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BMJ Publishing Group Ltd
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Tavistock Square
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T: **+44 (0)20 3655 5889**
E: **ard@bmj.com**
Twitter: **@ARD_BMJ**
ISSN: 0003-4967 (print)
ISSN: 1468-2060 (online)

ARD is published by BMJ Publishing Group Ltd typeset by Exeter Premedia Services Private Ltd, Chennai, India and printed in the UK on acid-free paper.

Annals of the Rheumatic Diseases, ISSN 0003-4967 (USPS 2152) is published monthly by BMJ Publishing Group Ltd, BMA House, Tavistock Square, WC1H 9JR London. Airfreight and mailing in the USA by agent named World Container Inc, 150-15, 183rd Street, Jamaica, NY 11413, USA. Periodicals postage paid at Brooklyn, NY 11256. US Postmaster: Send address changes to Annals of the Rheumatic Diseases, World Container Inc, 150-15, 183rd Street, Jamaica, NY 11413, USA. Subscription records are maintained at BMA House, Tavistock Square, WC1H 9JR London. Air Business Ltd is acting as our mailing agent.

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Antiphospholipid antibodies and COVID-19 thrombotic vasculopathy: one swallow does not make a summer

Pier Luigi Meroni [1], Maria Orietta Borghi [1,2]

The high morbidity and mortality of COVID-19 have been associated with the thrombotic microangiopathy described in the patients in addition to the increased prevalence of thrombosis affecting medium/large arterial and venous vessels.[1 2] Initial reports demonstrating prolonged activated partial thromboplastin times (aPTT) and positivity for antiphospholipid antibody (aPL) assays raised the issue of whether common pathogenic mechanisms were shared by the antiphospholipid antibody syndrome (APS) and COVID-19.[3 4] In particular, the systemic thrombotic microangiopathy and the increased circulating levels of proinflammatory cytokines underlined the similarity between catastrophic APS (CAPS) and COVID-19.[5 6]

The similarities between APS/CAPS and COVID-19 are even more complex and intriguing as summarised in table 1. A proinflammatory environment that includes the activation of the complement system has been reported in all these conditions, although at different degrees. The involvement of several cell types playing a role in the coagulation cascade, such as platelets, monocytes and neutrophils, has been described which is closely associated with the proinflammatory and prothrombotic phenotypes.[7 8] In particular, an endothelial perturbation is generally thought to be a common denominator in these diseases and several authors described it with the term 'endothelitis' in the COVID-19.[9–11]

Both proinflammatory cytokine (eg, interleukin-6) and complement activation products (ie, C5a and C5b9) were thought to play a role in mediating the endothelitis together with a direct effect of SARS-CoV-2 on the endothelium.[10–12] However, the SARS-CoV-2 endothelial tropism is still a matter of debate despite the presence of the entry molecule (ie, ACE2) on the endothelial surfaces.[13] So, it is not surprising that additional potential mediators of endothelial perturbation have been suggested. In particular, aPL came into the limelight because of their well-known ability to bind and activate endothelium in the APS.[14]

aPL can be formally identified by functional PL-dependent coagulation assay (ie, the so-called lupus anticoagulant (LA) test) and by solid phase methods that detect antibodies against beta2 glycoprotein I (β2GPI) (ie, anticardiolipin and anti-β2GPI assays) or prothrombin complexed with phosphatidylserine (ie, aPS/PT). These two last families of autoantibodies are responsible for the large majority of the positive LA.[14] The papers reporting positive aPL tests in patients with COVID-19 are quite heterogeneous regarding frequency and biochemical characteristics of these autoantibodies; in particular, their clinical impact on the disease did not emerge in a recent meta-analysis and systematic review.[15]

Some variables can affect the reproducibility of the functional LA assay, and specific caveats have been underlined by the international scientific societies to avoid misinterpretation. For example, concomitant anticoagulant therapy (eg, heparin) and systemic inflammation with high C-reactive protein plasma levels are well-known factors that can produce LA false-positive results.[16 17] On the other hand, aPL solid phase tests are not affected by anticoagulant therapy or inflammation mediators.

The positivity for LA in the absence of anti-β2GPI and aPS/PT is usually considered of low diagnostic and prognostic value in the setting of APS and systemic autoimmune rheumatic diseases.[18 19] Likewise, the high frequency of isolated positive LA (and prolonged aPTT) in most of the published COVID-19 papers casts doubts on the true presence of thrombophilic aPL in line with the general assumption that the association between aPL and thrombosis is doubtful in most of the COVID-19 series already published.[15]

Nevertheless, SARS-CoV-2 itself can be responsible for aPL production as reported in other viral and non-viral infections.[20] Moreover, the occurrence of concomitant infections in moderate/severe COVID-19 may contribute to aPL production as well. In line with the above-mentioned facts, the paper by Trahtemberg *et al*[21] correctly did not check for LA and raised the issue of the right pathological control group including in the study a series of intensive care unit (ICU) patients without SARS-CoV-2 infection but potentially susceptible to the usual comorbidities occurring in ICU patients. The study did not find any significant difference in the presence of a large panel of aPL between ICU patients with and without COVID-19. Such an approach further supports the conclusion that aPL does not seem to be the main player in the COVID-19 thrombophilic microangiopathy. The authors reported an association between aPL serology and more severe disease that, however, was independent of the COVID-19 status.

Moreover, additional findings are supporting the idea that aPL in COVID-19 may represent bystander rather than pathogenic autoantibodies. In fact, there is evidence that this aPL is transient, usually at medium/low titre and frequently of the IgM isotype only.[15] Moreover, the β2GPI-dependent aPL was not directed against the domain (D)1 immune-dominant epitope of the molecule but frequently against D4,5.[15 22] This profile is diametrically

Table 1 Pathogenic pathways reported in APS, CAPS and COVID-19

Pathogenic paths	APS	CAPS	COVID-19
Thrombotic microangiopathy	+/–	++	++
EC perturbation	+	++	++
Complement activation	+	+	++
NETosis	+/–	?	++
Proinflammatory cytokines	+/–	++	++
Impaired fibrinolysis[29 30]	+	?	++
aPL	++	++	+/–

aPL, antiphospholipid antibody; APS, antiphospholipid antibody syndrome; CAPS, catastrophic APS; EC, endothelial cell.

[1]Experimental Laboratory of Immunological and Rheumatologic Researches, Istituto Auxologico Italiano–Istituto di Ricovero e Cura a Carattere Scientifico, Milano, Italy
[2]Department of Clinical Sciences and Community Health, Università degli Studi di Milano, Milano, Italy

Correspondence to Professor Pier Luigi Meroni, Experimental Laboratory of Immunological and Rheumatologic Researches, Istituto Auxologico Italiano–Istituto di Ricovero e Cura a Carattere Scientifico, 20145 Milano, Italy; pierluigi.meroni@unimi.it

opposite to the persistent high-titre IgG against β2GPI D1 historically reported in autoimmune APS. High-titre anti-β2GPI D1 IgG has been closely associated with the vascular manifestations of the syndrome, was found in human tissue samples affected by APS thrombosis and displayed a thrombogenic effect in animal models at variance with aPL directed against other domains of the molecule.[14 23 24] Altogether, these findings are in line with the lack of a sound association between aPL and thrombosis reported in the majority of the studies,[15] and in the paper by Trahtemberg *et al*. In the same paper, the use of a solid phase assay that was suggested as a surrogate tool for LA further ruled out the presence of aPL theoretically responsible for LA and/or prolonged aPTT.[21]

It is important to keep in mind that patients with COVID-19 suffer from an acute form of systemic inflammation with complement activation, both responsible for endothelial perturbation.[8 9 11] In a similar situation, there is evidence that β2GPI can accumulate on the activated endothelium at high density, being much more available to the anti-β2GPI antibodies and ultimately favouring their pathogenic effect.[25] A comparable condition in which low titres of aPL can cause substantial damage was reported in obstetric APS, where high quantities of β2GPI are physiologically expressed in the placenta.[26] Therefore, while transitory low-titre aPL is likely to be clinically irrelevant in patients with COVID-19 as in other infections, their detection in a disease characterised by a strong inflammatory phenotype raises the issue of whether or not these antibodies may increase the ultimate thrombophilic risk and justify a prophylactic treatment. Accordingly, we could speculate that aPL may affect the clinical severity of the inflammatory disease in ICU patients regardless of the COVID-19 status as shown by Trahtemberg *et al*.[21]

While the use of prophylactic or therapeutic heparin therapy is widely accepted during the acute phase of the disease, this is still debated during the recovery period or even in the post-COVID-19 follow-up.[2] Until aPL positive, the patients can theoretically be at higher risk for thrombosis recurrences, and a prophylactic treatment be considered. Unfortunately, we do not have either large follow-up studies evaluating aPL-positive patients with COVID-19 or the best prophylactic regime for such kinds of patients.

If the hypothesis that SARS-CoV-2 is linked with an immune response against PL-binding proteins is true, then the other side of the coin should be represented by the risk of clinical manifestations or the increase in aPL titres in patients suffering from full-blown APS and concomitant SARS-CoV-2 infection. Besides few anecdotical case reports, there is no evidence that this is the case.[27 28]

The use of aPL test in patients with COVID-19 should be taken into consideration in the real life but critically assessed to avoid overinterpretation. For example, as previously discussed, the aPL characterisation in terms of persistence over time, isotype, titre and antigen specificity may help in discriminating between bystander antibodies and pathogenic ones. It is more difficult to draw definite conclusions from a clinical point of view: whether or not the aPL positivity can have a clinical significance to justify a specific treatment in the context of a disease characterised by the production of inflammatory mediators (eg, cytokines, complement activation products) potentially able to downregulate the threshold for endothelial activation.

Handling editor Josef S Smolen

Contributors PLM and MOB equally contributed in writing the editorial.

Funding The paper was supported in part by Ricerca Corrente 2020 to PLM of the Ministero della Salute, Italy.

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 required.

Provenance and peer review Commissioned; externally peer reviewed.

To cite Meroni PL, Borghi MO. *Ann Rheum Dis* 2021;**80**:1105–1107.

Received 21 June 2021
Accepted 15 July 2021
Published Online First 31 July 2021

Linked

► http://dx.doi.org/10.1136/annrheumdis-2021-220206

Ann Rheum Dis 2021;**80**:1105–1107.
doi:10.1136/annrheumdis-2021-220520

ORCID iDs
Pier Luigi Meroni http://orcid.org/0000-0002-3394-1451
Maria Orietta Borghi http://orcid.org/0000-0001-8967-9678

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Henrik Sjögren (1899–1986): the syndrome and his legacy

Roland Jonsson [1,2]

Handling editor Josef S Smolen

[1]Broegelmann Research Laboratory, Department of Clinical Science, University of Bergen, Bergen, Norway
[2]Department of Rheumatology, Haukeland Universitetssjukehus, Bergen, Norway

Correspondence to
Professor Roland Jonsson, Broegelmann Research Laboratory, Department of Clinical Science, University of Bergen, 5020 Bergen, Norway; roland.jonsson@uib.no

Received 22 March 2021
Accepted 5 May 2021
Published Online First 21 June 2021

To cite: Jonsson R. *Ann Rheum Dis* 2021;**80**:1108–1109.

Henrik Samuel Conrad Sjögren was born on 23 July 1899 in the small city of Köping, in the county of Västmanland, Sweden. He studied medicine at the Karolinska Institute and received his license as an authorised doctor in 1927 (figure 1). Henrik Sjögren's biography has been highlighted previously; one was in connection with the scientific meeting in Jönköping, Sweden celebrating the 100-year anniversary of his birth in 1999.[1]

In January 1930, at the Serafimer Hospital in Stockholm, Henrik Sjögren met a 49-year-old female patient who had suffered for about 6 years from pain in different joints, especially in her hands. However, the visit to Dr Sjögren was due to irritation of her eyes. Further, she reported problem about difficulties in eating and swallowing, due to lack of saliva. He had previously observed similar cases and that year he published in Hygiea, the Proceedings of the Swedish Medical Association, a paper in which he described another four cases.[2] In that article, he coined the symptom 'keratoconjunctivitis sicca' and where he also carefully described the method to stain the damaged cells in the conjunctiva and cornea by using 1% Bengal rose. The staining was similar to that seen in 'keratitis filiformis'.

At this time, Sjögren was well aware of that each separate condition had been described before, that is, keratitis filiformis by Leber already in 1882,[3] oral problems in the form of dry mouth (xerostomia) by Hadden in 1888[4] and their combination by the French dermatologist Henri Gourgerot in 1926.[5] In 1927, Muloch Houwer[6] described joint symptoms in connection with keratitis filiformis, and Houwer in fact found that one case report had been previously published by the German doctor Erich Fischer already in 1889.[7]

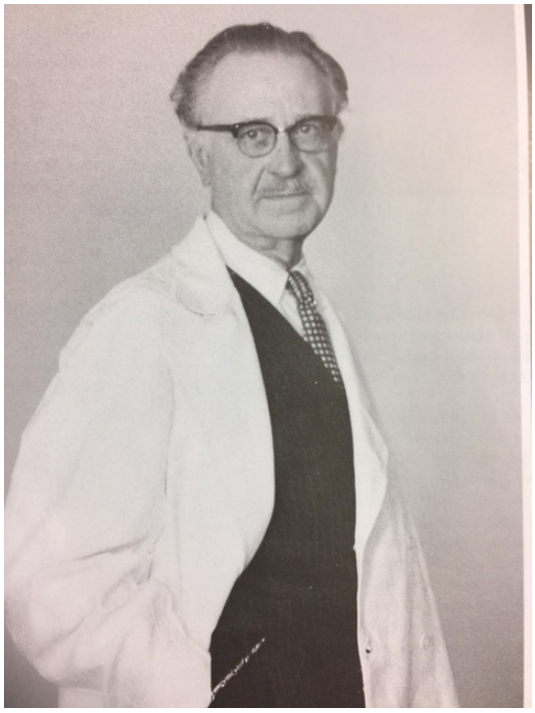

Figure 1 Portrait of Henrik Sjögren.

In 1931 Henrik Sjögren moved to another hospital in Stockholm, the Sabbatsberg Hospital, where he continued his clinical training as well as his research and studies of more cases under the designation "sicca syndrome".

In 1933, he had collected a total of 19 cases, all women aged 29–72 years. In these patients, he had conducted careful clinical and ophthalmological examinations, which included microscopic analysis of the lachrymal glands in 10 and parts of the conjunctivae/corneae in 12 of the patients. In one patient who died, an autopsy was performed, which included histopathological examination of the salivary glands.

The results were presented in the now classical doctoral dissertation 'Zur Kenntnis der Keratoconjunctivitis Sicca',[8] which Sjögren defended on the 8 May 1933. The thesis consisted of one pathological and one clinical part. He received credits for several items in the thesis but was also criticised for some aspects; this critique has by some been considered as unjustified. Consequently, this resulted in a mediocre grade of 1.5 on a 1–3 scale. This grade disqualified Sjögren from the 'docent' (associate professor) title and in fact ended his dream of an academic career. Not surprisingly, this was a great personal disappointment.

As one consequence of this not so successful outcome of the dissertation, Sjögren was obliged to consider a career outside academia. In 1935, he moved to Jönköping where he was appointed Head of the Eye Clinic at the County Hospital. However, his interest in the dry eye did not come to an end by these other duties. Indeed, he became a quite successful eye surgeon (figure 2). A more extensive historical perspective of these early years has also been presented.[9]

One important aspect with the increased awareness of Sjögren's syndrome was the translation of the thesis to English by Dr Bruce Hamilton, published in Sydney 1943.[10] Dr Hamilton became a close friend of Henrik Sjögren and invited him as a guest lecturer to Hobart University in Australia. This tour was combined with several visits and indeed an around the world tour including the USA.

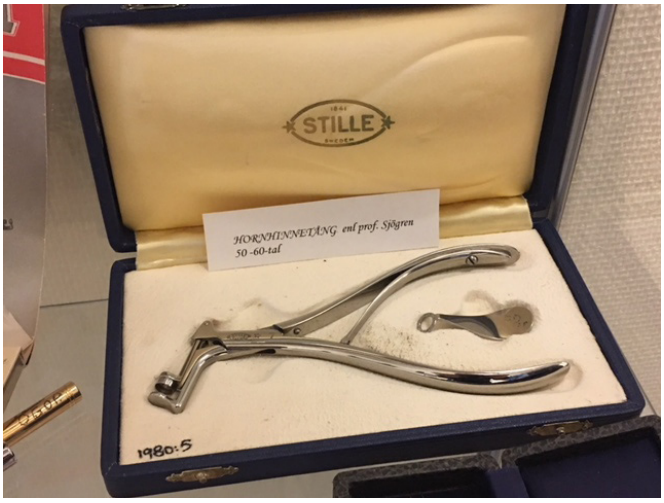

Figure 2 Instrument invented by Henrik Sjögren for corneal transplantations.

Henrik Sjögren's career subsequently was recognised when he in 1957 was awarded the title 'Docent' by the Faculty of Medicine at University of Gothenburg. Four years later, he received the honorary title 'Professor' by the Swedish Government, a quite rare appointment. This was the utmost acknowledgement of a successful career and international recognition.

Henrik Sjögren will long be remembered for his enthusiasm, his love of teaching and his important contribution not only to clinical medicine in general but also to oral medicine, ophthalmology and rheumatology. Sjögren's syndrome as a chronic inflammatory and autoimmune, rheumatic disease nowadays attracts and fascinates an increasing number of doctors and scientists in the search for a better understanding and more knowledge. Much has happened over the years on how the disease is recognised; for example, in 1965, it was suggested that Sjögren's syndrome could be divided into one primary and one secondary form.[11] Notably, a direct impact of Henrik Sjögren's doctoral thesis is the objective confirmation of keratoconjunctivitis sicca, one of the hallmarks of the most recent classification criteria.[12]

The increased activity and interest for the syndrome is illustrated by the regularly organised international symposia starting in 1986. This First International Sjögren's syndrome meeting was organised by Rolf Manthorpe just outside Copenhagen (with international advisors Haralampos Moutsopoulos and Norman Talal). Of note, Henrik Sjögren died in 1986, a few months after being named honorary president of this first meeting together with Jan Waldenström. These meetings have been circulated between Europe, Japan and USA, and the most recent meetings took place in Bergen 2015, Norway (sicca.org/isss2015) and Washington DC 2018 (https://hopkinscme.cloud-cme.com/Assets/hopkinscme/pdf/80040923schedule.pdf).

Additional activities related to Sjögren's syndrome are the recent EU and National Institute of Health (NIH)/National Institute of Craniofacial Research (NIDCR) supported contracts (Sjögren's International Collaborative Clinical Alliance (SICCA) (https://globalprojects.ucsf.edu/project/sj%C3%B6gren%E2%80%99s-international-collaborative-clinical-alliance-next-generation-studies-sicca) (2003–2020), HarmonicSS (https://www.harmonicss.eu) (2017–2020) and NEw Clinical Endpoints in primary Sjögren's Syndrome: an Interventional Trial based on stratifYing patients (NECESSITY) (https://www.necessity-h2020.eu) (2019–2024)). Furthermore, there are several major patient organisations which are actively supporting the patients—for example, European (http://sjogreneurope.org) and US based (https://www.sjogrens.org). Indeed, a clear illustration that Henrik Sjögren became one of the internationally most recognised Swedish medical doctors regarding this enigmatic and disabling rheumatic disease.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

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 required.

Provenance and peer review Commissioned; externally peer reviewed.

ORCID iD
Roland Jonsson http://orcid.org/0000-0002-9588-0260

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EULAR COVID-19 registry: lessons learnt and future considerations

Saskia Lawson-Tovey [1,2] Anja Strangfeld [3] Kimme L Hyrich [2,4] Loreto Carmona [5] Diana Rodrigues,[6] Laure Gossec,[7,8] Elsa F Mateus,[9,10] Pedro M Machado [11,12,13]

Handling editor Josef S Smolen

For numbered affiliations see end of article.

Correspondence to
Dr Pedro M Machado, Centre for Rheumatology & Department of Neuromuscular Diseases, University College London, London WC1 B5E, UK; p.machado@ucl.ac.uk

Received 9 March 2021
Revised 16 April 2021
Accepted 17 April 2021
Published Online First 27 April 2021

To cite: Lawson-Tovey S, Strangfeld A, Hyrich KL, *et al*. *Ann Rheum Dis* 2021;**80**:1110–1115.

INTRODUCTION

Future disease outbreaks of epidemic proportion are inevitable. Advance planning and preparation is essential to mitigate future public health risks; the WHO emphasises the importance of in-depth evaluation of response to and lessons learnt from a national/international pandemic.[1] Research is critical to an informed, evidence-based response, therefore establishing pandemic research study protocols, systems to manage and report data, and rapid response teams are considered key to well-prepared, accelerated research in public health emergencies.[2]

Establishing international data collection registries poses many challenges, which are only amplified in the urgent nature of a global pandemic. The aim of this manuscript is to reflect on the successes and challenges of the European Alliance of Associations for Rheumatology (EULAR) COVID-19 registry[3] to better understand how the rheumatology community (and other disease-specific communities) can be better prepared for rapid response research in the future. In particular, we consider the successes and challenges of the registry, what can be learnt from this experience, and what procedures and resources should be established and strengthened now in preparation for future pandemics.

HISTORY OF THE EULAR COVID-19 REGISTRY

In the early stages of the SARS-CoV-2 pandemic, a need was identified for data to address the lack of information on the relationship between COVID-19 outcomes and rheumatic and musculoskeletal diseases (RMDs) and their associated treatments. Generally, immunomodulatory/immunosuppressive treatments and comorbidities are associated with an increased risk of serious infection in people with rheumatic diseases,[4] which indicated that these patients may be at a higher risk of more severe COVID-19 infection. Conversely, some rheumatic disease treatments are being studied for the prevention or treatment of COVID-19 and its associated complications.[5]

To rapidly collect data on and learn about COVID-19 outcomes in this population, the COVID-19 Global Rheumatology Alliance (GRA)[6] set up a global provider-entered registry, 13 days after initial Twitter discussions prompted by COVID-19 initiatives in other diseases. Further details on the initial development of GRA core data variables are described elsewhere,[7 8] and similar initiatives are listed in table 1.

Due to General Data Protection Regulations[9] in the European Union, Europe needed a separate, parallel registry. As EULAR represents patients and health professionals in rheumatology, a COVID-19 taskforce, comprising members of the executive and different committees, patients and epidemiologists, was swiftly created to address the challenges of the pandemic and its impact on patients with RMDs. It was decided that this registry should fall under the EULAR COVID-19 taskforce; the EULAR COVID-19 registry was launched via a REDCap platform 3 days later, and a partnership established with the GRA. A registry steering committee was created, composed of clinical epidemiologists involved in other registries and/or EULAR taskforces or committees, two data scientists, a People with Arthritis/Rheumatism in Europe representative, and EULAR communications staff.

EULAR COVID-19 REGISTRY TODAY

The EULAR COVID-19 registry is an observational registry capturing physician-entered data on both adult and paediatric patients with a pre-existing RMD and SARS-CoV-2 infection. A timeline of key milestones for the EULAR COVID-19 registry is shown in figure 1. Data are entered voluntarily directly into the European data entry portal. In addition, as some countries were already collecting COVID-19 data, either within existing registries or in new COVID-19 registries (France, Germany, Italy, Portugal, Sweden and Switzerland), they were invited to share their data with the EULAR COVID-19 registry. Once formal data sharing agreements were complete, data import pipelines were set up between these national registries and EULAR. REDCap automatically created a bespoke data dictionary and data import template for the registry, which could be shared with the national societies to enable recreation of the same variables and data mapping. Some registries opted to do the mapping themselves, whereas others sent their data directly to the database management team at The University of Manchester for mapping.

Successes

Database development

In response to updated data and information on COVID-19, the steering committee regularly reviewed the database using feedback and existing EULAR guidelines on registry establishment[10] where appropriate. Changes were made if there was a clear need (i.e., adding new COVID-19 treatments or a new variable to capture cause of

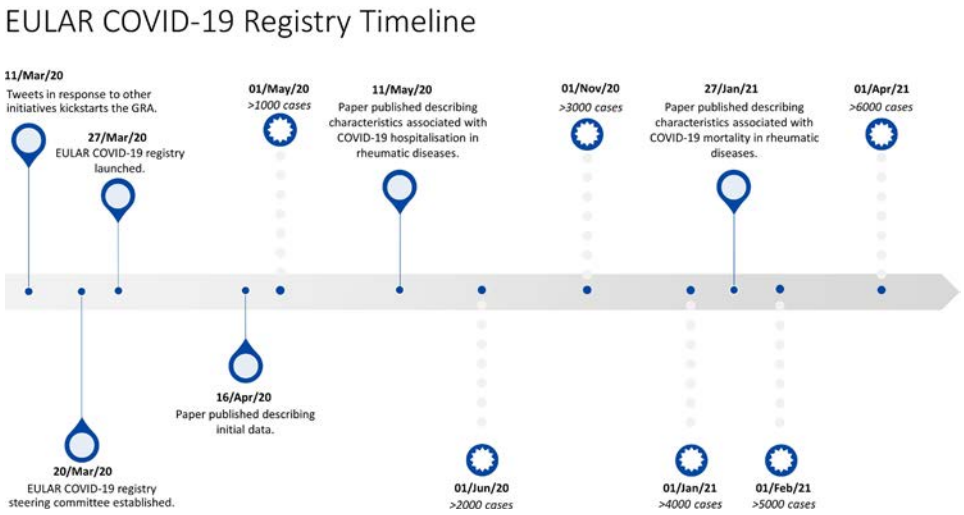

Figure 1 European Alliance of Associations for Rheumatology (EULAR) COVID-19 registry timeline. This figure shows key milestones reached by the EULAR COVID-19 registry from its inception until the present.

non-COVID-19-related death), which were then communicated to all national societies and the GRA. Additional data variables were also added after connecting with the European Scleroderma Trials and Research (EUSTAR) group to facilitate a combined analysis specific to patients with systemic sclerosis with COVID-19. COVID-19 vaccination questions were added once vaccines became available.

Having a steering committee made up of practicing clinicians, epidemiologists, data scientists, a communications expert and a patient partner ensured that we captured data and carried out analysis reflecting the needs of a broad spectrum of society. We met on a weekly basis for the first 6 months while we gained confidence with the challenges of running a European-wide registry and analysis effort in a rapidly changing situation. Subsequently, these meetings were reduced to a monthly basis supported by regular email communication.

Data acquisition

The prioritisation of COVID-19 by research ethics committees expedited the ethical review process of this registry in many jurisdictions. As the registry collects anonymous data, the UK Health Research Authority (and many others) considered it exempt from patient consent, making it easy to submit data. Furthermore, when submitting data, all providers accept that their own personal data are processed in accordance with the EULAR privacy notice.

There are currently 5824 cases in the registry, including 211 paediatric cases (as of 1 March 2021). The distribution of cases across Europe and the cumulative number of cases reported since the registry's inception are shown in figure 2. This includes 2519 (43%) cases reported directly into the database and 3305 (57%) cases imported from national registries. Rates of data acquisition fluctuated with the waves of SARS-CoV-2 infection seen across Europe, but the rate remains high with >500 cases directly reported in January 2021. Anonymous data collection in the form of a 5–10 min smartphone-compatible survey allowed clinicians to fit in data submission around their day-to-day work.

We leveraged the strength of existing EULAR connections to promote the EULAR COVID-19 registry. Where COVID-19 data collection was already established, new collaborations were formed with great success. Once data sharing was agreed with a national registry, the respective country was hidden from our live database and providers were redirected to the national society to submit data, thus supporting both local and international data collection, and preventing the upload of duplicate cases. National societies are also able to request an extract of their country's data without having to complete an application.

In recognition of participation, authorship was offered to national society leads and collaborator acknowledgements to clinicians who submitted a prespecified minimum number of cases depending on the analysis.

Data management/quality control

Simple measures were put in place to improve data quality from the outset. The majority of our fields were checkboxes

Table 1 List of initiatives collecting disease-specific data on COVID-19

Initiative	Medical area of interest
GRA	Rheumatic and musculoskeletal diseases
EULAR COVID-19	Rheumatic and musculoskeletal diseases
SECURE-IBD	Inflammatory bowel disease
SECURE-SCD	Sickle cell disease
COVID-HEP	Hepatology (liver disease or transplantation)
SECURE-LIVER	Liver disease
PsoProtect	Psoriasis
T1D Exchange	Type 1 diabetes
SECURE-AD	Atopic dermatitis
COVID-19 Dermatology Registry	Dermatology
CURE HIV-COVID	HIV
ASH RC COVID-19	Haematology
COVID-19 and Cancer Consortium	Cancer
PRIORITY	Pregnancy outcomes
Global Hidradenitis Suppurative COVID-19 Registry	Hidradenitis suppurativa

ASH RC COVID-19, American Society of Hematology Research Collaborative COVID-19 Registry for Hematology; COVID-HEP, COVID-19 in Patients with Liver Disease or Transplantation; CURE HIV, Coronavirus Under Research Exclusion HIV; EULAR, European Alliance of Associations for Rheumatology; GRA, Global Rheumatology Alliance; PRIORITY, Pregnancy Coronavirus Outcomes Registry; PsoProtect, Psoriasis Registry for Outcomes, Therapy and Epidemiology of COVID-19 Infection; SECURE-AD, Surveillance Epidemiology of Coronavirus Under Research Exclusion-Atopic Dermatitis; SECURE-IBD, Surveillance Epidemiology of Coronavirus Under Research Exclusion-Inflammatory Bowel Disease; SECURE-LIVER, Surveillance Epidemiology of Coronavirus Under Research Exclusion-Liver Disease; SECURE-SCD, Surveillance Epidemiology of Coronavirus Under Research Exclusion-Sickle Cell Disease; T1D Exchange, Type 1 Diabetes Exchange.

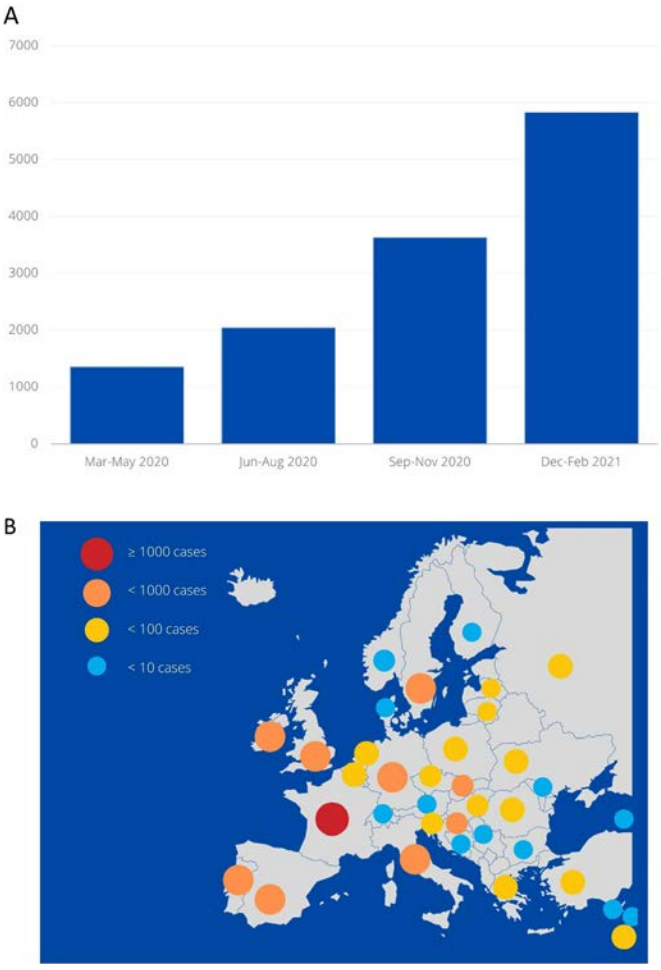

Figure 2 Cases reported to the European Alliance of Associations for Rheumatology COVID-19 registry as of 1 March 2021. (A) The cumulative number of cases over time. (B) The distribution of cases across Europe.

or dropdowns to limit inaccuracies frequently seen in free text. All other checkboxes in a field were disabled for selection if the provider had already selected a response of 'None' or 'Unknown'. Fields marked as required or with predefined ranges (e.g, minimum/maximum age of 0–120 years) would prompt the provider to fill/correct these fields before submission.

There were second level data quality control measures in place when cleaning the data for analysis. Dates were compared and sense checked and all free text entries were assessed to ascertain whether they could be recoded or if a reporter had clicked the correct checkboxes. If possible, cases were queried with the provider if a key variable was missing (e.g., age, COVID-19 outcome) and if the data were suspicious (e.g., a pregnant 80-year-old woman). Any fields potentially containing personal data were not shared with the GRA; this included details of the reporting clinician (except country) and any free text.

Outputs

One of our primary aims was to quickly disseminate our data and findings to the rheumatology community, hence, we committed to releasing regular summary reports on the EULAR COVID-19 registry website[3] while working on more substantial and complex analyses. These reports were weekly for the first 6 months of the pandemic and were subsequently reduced to monthly due to a reduction in cases over the summer of 2020.

By integrating our data with that of the GRA, we were able to produce a larger, more robust dataset. Stored on a secure platform at the University of California, San Francisco with accompanying statistical software, the ease of access to this combined global dataset and analysis platform facilitated stronger analyses by statisticians globally.

As of 1 March 2021, multiple papers[11–13] and abstracts have been produced using EULAR COVID-19 data, alongside numerous reviews and opinion pieces. Ongoing research includes combined analyses with the GRA, Childhood Arthritis Research and Rheumatology Alliance COVID-19 Global Paediatric Rheumatology Database, EUSTAR group, the Surveillance Epidemiology of Coronavirus Under Research Exclusion-Inflammatory Bowel Disease and the Psoriasis Registry for Outcomes, Therapy and Epidemiology of COVID-19 registries. Seven ancillary projects are also active after an open call for projects.

Our data, website and results have received high engagement from the rheumatology community, although social media engagement has declined throughout the pandemic (figure 3) . We produced infographics and lay versions of our reports and papers to provide easily accessible information to the patient

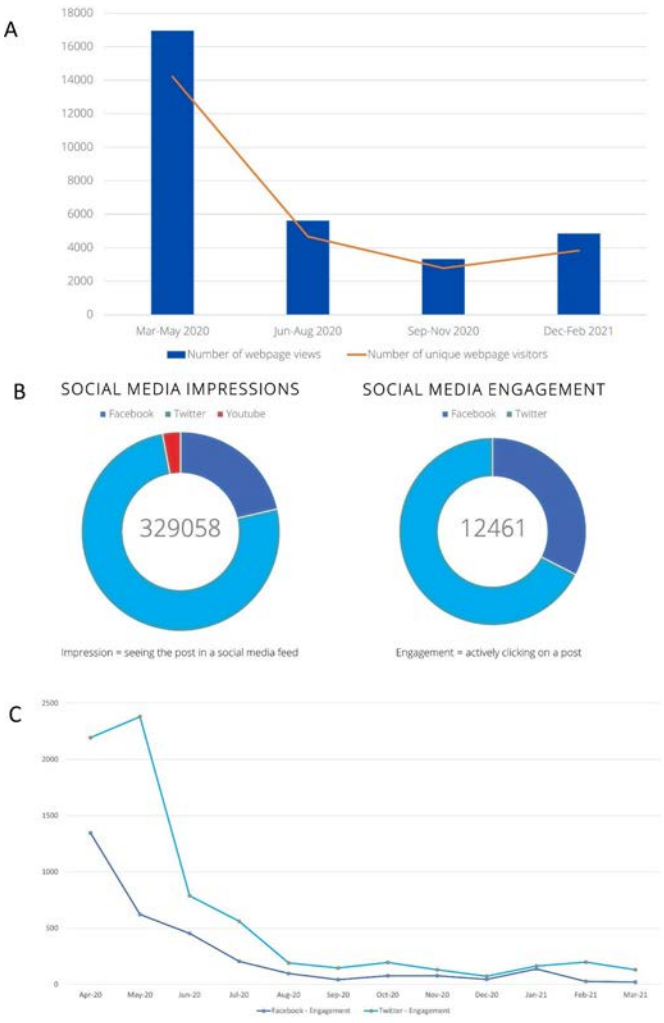

Figure 3 Web and social media analytics the European Alliance of Associations for Rheumatology (EULAR) COVID-19 registry as of 21 February 2021. (A) The number of EULAR COVID-19 registry webpage views and unique visitors over time. (B) The cumulative EULAR COVID-19 social media impressions and engagement levels. (C) The EULAR COVID-19 registry social media engagement over time.

Table 2 Proportion of missing and unknown data (N (%)) in the EULAR COVID-19 registry as of 1 March 2021

	Total N=5824	
Variable description	**Unknown**	**Missing**
General		
Date of case report	N/A	2 (0.03)
Age	N/A	0
Biological sex	N/A	0
Race/ethnic origin	209 (3.59)	1751 (30.07)
Comorbidities	92 (1.58)	250 (4.29)
Smoking status	1435 (24.64)	709 (12.17)
E-cigarette/vaping status	1649 (28.31)	1708 (29.33)
Seasonal influenza vaccination	1552 (26.65)	2399 (41.19)
Availability of lab tests	353 (6.06)	2366 (40.63)
COVID-19 measures		
Date of COVID-19 diagnosis	0	2 (0.03)
Method of COVID-19 diagnosis	168 (2.88)	27 (0.46)
COVID-19 diagnosis location	844 (14.49)	1636 (28.09)
COVID-19 infection acquisition	1394 (23.94)	1716 (29.46)
COVID-19 clinical symptoms *	53 (1.03)	61 (1.18)
COVID-19 treatment	139 (2.39)	1315 (22.58)
COVID-19 complications	188 (3.23)	2368 (40.66)
COVID-19 outcome		
COVID-19 outcome	203 (3.49)	2 (0.03)
Hospitalised	19 (0.33)	144 (2.47)
Interventions in hospital *	52 (2.63)	532 (26.90)
Approximate number of days from COVID-19 symptom onset to death*	N/A	111 (25.52)
Approximate number of days from COVID-19 symptom onset to resolution*	N/A	1506 (31.12)
Rheumatic disease		
Rheumatic disease diagnosis	0	0
Rheumatic disease activity	218 (3.74)	1592 (27.34)
Medication		
Immunomodulatory medication for rheumatic disease	21 (0.36)	307 (5.27)
Glucocorticoids at time of COVID-19 diagnosis	50 (0.86)	40 (0.69)
Glucocorticoid dose*	N/A	75 (4.23)
PD5 inhibitors	153 (2.63)	1994 (34.24)
ACE inhibitors	198 (3.40)	1887 (32.40)
Angiotensin receptor blockers	202 (3.47)	1925 (33.05)
Selective NSAIDs	212 (3.64)	1879 (32.26)
Non-selective NSAIDs	227 (3.90)	1412 (24.24)

Data are N (%) for all variables.
*Variable adjusted for database logic.
ACE, Angiotensin-converting enzyme; COVID-19, Coronavirus Disease 2019; EULAR, European Alliance of Associations of Rheumatology; NSAIDs, non-steroidal anti-inflammatory drugs; PD5, phosphodiesterase 5.

community hoping it would help alleviate patient anxiety around COVID-19 risk for patients with RMD.

Challenges

Database development

As our data needed to easily integrate into a global dataset, at times we were limited in the changes we could make to the database. The core data variables were put together very quickly at the start of the pandemic; had we had prior experience in a pandemic and more time and knowledge of what was required, we would have done some things differently. It became clear during analysis that fields such as date of last medication administration and further specific rheumatic disease measures would have been very useful and pertinent to the outcomes we were assessing, although we considered these against reporter time, data availability and the challenges of capturing outcomes across the entire spectrum of rheumatology.

Providers had an option to report any further relevant information in free text boxes—this led to some large paragraphs of text and full copies of patient case notes and correspondence. While we used some of this information to clean the data or evaluate the database, we rarely used this information in the analyses.

Data acquisition

Reporting bias towards more serious COVID-19 cases was evident from the start as we have a substantially higher proportion of hospitalised and deceased cases compared with the general population. Delays in mass testing availability in many European countries and cancellation of routine outpatient medical appointments would mean that some mild (or asymptomatic) SARS-CoV-2 infections may not have been detected or brought to the attention of the rheumatologist. Therefore, estimated rates of hospitalisation and death within the RMD population cannot be generated and the results cannot be used to infer any direct causal associations between the variables studied and outcome.

Fatigue among reporters was also evident; during the second European wave of SARS-CoV-2 infections, less clinicians directly reported cases than during the first. Some clinicians reported the survey was taking >10 min to complete as they had to trawl through the patient's case notes for the information.

Ethical approval procedures differed between countries and in some cases, the need for additional approvals delayed the ability to participate. It is also possible that national data collection efforts were missed if the relevant parties did not notice the request for collaboration with this registry.

Data management/quality control

As data collection is anonymous and cross-sectional, it is difficult to query data quality issues. We asked reporters to wait until the outcome was known and to record the auto-generated EULAR case ID, but this did not always happen or the IDs were incorrectly recorded. We decided to query only our most essential fields, as we were aware some providers might have difficulties accessing all the data we requested. Querying imported data was more complex and time-consuming, as we had to ask the national registry to query the original data provider; not all registries were able to do so. When uploading imported data, the existing plausibility checks could be bypassed (eg, age could be <0), increasing the need for second-line data quality measures.

Additionally, not all data were easily available to providers or collected by registries, either at all or in the same format. In some cases, this led to more complex data mapping or high levels of missingness in the EULAR COVID-19 dataset. One example is ethnicity—this is not regularly collected in Swedish medical data and local French data protection laws meant they were unable to provide us with this data. Another example is inflammatory rheumatic disease activity at time of COVID-19 infection. This was not recorded in the French registry who contributed ~25% of our cases—in all analyses where this variable was essential we had to either exclude these patients or impute missing data. The number of cases with unknown or missing data across most of our data items are shown in table 2.

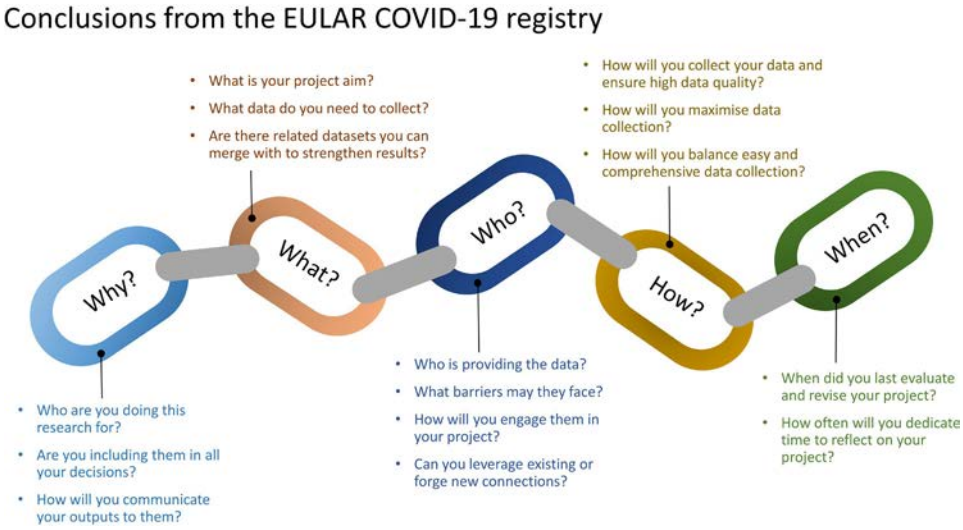

Figure 4 Key conclusions from the European Alliance of Associations of Rheumatology (EULAR) COVID-19 registry. This figure sums up our key conclusions drawn from setting up and running the EULAR COVID-19 registry.

CONCLUSIONS

The experience of setting up and managing this registry has emphasised the importance of the 'what, who and why' of data collection that we will all take forward to future projects. However, these considerations are not just applicable to rapid-response disease-specific research, but to all data collection projects in all specialties, regardless of region.

Arguably the most important is the why. Continuous involvement of patients and health professionals in our registry reminded us how essential it is to fully understand and address the questions and concerns of those who have a vested interest in the project's outcome.

What data we collect and who provides these data are inevitably intertwined. While we started the registry with a clear idea of what we thought essential to collect, this quickly changed when we realised data providers faced barriers such as siloed medical care records or ethical approval processes.

The balance between easy and comprehensive data collection is delicate. We created a quick, easy, anonymous survey while knowingly sacrificing a more robust, complex longitudinal data collection process. Ensuring the data also gives enough meaningful context around the outcomes one is analysing is, while easier to state in retrospect, vital.

There was an unspoken agreement within the rheumatic disease community, like many others, that the urgency of the pandemic made COVID-19 data collection a priority. We had high levels of engagement despite voluntary involvement and additional barriers to data collection; this may not be the case outside of such unique circumstances.

This registry demonstrated the strength in collaboration across Europe and we should look to strengthen these networks and pipelines further. As for the future of the EULAR COVID-19 registry, it now sits within the EULAR Virtual Research Centre,[14] which will act as a catalyst to build on these collaborations, for both COVID-19 and other RMD research.

We would encourage other registries/projects to undertake similar evaluations of their own situation, regardless of the project stage and include a diagram of our key conclusions in figure 4. There is much to be learnt from the incredible research that has occurred during this pandemic; failing to reflect and prepare in advance becomes all to evident when we are in the next one.

Author affiliations
[1]Centre for Genetics and Genomics Versus Arthritis, Centre for Musculoskeletal Research, The University of Manchester, Manchester, UK
[2]National Institute of Health Research Manchester Biomedical Research Centre, Manchester University NHS Foundation Trust, Manchester Academic Health Science Centre, Manchester, UK
[3]Epidemiology Unit, German Rheumatism Research Center (DRFZ Berlin), Berlin, Germany
[4]Centre for Epidemiology Versus Arthritis, The University of Manchester, Manchester Academic Health Science Centre, Manchester, UK
[5]Instituto de Salud Musculoesquelética, Madrid, Spain
[6]European Alliance of Associations for Rheumatology (EULAR), Kilchberg, Switzerland
[7]Sorbonne Université, INSERM, Institut Pierre Louis d'Epidemiologie et de Sante Publique, Paris, France
[8]Pitié-Salpêtrière hospital, AP-HP, Rheumatology department, Sorbonne Universite, Paris, Île-de-France, France
[9]Portuguese League Against Rheumatic Diseases (LPCDR), Lisbon, Portugal
[10]European Alliance of Associations for Rheumatology (EULAR) Standing Committee of People with Arthritis/Rheumatism in Europe (PARE), Kilchberg, Switzerland
[11]Centre for Rheumatology & Department of Neuromuscular Diseases, University College London, London, UK
[12]National Institute for Health Research (NIHR) University College London Hospitals Biomedical Research Centre, University College London Hospitals NHS Foundation Trust, London, UK
[13]Department of Rheumatology, Northwick Park Hospital, London North West University Healthcare NHS Trust, London, UK

Twitter Saskia Lawson-Tovey @saskiaamber, Loreto Carmona @carmona_loreto and Pedro M Machado @pedrommcmachado

Acknowledgements We wish to express our gratitude to all of our colleagues who have taken time to report data, especially in the darkest moments of the pandemic—we would not be able to call this endeavour a success without the generous contribution of all these colleagues. Additionally, we wish to thank the EULAR leadership and staff, and all the National Societies for supporting the Registry, particularly the representatives of national registries who have graciously taken time to collaborate and share their data with us: Rebecca Hasseli, Sascha Eulert, Eric Hachulla, Christophe Richez, Carlo Scirè, Lotta Ljung, Maria José Santos, Fernando Martins, Almut Scherer and Javier Bachiller. We would also like to thank colleagues from the COVID-19 Global Rheumatology Alliance, with whom EULAR has established a fruitful partnership, particularly their steering committee members: Philip Robinson, Jinoos Yazdany, Paul Sufka, Rebecca Grainger, Zachary Wallace, Suleman Bhana, Emily Sirotich, Jean Liew, Jonathan Hausmann and Wendy Costello.

Contributors SL-T drafted the first version of the manuscript. All authors revised the manuscript and approved the final version.

Funding The EULAR COVID-19 Database is financially supported by European Alliance of Associations for Rheumatology (EULAR).

Disclaimer The views expressed here are those of the authors and do not necessarily represent the views of the European Alliance of Associations for Rheumatology (EULAR), the (UK) National Health Service (NHS), the National

Institute for Health Research (NIHR), or the (UK) Department of Health, or any other organisation.

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Competing interests SL-T has nothing to disclose. AS reports personal fees from lectures for AbbVie, MSD, Roche, BMS, and Pfizer, all outside the submitted work. KLH reports she has received non-personal speaker's fees from Abbvie and grant income from BMS, UCB, and Pfizer, all unrelated to this manuscript, and is supported by the NIHR Manchester Biomedical Research Centre. LC has not received fees or personal grants from any laboratory, but her institute works by contract for laboratories among other institutions, such as Abbvie Spain, Eisai, Gebro Pharma, Merck Sharp & Dohme España, S.A., Novartis Farmaceutica, Pfizer, Roche Farma, Sanofi Aventis, Astellas Pharma, Actelion Pharmaceuticals España, Grünenthal GmbH, and UCB Pharma. LG reports personal consultant fees from AbbVie, Amgen, BMS, Biogen, Celgene, Gilead, Janssen, Lilly, Novartis, Pfizer, Samsung Bioepis, Sanofi-Aventis, UCB, and grants from Amgen, Lilly, Janssen, Pfizer, Sandoz, Sanofi, Galapagos, all unrelated to this manuscript. EFM reports that LPCDR received support for specific activities: grants from Abbvie, Novartis, Janssen-Cilag, Lilly Portugal, Sanofi, Grünenthal S.A., MSD, Celgene, Medac, Pharmakern, GAfPA; grants and non-financial support from Pfizer; non-financial support from Grünenthal GmbH, outside the submitted work. PMM has received consulting/speaker's fees from Abbvie, BMS, Celgene, Eli Lilly, Janssen, MSD, Novartis, Orphazyme, Pfizer, Roche and UCB, all unrelated to this manuscript, and is supported by the National Institute for Health Research (NIHR), University College London Hospitals (UCLH), Biomedical Research Centre (BRC).

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

ORCID iDs
Saskia Lawson-Tovey http://orcid.org/0000-0002-8611-162X
Anja Strangfeld http://orcid.org/0000-0002-6233-022X
Kimme L Hyrich http://orcid.org/0000-0001-8242-9262
Loreto Carmona http://orcid.org/0000-0002-4401-2551
Pedro M Machado http://orcid.org/0000-0002-8411-7972

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EPIDEMIOLOGICAL SCIENCE

EULAR Points to Consider (PtC) for designing, analysing and reporting of studies with work participation as an outcome domain in patients with inflammatory arthritis

Annelies Boonen ,[1,2] Polina Putrik ,[1,2] Mary Lucy Marques,[3,4] Alessia Alunno ,[5] Lydia Abasolo,[6] Dorcas Beaton,[7] Neil Betteridge,[8] Mathilda Bjørk ,[9] Maarten Boers ,[10] Boryana Boteva,[11] Bruno Fautrel ,[12,13] Francis Guillemin,[14,15] Elsa F Mateus,[16,17] Elena Nikiphorou ,[18,19] Márta Péntek,[20] Fernando Pimentel Santos,[21,22] Johannes L Severens,[23] Suzanne M M Verstappen,[24,25] Karen Walker-Bone,[26] Johan Karlsson Wallman ,[27,28] Marieke M ter Wee,[29,30] René Westhovens ,[31,32] Sofia Ramiro [33,34]

Handling editor Josef S Smolen

► Additional material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2020-219523).

For numbered affiliations see end of article.

Correspondence to
Dr Annelies Boonen, Dapt of Internal Medicine, Div of Rheumatology, Maastricht University Medical Centre+ Internal Medicine, Maastricht 6229 HX, The Netherlands; a.boonen@mumc.nl

Received 16 November 2020
Revised 21 March 2021
Accepted 23 March 2021
Published Online First 8 April 2021

To cite: Boonen A, Putrik P, Marques ML, *et al*. *Ann Rheum Dis* 2021;**80**:1116–1123.

ABSTRACT

Background Clinical studies with work participation (WP) as an outcome domain pose particular methodological challenges that hamper interpretation, comparison between studies and meta-analyses.

Objectives To develop Points to Consider (PtC) for design, analysis and reporting of studies of patients with inflammatory arthritis that include WP as a primary or secondary outcome domain.

Methods The EULAR Standardised Operating Procedures were followed. A multidisciplinary taskforce with 22 experts including patients with rheumatic diseases, from 10 EULAR countries and Canada, identified methodologic areas of concern. Two systematic literature reviews (SLR) appraised the methodology across these areas. In parallel, two surveys among professional societies and experts outside the taskforce sought for additional methodological areas or existing conducting/reporting recommendations. The taskforce formulated the PtC after presentation of the SLRs and survey results, and discussion. Consensus was obtained through informal voting, with levels of agreement obtained anonymously.

Results Two overarching principles and nine PtC were formulated. The taskforce recommends to align the work-related study objective to the design, duration, and outcome domains/measurement instruments of the study (PtC: 1–3); to identify contextual factors upfront and account for them in analyses (PtC: 4); to account for interdependence of different work outcome domains and for changes in work status over time (PtC: 5–7); to present results as means as well as proportions of patients reaching predefined meaningful categories (PtC: 8) and to explicitly report volumes of productivity loss when costs are an outcome (PtC:9).

Conclusion Adherence to these EULAR PtC will improve the methodological quality of studies evaluating WP.

Key messages

What is already known about this subject?

- Several systematic reviews of studies with work participation (WP) as a primary or secondary outcome domain have documented methodological deficiencies in the study design, analysis and reporting of results, hampering interpretation and pooling of data.

What does this study add?

- These Points to Consider (PtC) complement existing reporting guidelines, focusing on specificities of studies of patients with inflammatory arthritis that include WP as an outcome domain.
- The nine PtC address: study design, WP domains and instruments, data analysis and reporting of results.

How might this impact on clinical practice or future developments?

- Adherence to the PtC will improve the quality of studies on WP in patients with inflammatory arthritis, enabling comparisons across studies and meta-analyses.

INTRODUCTION

Earlier diagnosis and more effective treatment strategies have improved work outcomes in patients with inflammatory arthritis (IA), including presenteeism, sick leave and, to a lesser extent, employment rates. However, work participation (WP) remains lower compared with the general population.[1 2] For patients with IA, retaining work or (re)gaining a job is relevant to their life[3] and an important treatment goal.[4] From a societal perspective, participation in paid work contributes to each country's gross domestic product, and many (costly) innovations in IA can only approach cost-effectiveness when improvements in health are matched by improvements in long-term workforce participation.[5 6]

To bridge the WP gap with the general population, EULAR's current strategy states that 'by 2023, EULAR's activities and related advocacy will have

increased participation in work by people with rheumatic and musculoskeletal diseases (RMDs)'.[7] This requires actions within the healthcare system, but also at the level of workplaces and policies. To ensure efficient actions, high quality evidence from interventional and observational studies is needed.

WP studies face challenges that have been repeatedly highlighted in reviews of studies with WP as an outcome domain.[8 9] Identified issues relate to heterogeneity of definitions and measures to assess WP across studies. The role of contextual factors that modify or confound the outcome is often ignored. Sample size calculation specifically for the work outcomes and other methodological aspects are neglected and reporting of outcomes is often heterogeneous. To overcome such limitations that hamper correct interpretation, guidance for conducting and reporting studies with WP as an outcome are a first step. However, no such guidelines exist for studies on WP in RMDs.[10]

To fill this need, a EULAR taskforce was convened. The aim of the taskforce was to formulate Points to Consider (PtC) for the design, analysis and reporting of studies in patients with IA with work as a primary or secondary outcome domain. The target users of these PtC are researchers and any other persons that plan, conduct, analyse and critically appraise studies with WP as an outcome domain in patients with IA.

METHODS

Following approval by the EULAR Executive Committee, the convenor (AB) and methodologists (SR and PP) led a taskforce guided by the 2014 updated EULAR Standardised Operating Procedures, while being also aware of the Developers of Health Research Reporting Guidelines.[11]

At the first meeting, the taskforce decided the focus within IA would be on rheumatoid arthritis (RA), peripheral and axial spondyloarthritis (axSpA), psoriatic arthritis and adult patients with juvenile idiopathic arthritis. The definitions of *participation* and *employment*, central concepts to the current initiative, were specified following the WHO: *participation*: an active engagement in a life situation; *employment:* being employed or self-employed for a specific period in time (even as short as 1 day) to deliver products or services for compensation as wage, salary or in kind.[12 13] While outcomes such as employability, work (in) stability, and satisfaction with work can be relevant, they do not reflect active engagement in a production process (but the subjective experience) and thus are beyond the scope of these PtC. The taskforce also proposed to include unpaid work, as this is a relevant aspect of work participation for an even larger group of patients, and further emphasised that the PtC explicitly serve as an extension of existing reporting guidelines (eg, Consolidated Standards of Reporting Trials (CONSORT))[11 14 15] and assume adherence to them. The group agreed on 24 topics of concern across several methodological areas:study design; outcome domains; outcome measurement instruments; contextual factors; data analysis, reporting of results and work productivity costs (online supplemental table S2), and decided to perform two systematic literature reviews (SLRs) and two surveys. The first SLR included prospective studies with WP as an outcome domain in patients with IA and aimed at critically appraising methodological choices and heterogeneity across studies. The second SLR was an overview of reviews addressing SLRs of studies with WP as an outcome domain in chronic diseases other than IA, and focused on finding new aspects not yet identified by the taskforce or in IA studies. SLR findings have been published in an accompanying paper.[16] The first survey was conducted among professional organisations to identify other similar (unpublished) recommendations/guidelines beyond rheumatology. The second survey was conducted among experts on WP to identify other relevant methodological areas/topics (online supplemental tables S1 and S2). The SLRs and surveys resulted in 16 topics within four areas[1]: study design,[2] work outcome domains and measurement instruments,[3] data analysis and[4] reporting of results.

At the second meeting, the taskforce members formulated the PtC based on evidence from the two SLRs, findings of the surveys and expert opinion of taskforce members following a process of discussion and voting. Consensus was accepted if >75% of the members voted in favour of the PtC in the first (or >67% and >50% in a second and third) round. After the meeting, the levels of evidence derived from the SLRs following the standards of the Oxford Center for Evidence Based Medicine were added to each of the recommendations.[17] Finally, each taskforce member anonymously indicated the level of agreement (LoA) via email (numeric rating scale ranging from 0='do not agree at all' to 10='fully agree'). The mean and SD of the LoA as well as the percentage of taskforce members with an agreement ≥8 are presented.

Based on the gaps in evidence and the issues of controversy, a research agenda was formulated. The final manuscript was approved by the EULAR Executive Committee.

RESULTS

The taskforce agreed on two overarching principles and nine PtC (table 1).

Overarching principles

1. WP is important for people with inflammatory arthritis, their families and society as a whole.
2. There are unique methodological aspects around designing, analysing and reporting studies with WP as a primary or secondary outcome that require specific attention.

Points to consider

1. In studies with WP as primary or secondary outcome the study design, the study duration and the choice of WP outcome domains and measurement instruments should be considered in relation to the work-related study objective.

WP studies can serve a variety of *objectives*, such as developing risk-identification tools to predict adverse work outcomes, proving effectiveness of pharmacological or non-pharmacological interventions, assessing the impact of costs of work productivity loss in economic evaluations and so on. While each study objective requires a specific *design*, non-pharmacological interventions pose additional challenges related to contamination of the intervention, problems with double blinding, difficulty controlling for cointerventions, and long lag times for some outcomes. For these studies, strengths and weaknesses of various semiexperimental study designs should be weighted.[18] Next, careful consideration should be given to the *target population* as different WP outcomes may apply to distinct (sub)populations. For example, when the aim is to assess the impact of a certain treatment on employment, all persons below the age of retirement are the target, whereas for a study on the impact of treatment on sick leave, employed persons are the target. Additionally, some studies might wish to target specific patients, for example, those with short disease duration; with low educational level; doing manual work; or with low self-management skills, requiring specification of eligibility criteria. Further, interpretation of the work outcome(s) depends on the participation rate in

Table 1 EULAR Points to Consider when designing, analysing and reporting studies with work participation as a primary or secondary outcome domain: LoE, SoR and LoA

	LoE (0–5)	SoR	LoA (0–10) Mean (SD)	LoA (0–10) % with score ≥8
Overarching principles				
1. Work participation is important for people with inflammatory arthritis, their families and society as a whole.	n.a	n.a	9.6 (0.7)	100
2. There are unique methodological aspects around designing, analysing and reporting studies with work participation as an outcome that require specific attention.	n.a	n.a	9.5 (0.7)	100
Points to consider				
1. In studies with work participation as primary or secondary outcome the study design, the study duration and the choice of work participation outcome domains and measurement instruments should be considered in relation to the work-related study objective.	5	D	9.7 (0.6)	100
2. In studies with work participation as primary or secondary outcome, the power to detect meaningful effects deserves particular attention as work participation outcomes may not apply to the entire study population.	5	D	9.6 (0.8)	96
3. The work participation outcome domains (eg, work status, absenteeism, presenteeism) should be clearly defined and assessed with validated measurement instruments.	5	D	8.6 (0.8)	91
4. Key contextual factors (eg, job type, social security system), that is, contextual factors that are highly likely to confound or modify work participation outcomes, have to be identified upfront, considered in the study design and appropriately accounted for in the analysis.	5	D	9.1 (1.3)	87
5. Interdependence among different work participation outcome domains (eg, between absenteeism and presenteeism) should be taken into account in the analyses.	5	D	9.4 (0.8)	100
6. Populations included in the analysis of each work participation outcome domain should be specified and relevant characteristics described.	5	D	9.1 (1.3)	83
7. In longitudinal studies work status should be regularly assessed and changes reported.	5	D	9.3 (1.0)	91
8. Reporting both aggregated results (eg, mean/median) and proportions of individuals based on predefined meaningful categories (eg, no sick leave) should be considered.	5	D	9.3 (1.6)	91
9. In studies assessing costs of changes in work participation, volumes of work productivity (eg, days, hours) should also be reported.	5	D	9.3 (1.3)	91

LoE: 1–5 (5 indicating evidence from expert committee reports or opinions and/or clinical experience of respected authorities, and/or evidence extrapolated for quasi experimental or descriptive studies)[17]; SoR: A to D (D indicating troublingly inconsistent or inconclusive studies of any level).[18]
LoA, level of agreement; LoE, level of evidence; n.a, not applicable; SoR, strength of recommendation.

the general population. It is useful to reflect in the design phase whether population benchmarks for sick leave, work disability and employment status are important and feasible. Crucial in any design is the choice of the outcome domain(s) of interest and their *match with the objective and study duration*. While changes in presenteeism and sick leave can occur over short periods in time, longer term sick leave and, in particular, work disability require longer observation periods. Additionally, the taskforce urges researchers to ensure alignment of the *frequency of assessment of* WP outcomes to the recall of the measurement instruments and the study objective. For example, in a 24-week randomised controlled trial with a rapidly acting intervention, assessment of sick leave in the past 7 days (eg, using Work Productivity and Activity Impairment Index (WPAI)[16 19 20] at baseline and endpoint is useful, as the interest is to assess change in sick leave on a group level. Alternatively, when cumulative days of sick leave over time are of interest in an observational study with long follow-up, the recall (eg, past 3 months) should fit the duration of the inter-assessment period (in casu 3 months). Importantly, the taskforce emphasised that for studies with WP as a primary outcome, the choices on the issues above should be 'justified', not just 'considered'.

2. In studies with WP as primary or secondary outcome, the power to detect meaningful effects deserves particular attention as WP outcomes may not apply to the entire study population.

The majority of WP studies include work as a secondary objective.[16] As work outcomes often relate to a sub-sample of the population for which the initial sample size was calculated (eg, 18–64 years when work status is the outcome of interest; those employed when sick leave or presenteeism are studied), the number of patients eligible for the work outcome analyses drops, likely reducing the power to detect differences between groups. Researchers should consider this when designing the study or selecting a dataset.

3. The WP outcome domains (eg, work status, absenteeism, presenteeism) should be clearly defined and assessed with validated measurement instruments.

Heterogeneity or lack of definitions of the WP outcome domains are an important cause of incomparability and a risk for misinterpretation of findings across studies. While for some commonly used (sub)-domains (eg, employment) formal definitions have been proposed, operationalisation varies greatly across administrative entities (countries, regions, states, etc). As a consequence, researchers may have good reasons to use a specific or adjusted definition (eg, self-reported vs formal work disability). Nevertheless, a clear description of each WP outcome domain under study is warranted, and definitions should fit the research objective but also strike a balance between local usefulness and generalisability of the study findings (table 2).

To support measurement of WP outcome domains, Outcome Measures in Rheumatology (OMERACT) continuously updates the validity of *self-reported instruments* to assess presenteeism.[21] The taskforce specified that for presenteeism the study objective should guide the choice between single-item and multi-item/multidimensional instruments. Of note, specific aspects of measurement instruments including the recall period, disease attribution or the anchors for presenteeism or absenteeism (compared with your own best or to an average worker) are not specifically addressed in the above assessments of validity.

Table 2 Glossary of terms relevant for the current Points to Consider

Term	Source	Definition
Work participation	ICF	Active engagement in paid or unpaid work.
Contextual factor	ICF	In the bio-psycho-social *framework of health* contextual factors refer to variables that are part of the environment of the individual (eg, social attitudes, architectural characteristics, legal and social structures, as well as climate, etc) or characterise the individual him/herself (eg, gender, age, coping, lifestyle, social background, education, profession, past and current experiences). They influence occurrence and course of disease and determine how illness and disability is experienced by the individual.
	OMERACT	In the *framework of outcome assessment*, contextual factors are variables that are not the outcome of the study, but need to be recognised to understand the study results. They also include confounders and effect modifiers. They can be measurement affecting, outcome influencing or effect modifying.
Employment	ILO/WHO	An agreement to produce goods or services for a specific period in time for compensation by a salary, a wage or in kind. Different types of employment exist, among which is self-employment.
Part-time employment	ILO/WHO	When the hours of work are less than the 'normal' hours of work of a comparable full-time employment.
Sick leave	WIKI	Time off from work that workers can use to stay home to address their health and safety needs without losing pay.
Paid sick leave	ILO/WHO	A statutory requirement in many nations or organisations that comprise (universal) income substitutions for persons that have temporary time off from the employment contract due to illness or disability. Against this background sick leave consists of two components: leave from work due to sickness and cash benefits that replace the wage during the time of sick leave.
Presenteeism	Various	Refers to: 1. The behaviour of attending (paid) work while being ill. 2. The level of influence on the work process (productivity, efficiency, performance) experienced by the worker (ability, difficulty).
Work productivity		The amount of goods and services produced in a specific time frame/period in time.
Unemployment	ILO/WHO	Not being employed but looking for an employment.
Work disability	ILO	When an individual is unable to perform work-related tasks due to physical or mental impairments or disability. In many constituencies definitions of disability are identical with an administrative act of recognising a disability. This recognition as disabled becomes a prerequisite for the claiming of support on the basis of a physical or mental limitation or for litigation under an antidiscrimination law. Such support can comprise provisions for rehabilitation, special education, retraining, privileges in the securing and preserving of a place of employment, guarantee of subsistence through income, compensation payments and assistance with mobility, etc. Virtually every existing definition of disability thus mirrors a legal system and draws its meaning from this system. It is also a highly heterogeneous concept, making the search for a homogeneous definition a virtually impossible task.
Decent work	ILO	Decent work involves opportunities for work that are productive and deliver a fair income, security in the workplace and social protection for families, better prospects for personal development and social integration, freedom for workers to express concerns, organise and participate in the decisions that affect their lives and equality of opportunity for all women and men.
Unpaid work	WHO	Unpaid work activities include own-use production of services and volunteer work in households or organisations producing services for others.

ICF, International Classification of Functioning, Disability and Health; ILO, International Labour Organisation; OMERACT, Outcome Measures in Rheumatology.

Regarding recalling information, there is evidence that recall beyond 3 months for *sick leave* becomes inaccurate and that patients prefer a recall period of 1–4 weeks for presenteeism; patients suggests 4 weeks is more representative.[22 23] Attribution to overall health (opposed to IA-related) is preferred, as patients struggle to attribute restrictions to arthritis vs overall health, and it allows benchmarking with the general population. Of note, in several countries regulations are in place to link healthcare data to social security databases that include information on sick leave and work disability. While avoiding non-response and recall bias, such linkage of data is not without challenges. A pertinent example is that registration only starts when sick leave exceeds a number of prespecified days.

4. Key contextual factors (eg, job type, social security system), that is, contextual factors that are highly likely to confound or modify WP outcomes, have to be identified upfront, considered in the study design, and appropriately accounted for in the analysis.

There is ample evidence associating work-related environmental and personal contextual factors to WP outcomes, either as effect modifiers, or other types of covariates.[24] Contextual factors can be facilitators or barriers for WP.[25] For example, manual workers experience more impact from axSpA on presenteeism, but also experience more beneficial effect of bDMARDs on presenteeism.[26] Country of residence (likely reflecting social security regulations, including income substitution) is another contextual determinant of variation in employment and sick leave rates across countries,[27 28] and may cause effect modification of interventions.[29] OMERACT proposed a classification of 12 contextual factor domains potentially relevant for WP outcomes[30 31] (table 3). The choice of contextual factors, as well as the methodological approach to account for them (eg, stratification, post hoc analyses) should be prespecified in the study protocols. Whereas contextual factors refer—according to some definitions—to factors outside the disease (eg, job type),[32] also disease-related factors (eg, early vs established disease; type of joints involved) or factors within the work outcome continuum (eg, being partly work disabled) can be equally relevant as effect modifiers or covariates. On this line, jobs requiring hand dexterity might affect work outcomes more importantly in patients with small joint involvement compared with those with only back manifestations.

5. Interdependence among different WP outcome domains (eg, between absenteeism and presenteeism) should be taken into account in the analyses.

WP presents a continuum of subdomains which are *dependent* on each other, and may *compete over time*. For example, formal work disability cannot occur anymore after early retirement from paid work; and presenteeism cannot occur when a person is on sick leave (ie, absent form work). Dependency of outcome

Table 3 Proposal for classification of contextual factors relevant for studies with work participation as an outcome domain. Contextual factors can be facilitators or barriers

Personal contextual factors	Environmental contextual factors
Health*	Nature of work
Pain	Physical/mental demands
Fatigue	Job autonomy
Physical function	
Demographics	Workplace support/barriers
Age and gender	Assistance by coworkers
Education	Attitude of employer
Economic need	Workplace organisation
Income needs	Team dynamics at work
Quality of benefits	Compensation of absence (eg, replacement practices)
Personal appraisal of work	Workplace accommodation
Job satisfaction	Adaptive devices
Career perspectives	Modified hours/duties
Skills and abilities	Economic climate/labour regulations
Work-efficacy	Income compensation
Coping	Employment opportunities
Work-life balance	Workplace accommodation
Competing social roles	Adaptive devices
Quality of leisure	Modified hours/duties
	Non-workplace support/barriers
	Support from family
	Task assistance at home

*In the setting of clinical studies, health factors are relevant to interpret the study results and (contrary to the International Classification of Functioning, Disability and Health (ICF) definition) considered to represent personal contextual factors. In the ICF classification, contextual factors are by definition external to health factors. In the Outcome Measures in Rheumatology methodological definition, health factors can be covariates (effect modifiers, confounders).

domains can explain why an intervention that markedly reduces sick leave days, can lead to an increase in presenteeism. To account for dependencies, it is advised to always collect information on the (sub-)domains that are hierarchically higher (presenteeism depends on sick leave, sick leave depends on work status) on the work ability/productivity continuum, or conceptually related to the outcome (sub)-domain of interest (eg, absenteeism and presenteeism; retiring early or becoming work disabled). Authors need to report whether and how they dealt with this dependency.[16 33] For example, the WPAI deals formally with this issue by combining presenteeism and absenteeism into an overall work impairment scale.[34]

6. Populations included in the analysis of each WP outcome domain should be specified and relevant characteristics described.

WP outcomes are often performed in subsamples of the original study.[16] For example, a model exploring risk factors for work disability is to be analysed in the at-risk population below retirement age (usually 18–64 years old), while a model on risk factors for long-term sick leave or presenteeism addresses the employed population. Especially when measurement instruments report impact on paid as well as unpaid work (eg, WPAI), numbers and details of the employed and unemployed patients should be provided.[16] To facilitate the correct interpretation of the output of the analyses, the baseline demographic and disease characteristics of each (sub)-group should be described.

7. In longitudinal studies work status should be regularly assessed and changes reported.

Given the chronic, progressive character of IA, longitudinal studies are encouraged to assess changes in WP. Those changing their work status (especially, becoming work disabled) are likely prognostically different from the rest of the population. For example, if an improvement in sick leave of employed persons with early RA was observed over time, this may partly be due to patients with the highest disease impact—and thus sick leave—becoming work disabled over time. Therefore, in longitudinal studies transitions should be described, and either accounted for in analyses or discussed when interpreting the results.

8. Reporting both aggregated results (eg, mean/median) and proportions of individuals based on predefined meaningful categories (eg, no sick leave) should be considered.

In addition to mean and median values of continuous measures (such as sick leave days, level of presenteeism), also the proportion of patients attaining a specific meaningful (change in) outcome adds to insight of the WP outcome. For example, as presenteeism and absenteeism have often a skewed (or zero-inflated) distribution, it is informative to present also the proportion of patients that had no sick leave or presenteeism. Meaningful categorisation can also be based on what is used by the social security system (eg, proportion with specific number of sick leave days). For presenteeism, work has been done on the minimally important difference, but data do not seem robust and more work is needed before a generalisable threshold is proposed.[35]

9. In studies assessing costs of decreased WP, volumes of work productivity loss (eg, days, hours) should also be reported.

Productivity costs are a relevant aspect of WP but valuing loss of productivity in monetary terms (ie, costing) is complex and beyond the expertise of this taskforce. Nevertheless, the taskforce wanted to highlight a basic principle that should be fulfilled when researchers aim to proceed towards calculating costs of productivity loss. In any cost study, authors should first collect/report the *natural volumes of production loss* (usually *time*; days/hours) before providing the cost-estimates. In view of poor agreement between self-reported productivity loss while at work (presenteeism) and actual productivity loss, presenteeism costs should be considered in sensitivity analyses only.[16]

Research agenda

Areas or topics that were considered important by the taskforce experts but for which the level of uncertainty was too high to formulate a PtC were included in a research agenda (table 4).

DISCUSSION

Assessment of WP as an outcome domain in clinical studies has specific methodological challenges. The nine PtC aim to improve the quality of interventional and non-interventional studies and should eventually contribute to improving WP for patients with IA. Specifically, adherence to these methodological considerations should lead to unbiased results and facilitate meta-analyses.

A clear study objective constitutes a first and critical step of any WP outcome study, as it determines the target population, the outcome domains, the study duration, the frequency with which outcomes should be assessed in relation to the recall of the measurement instrument, and, finally, the contextual factors that should be accounted for. In addition, in the analysis and report the interdependence (and competition) between WP outcomes should receive specific attention. While these individual topics seem basic epidemiological knowledge, and some of them are (implicitly) part of the CONSORT[15 36] and Strengthening the Reporting of Observational Studies in Epidemiology[14] statements, they accumulate in work outcome studies and are frequently ignored in existing studies.[16]

Table 4 Research agenda

Topic	Questions
Unpaid work participation	How can unpaid work participation as an outcome domain be defined? Which measurement instruments are valid to assess the domain unpaid work (in IA)?
Contextual factors	How to *measure* contextual factor domains relevant for work participation? What is the *operational definition* of a 'key' contextual factor (eg, if it has proven to behave consistently as: (a) Relevant effect modifier of interventions in work outcome studies, or (b) Consistently relevant covariate of work outcomes in observational studies.)? To what extent are contextual factors specific to certain setting (eg, specific for a certain outcome are a certain intervention)?
Interdependence and integration of the work outcome domains	How to deal with interdependence or competition between work participation outcomes (work status, absenteeism and presenteeism)? Can we redesign work outcome measurement that integrates work disability, absenteeism and presenteeism?
Analyses of skewed data	What is the comparative accuracy of methods to deal with different types of skewed or zero-inflated data?
Decent work and healthy workplaces	What is a healthy work and what is a healthy workplace? How can we measure it? What are the health effects of *not* taking sick leave and *not* adjusting productivity while at work (presenteeism)?

IA, inflammatory arthritis.

The taskforce identified and discussed some areas or topics where no consensus could be reached due to lack of evidence and placed these in the research agenda. In the first taskforce meeting, it was proposed to broaden the scope of PtC to studies with unpaid work as an outcome domain clearly impacted by IA. However, the absence of appropriate definitions and absence of evidence from both SLRs, led the taskforce to urgently recommend more research focus on unpaid work. The lack of evidence on specific methodological issues (eg, contextual factors, skewness or interdependence of outcomes) prevented more specific statements on these issues, which were also added to the research agenda.

The taskforce would like to emphasise that while important, improvement of WP, employment, reduced sick leave or presenteeism should never be reached at the expense of long-term health or even life satisfaction. Rather, the final goal should be to support patients in healthy and sustainable work, and days off work or adjustments in work productivity can be tools to reach this goal. Defining and measuring 'healthy and sustainable work' is added as a challenge to our research agenda. Reaching these goals will not only depend on efforts within the healthcare system to support patients to stay at work but will also require supportive employers, behavioural changes towards workers with a chronic disease and policies for healthy workplaces and support systems for persons with chronic diseases. This underpins the urgency of EULAR's strategic goal to improve work circumstances of people with RMDs.[7] Patient representatives found it challenging to take an active role in the discourse of complex methodological issues, but were instrumental in reinforcing the discussions on unpaid work, healthy work and context, ensuring these aspect were included in statements or research agenda.

In conclusion, guidance is now available to improve interpretation and comparison of studies in IA with WP as an outcome domain. We expect the PtC will facilitate improved conduct of WP outcome studies.

Author affiliations
[1]Division of Rheumatology, Maastricht University Medical Centre+ Internal Medicine, Maastricht, The Netherlands
[2]Universiteit Maastricht Care and Public Health Research Institute, Maastricht, The Netherlands
[3]Rheumatologist, Leiden University Medical Center, Leiden, The Netherlands
[4]Rheumatology, Centro Hospitalar e Universitario de Coimbra EPE, Coimbra, Portugal
[5]Rheumatology Unit, University of Perugia Department of Medicine, Perugia, Italy
[6]Department of Rheumatology, Instituto de Investigation Sanitaria San Carlos, Hospital Clinico Universitario San Carlos, Madrid, Spain
[7]Mobility Program Clinical Research Unit, St Michael's Hospital Li Ka Shing Knowledge Institute, Toronto, Ontario, Canada
[8]Neil Betteridge Associates, London, UK
[9]Pain and Rehabilitation Center, and Department of Health, Medicine and Caring Sciences, Linköping University, Linkoping, Sweden
[10]Department of Epidemiology and Data Science; Amsterdam Rheumatology and Immunology Center, Amsterdam University Medical Centers, Vrije Universiteit, Amsterdam, Amsterdam, The Netherlands
[11]Patients with Arthritis and Rheumatism (PARE) working group, European League Against Rheumatism, Zurich, Switzerland
[12]PEPITES teams, Pierre Louis Institute for Epidemiology and Public Health, Inserm UMR 1136, Paris, France
[13]Rheumatology Dept, Pitié Salpetriere Hospital, Sorbonne University / Assistance Publique- Hôpitaux de Paris, Paris, Fance
[14]APEMAC, Université de Lorraine, Nancy, France
[15]CIC Epidémiologie Clinique, CHRU Nancy, Inserm, Université de Lorraine, Nancy, France
[16]Portuguese League Against Rheumatic Diseases (LPCDR) and Comprehensive Health Research Centre (CHRC), Lisbon, Portugal
[17]People with Arthritis and Rheumatism (PARE), European League Against Rheumatism, Zurich, Switzerland
[18]Centre for Rheumatic Diseases, King's College of London, London, UK
[19]Rheumatology Department, King's College Hospital, London, UK
[20]Health Economics Research Center, University Research and Innovation Center, Óbuda University, Budapest, Hungary
[21]Rheumatology Department, Centro Hospitalar de Lisboa Ocidental EPE Hospital de Egas Moniz, Lisboa, Portugal
[22]NOVA Medical School, Lisboa, Portugal
[23]Erasmus School of Health Policy & Management and iMTA, Institute for Medical Technology Assessment, Erasmus University Rotterdam, Rotterdam, The Netherlands
[24]Centre for Epidemiology Versus Arthritis, Centre for Musculoskeletal Research, Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, UK
[25]Manchester University NHS Foundation Trust, Manchester Academic Health Science Centre, NIHR Manchester Biomedical Research Centre, Manchester, UK
[26]MRC Versus Arthritis Centre for Musculoskeletal Health and Work, MRC Life course Epidemiology Unit, Southampton General Hospital, Southampton, UK
[27]Department of Clinical sciences, Lund University, Lund, Sweden
[28]Department of Rheumatology, Skåne University Hospital, Lund, Sweden
[29]Department of Epidemiology and Data Science, Amsterdam Public Health, Amsterdam UMC, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands
[30]Department of Rheumatology and immunology, AI&I, Amsterdam UMC, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands
[31]Dept of Rheumatology, KU Leuven University Hospitals Leuven, Leuven, Belgium

[32]Skeletal Biology and Engineering Research Centre, KU Leuven Department of Development and Regeneration, Leuven, Belgium
[33]Rheumatology, Leiden University Medical Center, Leiden, The Netherlands
[34]Rheumatology, Zuyderland Medical Centre Heerlen, Heerlen, The Netherlands

Twitter Neil Betteridge @Neil_Betteridge and Elena Nikiphorou @ElenaNikiUK

Acknowledgements We wish to express our gratitude to all experts participating in the survey, several of which consented to be included formally in the acknowledgements: Ailsa Bosworth, MBE, National Patient Champion, National Rheumatoid Arthritis Society, UK; Ann Bremander, PT, PhD, Department of Regional Health Research, University of Southern Denmark, Odense, Denmark and Section of Rheumatology, Department of Clinical Sciences, Lund University, Lund, Sweden; Arthur Kavanaugh, MD, Professor of Medicine, University of California, San Diego, USA; Atul Deodhar, MD, Professor of Medicine, Oregon Health & Science University, Portland, USA; Casper Webers, MD, Department of Internal Medicine, Division of Rheumatology, Maastricht University Medical Center, Maastricht, the Netherlands; Désirée van der Heijde, MD, PhD, Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands; Florian Berghea, Associate Professor, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania; Imre Boncz, MD, MSc, PhD, Institute for Health Insurance, Faculty of Health Sciences, University of Pécs, Pécs, Hungary; Ingemar Petersson, MD, PhD, Head of Research-Skåne University Care Trust (Lund and Malmö), Sweden; Jette Primdahl, Professor, Danish Hospital for Rheumatic Diseases, Sonderborg, Denmark and Department of Regional Health Research, University of Southern Denmark, Odense, Denmark; Kari Puolakka, MD, PhD, Terveystalo, Lappeenranta, Finland; Karthik Mani, OTD, OTR/L, National Board for Certification in Occupational Therapy (NBCOT), Maryland, USA; Katie Druce, MSc, PhD, Centre for Epidemiology Versus Arthritis, Division of Musculoskeletal and Dermatological Sciences, University of Manchester, UK; Laszlo Czirjak, PTE, Department of Rheumatology and Immunology, Medical School, University of Pécs, Pécs, Hungary; László Gulácsi, MD, MSc, PhD, Department of Health Economics, Corvinus University of Budapest, Hungary; Lennart R. A. van der Burg, MD, Department of General Practice and Care and Public Health Research Institute (CAPHRI), Maastricht University, Maastricht, The Netherlands; Manouk de Hooge, PhD, Department of Rheumatology, Ghent University Hospital and VIB Center of Inflammation Research, Ghent University, Ghent, Belgium; Michiel Reneman, MD, PhD, University Medical Center Groningen, The Netherlands; Miranda van Lunteren, MSc, Leiden University Medical Center, Leiden, the Netherlands; Pál Géher, MD, Semmelweis Univ. Budapest, Hungary; Peter C Taylor MA, PhD, FRCP, University of Oxford, UK; Peter V Balint, MD, PhD, FRCP (Glasgow), 3rd Rheumatology Department, National Institute of Rheumatology and Physiotherapy Budapest, Hungary; Ross Wilkie, Senior Lecturer in Public health and Epidemiology, School for Primary, Community and Social Care, Keele University, Keele, Staffordshire, UK; Yeliz Prior, PhD, University Salford and Advanced Clinical Specialist Occupational Therapist at the Mid Cheshire NHS Hospitals Trust, Crewe, UK; Zoltan Szekanecz, MD PhD, Division of Rheumatology, University of Debrecen Faculty of Medicine, Debrecen, Hungary. We are very grateful to Louise Falzon for the literature searches that served the systematic literature reviews.

Contributors AB, SR, PP, MLM and AA designed the study; MLM and AA collected the data; all authors participated in the interpretation of the data; AB, SR, PP, MLM and AA prepared the manuscript; all authors critically appraised and approved the final manuscript.

Funding This study was funded by European League Against Rheumatism (EPI021); SMMV is supported by the NIHR Manchester Biomedical Research Centre and Versus Arthritis (grant number 20380).

Competing interests AB: research grants from Abbvie and Celgene and honoraria for consultancy or speakers honoraria from UCB, Biogen, Sandoz, Eli Lilly and Galapagos (all to her department); EN has received speaker honoraria/participated in advisory boards for Celltrion, Pfizer, Sanofi, Gilead, AbbVie and Lilly; FPS: research grant from AbbVie, Novartis; consultancy or speaking fees: AbbVie, Angelini, AstraZeneca, Biogen, Eli Lilly, Laboratórios Vitória, MSD, Novartis, Tecnimed, UCB; SR: research grant from MSD; consultancy or speaking fees: AbbVie, Eli Lilly, MSD, Novartis, Sanofi, UCB; JKW: consultancy fees from Celgene, Eli Lilly, Novartis; RW: consultancy and speakers bureau Celltrion and Galapagos/Gilead.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data sharing not applicable as no datasets generated and/or analysed for this study. No datasets were generated and/or analysed for this study.

ORCID iDs
Annelies Boonen http://orcid.org/0000-0003-0682-9533
Polina Putrik http://orcid.org/0000-0002-9342-1861
Alessia Alunno http://orcid.org/0000-0003-1105-5640
Mathilda Bjørk http://orcid.org/0000-0002-1607-187X
Maarten Boers http://orcid.org/0000-0002-6969-283X
Bruno Fautrel http://orcid.org/0000-0001-8845-4274
Elena Nikiphorou http://orcid.org/0000-0001-6847-3726
Johan Karlsson Wallman http://orcid.org/0000-0002-4915-2924
René Westhovens http://orcid.org/0000-0002-3432-3073
Sofia Ramiro http://orcid.org/0000-0002-8899-9087

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CLINICAL SCIENCE

Glucocorticoid discontinuation in patients with early rheumatoid and undifferentiated arthritis: a post-hoc analysis of the BeSt and IMPROVED studies

Johanna Maria Maassen [1], Raquel Dos Santos Sobrín [2], Sytske Anne Bergstra [1], Robbert Goekoop [3], Tom W J Huizinga [1], Cornelia F Allaart[1]

Handling editor Josef S Smolen

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2021-220403).

[1]Rheumatology, Leiden University Medical Center, Leiden, The Netherlands
[2]Rheumatology, Clinical University Hospital of Santiago de Compostela, Santiago de Compostela, Spain
[3]Rheumatology, HagaZiekenhuis, Den Haag, The Netherlands

Correspondence to
Dr Johanna Maria Maassen, Rheumatology, Leiden University Medical Center, Leiden 2333 ZA, The Netherlands; j.m.maassen@lumc.nl

JMM and RDSS contributed equally.

Received 19 March 2021
Accepted 18 May 2021
Published Online First 28 May 2021

To cite: Maassen JM, Dos Santos Sobrín R, Bergstra SA, *et al*. *Ann Rheum Dis* 2021;**80**:1124–1129.

ABSTRACT

Objectives To evaluate the success rate of glucocorticoid discontinuation and to study which factors are associated with successful discontinuation.

Methods Data from two treat-to-target studies, BeSt (target Disease Activity Score (DAS) ≤2.4) and IMPROVED (target DAS <1.6), were evaluated for all patients initially treated with a tapered high dose of prednisone with conventional synthetic disease-modifying antirheumatic drugs. Prednisone was discontinued when DAS ≤2.4 was maintained for 28 weeks in BeSt and as soon as DAS was <1.6 in IMPROVED. Discontinuation was considered successful if the target was maintained at the next visit. Logistic regression analyses were performed to identify predictors of successful discontinuation. A mixed effects logistic regression model was used to assess whether primary versus secondary discontinuation was as successful.

Results In the BeSt study, 40% (47 of 93) of patients flared after primary prednisone discontinuation, and of the other 60% (56 of 93), 38% had to restart later. Of those who restarted (secondary discontinuation), 47% (17 of 35) again flared. In IMPROVED, after primary discontinuation 39% (158 of 400) flared, and of the other 61% (242 of 400), 40% had to restart later. After secondary discontinuation 49% (68 of 139) flared. Only in IMPROVED a secondary attempt was less successful (BeSt OR 0.71, p=0.45; IMPROVED OR 0.60, p=0.01). A lower DAS both at baseline and stop visit and male gender (in IMPROVED) were associated with successful primary discontinuation.

Conclusion Primary glucocorticoid discontinuation resulted in direct loss of disease control in approximately 40% and secondary in 50% of patients. 'Standard' baseline characteristics seem insufficient to personalise the duration of temporary glucocorticoid bridging, but the DAS at the time of discontinuation might provide guidance.

Key messages

What is already known about this subject?

- Glucocorticoids are widely used as effective bridging therapy in rheumatoid arthritis, but should ideally be tapered and discontinued as soon as possible.

What does this study add?

- This study reports on the outcomes of protocolised discontinuation of glucocorticoids in two treat-to-target trials and shows that in about 40% of patients direct loss of disease control occurs despite continuation of conventional synthetic disease-modifying antirheumatic drugs (csDMARDs).
- Restart of glucocorticoids, although effective in reachieving the treatment target, was followed by flare on discontinuation again in 50%.
- Baseline patient characteristics seem insufficient in predicting which patient can successfully discontinue glucocorticoids.

How might this impact on clinical practice or future developments?

- Initial benefits of early achievement of treatment target may be lost after discontinuation of bridging with glucocorticoids due to poor efficacy of the remaining csDMARDs.
- Alternative effective treatment should be at the ready if discontinuation of glucocorticoids is considered.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic and disabling systemic disease for which currently many therapeutic options are available. Early recognition of disease,[1] fast introduction of treatment and subsequent treatment-to-target with rapid drug escalation characterise the current management of RA, leading to successful suppression of disease activity in an increasing number of patients.[2 3] Glucocorticoids (GC) are widely used as part of the (initial) treatment strategy for RA, as bridging therapy in combination with slower-acting conventional synthetic disease-modifying antirheumatic drugs (csDMARDs).[4–6] It has been shown that initial treatment with a combination of csDMARDs and GCs is more successful than csDMARDs alone, resulting in more rapid clinical and functional improvement and less radiographic damage progression.[7–11] Given the risk of various negative effects of prolonged use of GC, current international recommendations advise tapering initial GC as soon as clinically feasible, while continuing other disease-modifying antirheumatic drugs (DMARDs).[6 12] Therefore, as part of a

treat-to-target strategy, tapering GC treatment should be considered as soon as a treatment target of remission, or at least of low disease activity (LDA), is achieved. Currently data regarding the proportion of patients who maintain remission and/or LDA after complete GC discontinuation are lacking. Moreover, when GC therapy is restarted due to a disease flare, it is unclear whether a second attempt to taper and stop GC therapy is likely to be successful, nor is it clear whether there are predictors for successful discontinuation.

Therefore the objective of the current study was to assess the rate of successful GC discontinuation (oral prednisone) at the first or second attempt and to evaluate patient characteristics associated with successful discontinuation in two treat-to-target studies (BeSt[8] and IMPROVED[13]).

METHODS

Data sources

BeSt study

In the Behandel-Strategieën, "treatment strategies" (BeSt) study (extensively described elsewhere[8 14]), 508 patients with early RA (according to the 1987 American College of Rheumatology (ACR) classification criteria) with symptom duration ≤2 years and active disease (≥6 of 66 swollen joints, ≥6 of 68 tender joints, and erythrocyte sedimentation rate ≥28 mm/hour and/or a Visual Analogue Scale (VAS) global health (GH) score ≥20 mm) were randomised to four treatment strategy arms. Disease activity was evaluated every 3 months, and patients were treated to target LDA (Disease Activity Score (DAS) ≤2.4). For the current analyses all patients in arm 3 (n=133) were selected. The initial treatment was methotrexate (MTX) 7.5 mg/week, sulfasalazine (SSZ) 2000 mg/day and prednisone 60 mg/day tapered to 7.5 mg/day in 6 weeks. If DAS remained ≤2.4, prednisone was tapered from week 28 to 5 mg/day and next in 7 weeks, to zero at week 35 (primary prednisone discontinuation). If treatment was initially escalated due to a DAS >2.4 (first increase of MTX to 25 mg/week; if DAS is still >2.4, change SSZ to ciclosporin), prednisone 7.5 mg/day was prolonged until for at least 6 consecutive months of LDA, then prednisone was tapered to 5 mg/day and next to zero in 7 weeks (delayed primary prednisone discontinuation). If after or during prednisone discontinuation a flare occurred (DAS >2.4), prednisone was restarted at 7.5 mg/day once, and discontinued the second time if LDA was maintained again for 6 consecutive months (secondary prednisone discontinuation).

IMPROVED study

In the Induction therapy with MTX and Prednisone in Rheumatoid Or Very Early arthritic Disease (IMPROVED) study (extensively described elsewhere[13]), 610 patients with early RA (symptom duration ≤2 years) fulfilling the 2010 American College of Rheumatology/European League Against Rheumatism classification criteria or undifferentiated arthritis were initially treated with a combination of MTX (gradually intensified in the first 6 weeks from 7.5 mg/week to 25 mg/week) and prednisone 60 mg/day tapered to 7.5 mg/day in 6 weeks. Disease activity was evaluated every 4 months, aiming at remission (DAS <1.6). In patients who were in (early) remission at 4 months, treatment with prednisone tapered (in 3 weeks) and then stopped (primary prednisone discontinuation). If remission persisted at 8 months, MTX was tapered next. In case of a disease flare after or during prednisone discontinuation, prednisone 7.5 mg/day was restarted once, and if DAS <1.6 was again maintained for 4 months prednisone was tapered and stopped the second and final time (secondary prednisone discontinuation). Patients who did not achieve DAS <1.6 at 4 months were randomised to two arms: (1) MTX 25 mg/week, prednisone (continued at 7.5 mg/day), SSZ 2000 mg/day and hydroxychloroquine 400 mg/day; or (2) MTX 25 mg/week plus adalimumab 40 mg/2 weeks. For patients in arm 1 who achieved DAS <1.6 at 8 months, prednisone was tapered to zero (delayed primary prednisone discontinuation). If DAS increased to ≥1.6 after discontinuation, prednisone 7.5 mg/day was restarted once, and stopped again for the second and final time if DAS <1.6 was regained (secondary prednisone discontinuation).

Study design

In this post-hoc analyses we evaluated prednisone discontinuation in BeSt (arm 3) and IMPROVED separately for patients discontinuing prednisone according to treatment target and timing of the corresponding study protocol. Successful discontinuation was defined as having tapered prednisone to zero with maintenance of the set treatment target and without the necessity to restart prednisone per immediate and/or restart or escalate other treatment(s). The success of a discontinuation attempt was evaluated at the first visit after discontinuation.

Statistical analyses

Descriptive statistics were used to determine the frequency of successful discontinuation. Logistic regression analyses were performed to identify predictors of successful discontinuation. Stepwise exploratory backward (primary) and forward (sensitivity) logistic regression analyses were performed with the following possible predictors: age, gender, symptom duration in weeks, DAS, autoantibody positivity (rheumatoid factor positivity and/or anticitrullinated protein antibody positivity) all at baseline and DAS at the visit of discontinuation initiation ('stop visit'). Predictors with a p value <0.20 were included in the final model. As a sensitivity analysis, we performed logistic regression analyses in patients from the IMPROVED study who retrospectively would have fulfilled the inclusion criteria of the BeSt study. Finally, a mixed effects logistic regression model with success of prednisone discontinuation as the dependent variable and primary versus secondary discontinuation attempt as independent variable corrected for the DAS at the stop visit was executed to estimate the difference in successful prednisone discontinuation between a primary and a secondary attempt. To account for the correlation between a primary and secondary attempt within patients, analyses were clustered by patient. Primary and delayed primary prednisone discontinuations were analysed together ('overall primary') as well as all secondary discontinuations. Missingness in the data was low (<5%) and therefore a complete case analysis was done.[15] Because of the exploratory nature of this post-hoc analysis, no correction for multiple testing was applied. P values <0.05 were considered statistically significant. Statistical analyses were performed with Stata SE V.16.0.

RESULTS

Prednisone discontinuation in the BeSt study

All 133 patients enrolled in arm 3 of the BeSt study initiated treatment with MTX, SSZ and prednisone. For the current post-hoc analyses, follow-up data were available for 131 patients (online supplemental figure 1). In 38 of 131 (29%) patients, prednisone discontinuation as referred to in this analysis was never attempted or could not be evaluated.

Table 1 Patient characteristics for overall primary prednisone discontinuation in arm 3 of the BeSt study

Characteristics	n	Total n=93	No success n=37	Success n=56
Age, years*	93	54 (14)	52 (15)	55 (12)
Gender, female, %	93	65	78	55
Symptom duration BL, weeks†	93	23 (15–58)	23 (17–51)	24 (14–59)
DAS at BL*	93	4.3 (0.9)	4.3 (0.8)	4.2 (0.9)
SJC at BL*	93	14 (6)	15 (6)	14 (6)
RAI at BL*	93	14 (6)	14 (7)	13 (6)
ESR at BL*	93	35 (22)	32 (20)	36 (23)
VAS GH at BL*	93	49 (22)	45 (20)	51 (23)
DAS at stop visit*	93	1.6 (0.6)	2.0 (0.3)	1.4 (0.6)
RF, positive, %	93	66	59	70
ACPA, positive, %	90	53	61	48
Erosions, present at BL, %	91	74	76	72

*Mean (±SD).
†Median (IQR).
ACPA, anticitrullinated protein antibodies; BL, baseline; DAS, Disease Activity Score; ESR, erythrocyte sedimentation rate; GH, global health; RAI, Ritchie articular index (0–78); RF, rheumatoid factor; SJC, swollen joint count (0–44); VAS, Visual Analogue Scale (0–100).

Primary prednisone discontinuation

After 28 weeks, 78 of 131 (60%) patients initiated prednisone, tapering to zero, and 15 of 131 (16%) more patients tapered and discontinued prednisone later in the disease course. Overall primary prednisone discontinuation was successful in 60% (56 of 93), and 35 of 93 (38%) flared immediately and 2 of 93 (2%) were not able to discontinue prednisone. Successful primary discontinuation occurred more often in male patients (45% vs 22%) and if the DAS at the stop visit was lower (1.4, SD 0.9 vs 2.0, SD 0.3) (table 1, online supplemental table 1). This was confirmed in the univariable logistic regression (table 2). In the final multivariable model, a lower DAS at the stop visit ($p<0.01$) was associated with successful primary prednisone discontinuation (table 2, online supplemental tables 2 and 3). Restart of prednisone following a 'prednisone free period' after initial successful discontinuation occurred in 36 of 56 (64%) (38% of all the patients attempting primary discontinuation and 27% of the whole cohort).

Secondary prednisone discontinuation

Thirty-five patients attempted to discontinue prednisone the second time (secondary prednisone discontinuation), which was successful in 19 of 35 (54%). No differences were found in characteristics between patients with successful versus unsuccessful secondary discontinuation, confirmed by univariable and multivariable logistic regression analyses (online supplemental tables 4 and 5).

Prednisone discontinuation in the IMPROVED study

In the IMPROVED study, in 210 of 610 (34%) patients, prednisone discontinuation as referred to in this analysis was never attempted or could not be studied.

Primary prednisone discontinuation

Primary prednisone discontinuation occurred in 372 patients who were in remission at 4 months and an additional 28 patients who attempted delayed primary prednisone discontinuation. Overall primary prednisone discontinuation was successful in 61% (242 of 400) of patients. Of 400 patients, 35 (8%) were not able to discontinue prednisone and 123 (31%) flared immediately after discontinuation. Primary prednisone discontinuation was more often successful in male patients (43% vs 28%), in patients with lower DAS at baseline (2.9, SD 0.8 vs 3.2, SD 0.9), and in those with corresponding lower baseline tender and swollen joint counts and lower VAS GH (table 3). Also, the DAS at the stop visit was lower in patients who successfully discontinued (0.9, SD 0.4 vs 1.1, SD 0.4) (table 3). This was confirmed by univariable logistic regression analysis (table 4). In the multivariable analyses, male gender ($p=0.03$), lower DAS at baseline ($p=0.02$) and lower DAS at the stop visit ($p<0.01$) were significantly associated with successful primary prednisone discontinuation (table 4, online supplemental tables 7 and 8). Restart of prednisone following a 'prednisone free period' after initial successful discontinuation occurred in 98 of 242 (40%). A second prednisone course after initial discontinuation (independent of the primary stop reason) occurred in 209 of 610 (34%) patients.

Secondary prednisone discontinuation

In total 139 patients attempted secondary prednisone discontinuation when remission was reached again, which was successful in 71 of 139 (51%) patients. The DAS at the stop visit was lower in patients with successful secondary prednisone discontinuation after unsuccessful primary discontinuation. Patients with successful secondary discontinuation who had flared after successful primary discontinuation had older age, shorter symptom duration at baseline and lower DAS at the stop visit (online supplemental table 9). In the overall univariable and multivariable logistic regression analyses, a lower DAS at the

Table 2 Logistic univariable and multivariable logistic regression analyses evaluating which factors are associated with overall successful primary prednisone discontinuation in arm 3 of the BeSt study

	Univariable model		Final multivariable model* n=91, R^2=0.2390	
Variable	OR (95% CI)	P value	OR (95% CI)	P value
Age, year	1.02 (0.98 to 1.05)	0.32		
Gender, female	0.34 (0.13 to 0.88)	0.03		
Symptom duration BL, weeks	1.00 (0.99 to 1.01)	0.94		
DAS at BL	0.91 (0.56 to 1.47)	0.69		
DAS at stop visit	0.05 (0.01 to 0.21)	<0.01	0.05 (0.01 to 0.22)	<0.01
Autoantibody, positive	0.89 (0.34 to 2.32)	0.81		

*The final multivariable logistic regression model was based on a stepwise backward selection of predictors (see online supplemental file 1).
BL, baseline; DAS, Disease Activity Score.

Table 3 Patient characteristics for overall primary prednisone discontinuation in the IMPROVED study

Characteristics	n	Total n=400	No success n=158	Success n=242
Age, years*	400	52 (14)	52 (13)	52 (14)
Gender, female, %	400	63	72	57
Symptom duration BL, weeks†	394	16 (8–30)	16 (8–32)	16 (9–30)
DAS at BL*	398	3.0 (0.8)	3.2 (0.9)	2.9 (0.8)
SJC at BL*	399	7 (6)	8 (6)	6 (6)
RAI at BL*	399	6 (4)	7 (4)	6 (4)
ESR at BL*	400	29 (24)	31 (25)	28 (23)
VAS GH at BL*	398	42.6 (23.2)	47.2 (21.6)	39.7 (23.8)
DAS at stop visit*	400	1.0 (0.4)	1.1 (0.4)	0.9 (0.4)
RF, positive, %	386	60	63	58
ACPA, positive, %	391	60	57	62
Erosions, present at BL, %	391	15	16	15

*Mean (±SD).
†Median (IQR).
ACPA, anticitrullinated protein antibodies; BL, baseline; DAS, Disease Activity Score; ESR, erythrocyte sedimentation rate; GH, global health; RAI, Ritchie articular index (0–78); RF, rheumatoid factor; SJC, swollen joint count (0–44); VAS, Visual Analogue Scale (0–100).

stop visit was found to be associated with successful discontinuation (online supplemental table 10).

In the sensitivity analyses in 175 IMPROVED patients who would have met the inclusion criteria for the BeSt study, primary prednisone discontinuation was successful in 46% (47 of 102), followed by immediate flare in 55 of 102 (54%). In 73 of 175 (41%) patients, discontinuation of GCs as referred to in this study was not attempted. Secondary discontinuation was successful in 18 of 39 (54%). In both univariable and multivariable analyses, a lower DAS at the stop visit was associated with successful primary (OR 0.12, 95% CI 0.03 to 0.39, p<0.01) and secondary (OR 0.04, 95% CI 0.003 to 0.50, p=0.01) discontinuation.

Success of primary versus secondary discontinuation attempts

When comparing the success of primary and secondary prednisone discontinuation, the odds of successful discontinuation were lower in secondary versus primary attempts in both studies, but only significantly so in the IMPROVED study (BeSt OR 0.71, p=0.45; IMPROVED OR 0.60, p=0.01).

DISCUSSION

Compared with slow-acting csDMARDs alone, a combination with GCs as part of the initial treatment in early RA has proven to be more effective in rapidly suppressing disease activity, which is important for improvement of functional ability and prevention of damage.[8 16] International recommendations state that 'glucocorticoids should primarily be used as bridging therapy until csDMARDs exhibit their efficacy' and 'tapered as rapidly as clinically feasible'.[6 12] In the current post-hoc analysis of two studies, where a tapered high dose of prednisone was part of the initial treatment, we evaluated how often, and in which patients, initial GCs could successfully be stopped while csDMARD(s) were continued. We found that, regardless of whether discontinuation was based on having achieved LDA after 28 weeks (arm 3 of BeSt) or remission after 4 months (IMPROVED), in about 40% of patients GCs were not discontinued or the treatment target was not maintained at the next visit on csDMARDs alone. A further 25% in the IMPROVED and 38% in BeSt restarted prednisone later in time after an initial successful discontinuation. Independent of the reason for initial discontinuation, restart of prednisone was needed in 27% of the BeSt and 36% of the IMPROVED patients. Some patients never met the treatment target to discontinue prednisone and had to proceed to other treatment steps.

After initial GC discontinuation, 27% of patients in the BeSt study and 34% of patients in the IMPROVED study had to restart prednisone due to a disease flare, which was allowed only once before the protocol dictated other treatment steps to be taken. If the treatment target was met again, prednisone had to be discontinued the second time ('secondary discontinuation'). In about 50% of patients a second attempt to discontinue GCs was successful without a disease flare. Thus, there was a circa 10% difference in success between a primary and a secondary discontinuation, but this difference was only significant in the IMPROVED study.

Direct comparison with other trials with protocolised GC discontinuation from the initial therapy is difficult due to differences in patient population, tapering protocol, treatment target and reported outcomes. The Combinatietherapie Bij Reumatoide Artritis (COBRA) trial, including similar patients and using the treatment scheme that was copied in arm 3 of the BeSt study, tapered prednisone from week 28 and discontinued by week 35. Increasing or restarting prednisone was only allowed if there was a joint count increase of five active joints or an increase from zero to three active joints. The study reports 21 of 76 (28%) patients being in (probable) remission by week 28 and 'almost all' losing remission after prednisone was discontinued.[11] In the

Table 4 Logistic univariable and multivariable logistic regression analyses evaluating which factors are associated with overall successful primary prednisone discontinuation in the IMPROVED study

	Univariable model		Final model* n=380, R^2=0.0828	
Variable	OR (95% CI)	P value	OR (95% CI)	P value
Age, year	0.99 (0.99 to 1.01)	0.99		
Gender, female	0.52 (0.34 to 0.80)	<0.01	0.59 (0.37 to 0.94)	0.03
Symptom duration BL, weeks	1.00 (0.99 to 1.01)	0.96		
DAS at BL	0.66 (0.52 to 0.85)	<0.01	0.72 (0.55 to 0.94)	0.02
DAS at stop visit	0.23 (0.13 to 0.41)	<0.01	0.22 (0.12 to 0.41)	<0.01
Autoantibody, positive	0.83 (0.52 to 1.31)	0.41		

*The final multivariable logistic regression model was based on a stepwise backward selection of predictors (see online supplemental file).
BL, baseline; DAS, Disease Activity Score.

COBRA light study, including patients with slightly earlier and milder RA than the original COBRA trial, the COBRA treatment scheme was compared with a scheme with a lower dose of prednisone (initiating 30 mg/day and tapering to 7.5 mg/day in 9 weeks in combination with MTX without SSZ). At week 26, 49% of patients in the original COBRA arm and 41% of patients in the COBRA light arm were in remission and stopped prednisone at week 35.[17] At either week 26 or 39, 59% of patients in the original COBRA arm and 75% in the COBRA light arm were not in remission, and 38% vs 49% were not in LDA (ie, unable/unsuccessful prednisone discontinuation).[18] Restart of prednisone was not allowed in this study. The Care in early RA (CareRA) trial, with almost similar patients as the COBRA light study, also used the original COBRA treatment scheme and the COBRA light scheme (but tapered over 6 weeks), both discontinuing prednisone at week 35. No data on early success of prednisone stopping are published, but at week 52, regardless of the initial prednisone scheme, percentages of patients not in remission were similar (circa 38%) as were percentages of patients not in LDA (circa 23%).[5] Several cohort studies have reported on large percentages of patients who continue on or come back to GCs for long periods of time.[19–21] This reluctance or failure to stop GCs may be due to various conditions and circumstances, but insufficient benefit from available DMARDs and lack of access to more effective (expensive) medication are likely to be the most significant.[22] In our studies, as in the Dutch routine practice, access to subsequent treatment options was guaranteed. Nevertheless, protocol violations occurred in both trials, although we have no details on specific reasons.[23]

We found that a lower DAS at the stop visit was a stable and reproducible factor associated with maintenance of disease control after GC discontinuation. Baseline characteristics that were found to be associated with successful GC discontinuation in the BeSt study could not be confirmed in the IMPROVED study, or vice versa, although male patients seem to fare somewhat better than female patients. In light of the differences in patient population between the two trials, a sensitivity analysis was performed selecting only those patients in the IMPROVED study that in retrospect would have met the BeSt inclusion criteria with similar results on associated factors for successful discontinuation. Future studies may try to explore prediction models including not only clinical data but also genomic data or biomarkers.

This post-hoc study has some limitations. There were a limited number of patients in the regression analyses, particularly with regard to secondary discontinuation. This limited the power to identify predictors, and the overall predictive ability of the models was low. Since different variable selection strategies have different limitations,[24] we performed both backward and forward multivariable analyses and chose a p value <0.20 as a cut-off to include to lower the chance of missing potentially relevant predictors. Data were derived from two clinical trials with a (strict) treat-to-target protocol. Therefore high-quality data were available from a large number of variables with little missing data. Nevertheless, results of clinical trials may, due to the selection of patients fulfilling specific inclusion and exclusion criteria and the choice of treatment, not be directly generalisable to daily practice. It should be considered that the initial therapy in arm 3 of the BeSt trial included SSZ, next to MTX and prednisone, and patients with undifferentiated arthritis were included in the IMPROVED study. Both might lead to an overestimation of the percentages of successful GC discontinuation. We evaluated the result of GC discontinuation at the next available study visit, 3 or 4 months after GC discontinuation. This provides valuable information on whether a rapid treatment adjustment is needed after trying to stop GC, according to a treat-to-target strategy. Late flares could not solely be related to GC discontinuation, since in both studies, as long as the treatment target was met, subsequent tapering and discontinuation steps of the remaining drugs were required, which in turn may have triggered flares after initially stopping GCs.

To conclude, our results show that, despite the rapid clinical improvement achieved by GCs, once discontinued the efficacy of MTX up to 25 mg/week (in IMPROVED) with additional SSZ 2000 mg/day (in BeSt) proved immediately insufficient to maintain the achieved level of disease control in about 40% of our patients. Approximately 30% of patients experienced a flare sometime after GC discontinuation and had to temporarily restart. Of those patients, 50% could successfully discontinue in a second instance. Characteristics identifying patients who can discontinue GC bridging without significant impact on disease activity could not explicitly be identified, although a lower DAS at the stop visit was associated with successful discontinuation. Our results suggest that, to avoid deterioration or prolonged use of GCs, patients with insufficient disease control on csDMARDs alone require timely change to more effective antirheumatic drugs.

Acknowledgements We would like to thank all patients for their contribution as well as the following rheumatologists who participated in the BeSt study group and in the IMPROVED study group: J van Aken (currently Spaarne Gasthuis, Hoofddorp); WM de Beus (retired); C Bijkerk (Reinier de Graaf Gasthuis, Delft); MHW de Bois (Medical Center Haaglanden, The Hague); H Boom (currently Spaarne Gasthuis, Hoofddorp); M de Buck (Medical Center Haaglanden, The Hague); G Collée (retired); BAC Dijkmans (retired); JAPM Ewals (retired); F Fodili (currently Reumazorg Zuid West Nederland, Roosendaal); AH Gerards (currently Franciscus Gasthuis and Vlietland, Schiedam); YPM Goekoop-Ruiterman (Haga Hospital, The Hague); BAM Grillet (retired); KH Han (retired); JB Harbers (currently Reumazorg Zuid West Nederland, Roosendaal); AL Huidekoper (Medical Center Haaglanden, The Hague); MV van Krugten (currently Reumazorg Zuid West Nederland, Terneuzen); L Lard (Medical Center Haaglanden, The Hague); H van der Leeden (retired); MF van Lieshout-Zuidema (currently Spaarne Gasthuis, Hoofddorp); A Linssen (retired); MC Lodder (currently Spaarne Gasthuis, Haarlem); PAHM van der Lubbe (currently Franciscus Gasthuis and Vlietland, Schiedam); C Mallée (retired); M van Oosterhout (Groene Hart Hospital, Gouda); AJ Peeters (retired); N Riyazi (Haga Hospital, The Hague); HK Ronday (Haga Hospital, The Hague); D van Schaardenburg (VU Medical Center, Amsterdam); AA Schouffoer (Haga Hospital, The Hague); PEH Seys (retired); GM Steup-Beekman (Medical Center Haaglanden, The Hague); PBJ de Sonnaville (retired); I Speyer (Medical Center Haaglanden, The Hague); KSS Steen (currently Spaarne Gasthuis, Haarlem); JPh Terwiel (retired); AE Voskuyl (VU Medical Center, Amsterdam); ML Westedt (retired); S ten Wolde (currently Spaarne Gasthuis, Haarlem); and D van Zeben (currently Franciscus Gasthuis and Vlietland, Rotterdam). We would also like to thank all other rheumatologists and trainee rheumatologists who enrolled patients in these studies, and all research nurses for their contributions.

Contributors JMM, RDSS, SAB and CFA contributed to the design, analysis and interpretation of the data. RG, TWJH and CFA contributed to data acquisition. JMM and RDSS drafted the manuscript. All authors critically revised the manuscript and read and approved the final version of the manuscript to be published.

Funding The BeSt study was designed by the investigators and supported by a government grant from the Dutch Insurance Companies, with additional funding from Schering-Plough and Janssen. Data collection, trial management, data analysis and preparation of the manuscript were performed by the authors. The IMPROVED study was designed by the investigators and financially supported by AbbVie in the first year. Trial management, data collection, data analysis and preparation of the manuscript were performed by the authors.

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval Approval for the BeSt and IMPROVED studies was granted by the medical ethics committees of each participating centre.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. The datasets used and/or analysed during the present study are available from the corresponding author on reasonable request.

ORCID iDs
Johanna Maria Maassen http://orcid.org/0000-0002-9591-5398
Raquel Dos Santos Sobrín http://orcid.org/0000-0002-2738-9752
Sytske Anne Bergstra http://orcid.org/0000-0002-7136-5248
Robbert Goekoop http://orcid.org/0000-0002-4589-6731
Tom W J Huizinga http://orcid.org/0000-0001-7033-7520

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CLINICAL SCIENCE

Efficacy and safety of tofacitinib versus baricitinib in patients with rheumatoid arthritis in real clinical practice: analyses with propensity score-based inverse probability of treatment weighting

Yusuke Miyazaki ,[1] Kazuhisa Nakano,[1] Shingo Nakayamada,[1] Satoshi Kubo ,[1] Yoshino Inoue,[1] Yoshihisa Fujino,[2] Yoshiya Tanaka [1]

Handling editor Josef S Smolen

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2020-219699).

[1]The First Department of Internal Medicine, University of Occupational and Environmental Health, Japan, Kitakyushu, Japan
[2]Department of Environmental Epidemiology, University of Occupational and Environmental Health, Japan, Kitakyushu, Japan

Correspondence to
Professor Yoshiya Tanaka, First Department of Internal Medicine, University of Occupational and Environmental Health Japan, Kitakyushu 807-8555, Japan; tanaka@med.uoeh-u.ac.jp

Received 12 December 2020
Revised 22 March 2021
Accepted 25 March 2021
Published Online First 7 April 2021

ABSTRACT

Objectives The differences of efficacy between each Janus kinase (JAK) inhibitors have not been clarified in the patients with rheumatoid arthritis (RA) in clinical practice. Here, we compared the efficacy between tofacitinib (TOFA) and baricitinib (BARI) in clinical practice.

Methods The efficacy of TOFA (n=156) in patients with RA was compared with BARI (n=138). Selection bias was reduced to a minimum using propensity score-based inverse probability of treatment weighting (IPTW). The Clinical Disease Activity Index (CDAI) trajectory for patients who started TOFA or BARI was analysed using growth mixture modelling (GMM).

Results No significant difference was observed in patient characteristics between the TOFA and BARI groups in after adjustment by propensity score-based IPTW. The BARI group had a significantly higher rate of CDAI remission at week 24 after the introduction of JAK inhibitors than the TOFA group. The treatment-resistant group defined by GMM, comprising patients who did not achieve low disease activity at week 24, was more likely to include those who had received many biological disease-modifying antirheumatic drugs (bDMARDs) before the introduction of JAK inhibitors and those who received TOFA. Among patients with RA who received TOFA, those who had received ≥4 bDMARDs before the introduction of TOFA were more likely to be classified into the treatment-resistant group.

Conclusions BARI showed a similar safety profile and better clinical outcome when compared with TOFA after reduction to a minimum of selection bias. However, these were observed in a small population. Accordingly, further investigation is required in an accurately powered head-to-head trial.

Key messages

What is already known about this subject?

- Randomised controlled trials have confirmed the efficacy of tofacitinib (TOFA) and baricitinib (BARI) monotherapies in patients with rheumatoid arthritis (RA) who are methotrexate (MTX) naïve and those who achieve MTX-inadequate response (IR), tumour necrosis factor inhibitor-IR and biological disease-modifying antirheumatic drug (bDMARD) IR.

What does this study add?

- When the Clinical Disease Activity Index (CDAI) after 24 weeks of treatment was compared between TOFA or BARI after reduction of the selection bias to a minimum and adjustment for patient characteristics by propensity score-based inverse probability of treatment weighting, BARI was more effective.
- Trajectory analysis of the changes in CDAI for TOFA and BARI divided the patients into three trajectory groups.
- Among the three groups was a treatment-resistant group that did not achieve low disease activity at week 24 after the introduction of Janus kinase inhibitors and was more likely to include patients with RA treated with TOFA, particularly those resistant to multiple bDMARDs.

How might this impact on clinical practice or future developments?

- Results suggest that TOFA may be less effective in patients resistant to multiple bDMARDs, while BARI may be more effective after 24 weeks of treatment.

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic inflammatory disease that causes progressive bone and joint destruction and irreversible physical dysfunction.[1–3] In the last 20 years, a paradigm shift has occurred in the treatment of RA with the advent of biological disease-modifying antirheumatic drugs (bDMARDs).[4] However, owing to their high molecular weight, bDMARDs can be administered only via the parenteral route and are associated with secondary failure.[5] To address these issues, Janus kinase (JAK) inhibitors, which are orally administered low molecular weight compounds, have been used. Among the JAK inhibitors currently available, tofacitinib (TOFA) and baricitinib (BARI) have been widely used in many regions for RA treatment. TOFA is a selective inhibitor of JAK1 and JAK3, and its inhibitory effect on JAK2 and tyrosine kinase (TYK) 2 is limited.[6] BARI is a selective inhibitor of JAK1 and JAK2 and exhibits a moderate inhibitory activity against TYK2, while its inhibitory activity against JAK3 is limited.[7] Randomised

To cite: Miyazaki Y, Nakano K, Nakayamada S, *et al*. *Ann Rheum Dis* 2021;**80**:1130–1136.

controlled trials have shown that TOFA and BARI monotherapies are effective in patients with RA who are methotrexate (MTX) naïve[8 9] or have MTX-inadequate response (IR),[10 11] tumour necrosis factor inhibitor-IR[12 13] and bDMARDs-IR.[14] In vitro studies have revealed variations in the pharmacological effects of JAK inhibitors at the cellular level.[15 16] However, such variations have not been investigated in real-world clinical practice. Therefore, the selection of JAK inhibitor for RA treatment based on patient type remains a major concern.

In the present study, we compared the efficacy and safety of TOFA and BARI in real-world clinical practice after reduction to a minimum of the selection bias, using propensity score-based inverse probability of treatment weighting (IPTW) and adjustment for confounding patient characteristics. Growth mixture modelling (GMM) is a method of analysis to identify trajectory groups into which longitudinal changes in factors can be classified.[17] This method allows the identification of characteristics of each trajectory group and analysis of factors affecting trajectories. We analysed the trajectories of changes in disease activity in patients receiving TOFA or BARI using GMM and evaluated clinical characteristics of their responses to both the drugs.

MATERIALS AND METHODS

Patients and study design

Patients were recruited from the FIRST registry, a registry study of patients with RA receiving molecularly targeting antirheumatic drugs at multiple institutions affiliated to our university hospital, the key station.[18–20] RA was diagnosed when patients met the 2010 American College of Rheumatology (ACR)/European League Against Rheumatism classification criteria or the 1987 ACR classification criteria.[21 22] The observation period of the study was 24 weeks.

Treatment with JAK inhibitors

TOFA or BARI was administered to patients with RA in whom disease activity could not be controlled by standard doses of MTX or conventional synthetic DMARDs (csDMARDs) or in patients with RA for whom csDMARDs, including MTX, could not be used. There was no major difference observed in the proportion of patients who were allocated to TOFA or BARI at each site; no major difference was noted in the selection of JAK inhibitors at each site. Dose of JAK inhibitor was shown in online supplemental material.

Clinical efficacy and outcome

The primary outcome was rate of remission at week 24 in each group, measured by the Clinical Disease Activity Index (CDAI).[23 24] CDAI remission was defined as a score of ≤2.8 and low disease activity (LDA) was defined as a score of ≤10.0. Additional secondary outcomes included disease activity, retention rate and safety at week 24. The analyses were performed by the last observation carried forward (LOCF) and non-responder imputation (NRI) was also used to evaluate CDAI, Simplified Disease Activity Index (SDAI) and Health Assessment Questionnaire (HAQ) remission rates.

Safety

The incidence and severity of all adverse events were recorded. The National Institutes of Health Common Terminology Criteria for Adverse Events (V.3.0) were used to describe adverse events and laboratory abnormalities.

Propensity score-based IPTW

To adjust for baseline patient characteristics between the two groups, the calculated propensity scores were weighted using the 'ratio of patients receiving BARI to all patients/propensity score' in the BARI group and the 'ratio of patients receiving TOFA to all patients/1 propensity score' in patients treated with TOFA as the weighting coefficient on stability. Details of the procedure of calculating propensity score are shown in online supplemental material.

Growth mixture modelling

To understand patient response patterns after receiving TOFA and BARI, GMM was applied to classify patients into different subgroups based on CDAI trajectories.[25] GMM was performed with STATA V.16.0 (StataCorp LLC, College Station, Texas, USA).[17] Details of the procedure of GMM are shown in online supplemental material.

Other statistical analyses

Patient characteristics are expressed as mean±SD, median (IQR) or number (%) of patients. Kaplan-Meier method was used to assess the retention rates, and the differences between the TOFA and BARI groups were analysed by the log-rank test. Student's t-test, Mann-Whitney's U test or one-way analysis of variance (ANOVA) were used for between-group comparisons, and the Pearson's χ^2 test was used for the comparison of categorical variables. All reported p values are two-sided and were not adjusted for multiple testing. The level of significance was $p<0.05$. All analyses were conducted using JMP V.14.0 (SAS Institute, Cary, North Carolina, USA) and SPSS software V.25.0.

Table 1 Safety and laboratory data, weeks 0–24

Variables	TOFA, n=156	BARI, n=138	P value
Safety data			
Serious adverse events, n (%)	10 (6.4%)	4 (2.9%)	0.15
Any adverse event after start of therapy, n (%)	56 (35.9%)	31 (22.5%)	0.04
Infection, n (%)	37 (23.7%)	23 (16.7%)	0.13
Herpes zoster, n (%)	2 (1.3%)	5 (3.6%)	0.18
Serious infection, n (%)	2 (1.3%)	2 (1.5%)	0.90
Cancer, n (%)	0 (0.0%)	0 (0.0%)	1.00
Major adverse cardiovascular event, n (%)	0 (0.0%)	0 (0.0%)	1.00
Venous thromboembolism, n (%)	0 (0.0%)	0 (0.0%)	1.00
Laboratory data—median change from baseline			
Haemoglobin (g/L)	1.5 (−4.0–8.8)	0.5 (−7.0–8.0)	0.21
Neutrophils (/μL)	−857 (−2057–268)	−817 (−2170–47)	0.67
Lymphocytes (/μL)	111 (−172–458)	232 (−134–621)	0.10
Alanine aminotransferase (IU/L)	2 (−4–9)	5 (−1–10)	0.09
Creatinine (mg/dL)	0.06 (0.00–0.12)	0.08 (0.03–0.13)	0.11
Creatine phosphokinase (IU/L)	30 (4–65)	39 (4–70)	0.51

Adverse events, infection or laboratory abnormalities leading to permanent discontinuation of the JAK inhibitor are designated as serious adverse events.
The data shown are numbers and percentages of patients with adverse events.
Laboratory values are reported as the least-squares-mean change from baseline at week 24.
BARI, baricitinib; CDAI, Clinical Disease Activity Index; JAK, Janus kinase; TOFA, tofacitinib.

Table 2 Patient characteristics in the TOFA and BARI groups before and after IPTW

	Before IPTW			After IPTW		
Variables	**TOFA, n=156**	**BARI, n=138**	**P value**	**TOFA, n=153***	**BARI, n=141***	**P value**
Age (years)	58.9±13.2	57.2±13.6	0.25	58.2±13.4	58.2±13.3	0.96
Sex, n (% female)	129 (82.7)	109 (79.0)	0.16	126 (82.4)	115 (81.6)	0.86
Disease duration (month)	96 (35–192)	77 (24–158)	0.16	118.5±103.7	122.3±120.0	0.77
Treatment history						
MTX use at baseline, n (%)	117 (75.0)	94 (68.1)	0.20	112 (73.2)	103 (73.1)	0.53
Dose, mg/w	12.3±3.5	11.7±3.7	0.18	12.2±3.5	12.0±3.6	0.68
Glucocorticoid use at baseline, n (%)	20 (12.8)	33 (23.9)	0.02	25 (16.3)	24 (17.0)	0.88
Dose, mg/day	5.0 (2.5–7.5)	7.5 (5.0–10.0)	0.14	5 (2.5–6.4)	7.5 (2.5–10.0)	0.26
bDMARD naïve, n (%)	37 (23.7)	45 (32.6)	0.09	43 (28.1)	40 (28.4)	0.52
Number of previous bDMARDs use, n 1/2/3/4/≥5	38/30/26/19/6	37/22/17/8/9	0.24	36/27/25/17/5	38/24/20/9/10	0.60
JAK inhibitor dose, n (%)	10 mg=140 (89.7)	4 mg=122 (88.4)		10 mg=135 (90.8)	4 mg=122 (86.5)	
	5 mg=16 (10.3)	2 mg=16 (11.6)		5 mg=10 (9.2)	2 mg=19 (13.5)	
28-tender joint count	9.2±6.1	9.2±6.7	0.98	9.2±6.2	9.0±6.5	0.72
28-swollen joint count	7.0±4.6	7.8±5.8	0.21	7.3±4.6	7.3±5.3	0.89
GH, VAS 0–100 mm	53.3±24.1	52.8±24.6	0.86	52.9±23.3	52.7±24.3	0.95
EGA, VAS 0–100 mm	44.9±20.8	47.4±22.0	0.31	45.9±20.7	45.6±21.4	0.92
Pain VAS 0–100 mm	52.4±24.6	51.1±26.6	0.67	52.2±24.0	51.8±25.6	0.91
DAS28-ESR	5.3±1.3	5.2±1.3	0.34	5.4±1.3	5.2±1.3	0.29
SDAI	27.6±12.9	27.5±13.3	0.94	27.8±12.8	26.9±12.6	0.53
CDAI	25.8±11.7	26.1±12.7	0.88	26.3±11.7	25.4±11.9	0.81
HAQ-DI	1.3±0.8	1.2±0.8	0.12	1.3±0.8	1.2±0.7	0.90
EQ-5D	0.6±0.1	0.6±0.1	0.43	0.6±0.1	0.6±0.1	0.86
CRP (mg/dL)	0.4 (0.1–1.8)	0.4 (0.1–1.3)	0.64	0.4 (0.1–1.7)	0.4 (0.1–1.6)	0.76
ESR (mm/hour)	39.4±30.1	38.2±30.1	0.73	40.4±31.5	39.4±30.9	0.80
Rheumatoid factor positive, n (%)	121 (77.6)	107 (77.5)	1.00	118 (77.1)	107 (75.9)	0.89
Rheumatoid factor (U/mL)	69.2 (18.6–157.9)	51.4 (13.7–150.7)	0.39	70.3 (18.1–170.4)	46.5 (11.4–121.9)	0.85
Anti-CCP antibody, n (%)	118 (75.6)	98 (71.0)	0.43	115 (75.2)	99 (70.2)	0.36
Anti-CCP antibody (U/mL)	41.7 (5.2–265.8)	76.2 (2.6–397.1)	0.56	45.2 (5.3–272.1)	70.6 (1.9–386.4)	0.78
MMP-3 (ng/mL)	93 (55–264)	118 (51–229)	0.82	86 (53–252)	111 (50–234)	0.80

Data are mean±SD, median (IQR) or number (%) of patients.
*The number of subjects changed after IPTW in the calculation; however, the actual number of subjects did not change.
BARI, baricitinib; bDMARDs, biological disease-modifying antirheumatic drugs; CCP, cyclic citrullinated peptide; CDAI, Clinical Disease Activity Index; CRP, C reactive protein; DAS, Disease Activity Score; EGA VAS, Evaluator Global Assessment of Disease Activity Visual Analogue Scale; EQ-5D, EuroQol 5 dimension; ESR, erythrocyte sedimentation rate; HAQ-DI, Health Assessment Questionnaire-Disability Index; IPTW, inverse probability of treatment weighting; JAK, Janus kinase; MMP-3, matrix metalloproteinase 3; MTX, methotrexate; SDAI, Simplified Disease Activity Index; TOFA, tofacitinib; GH VAS, patient's global assessment of disease activity visual analogue scale.

RESULTS

Comparison of the efficacy and safety of TOFA and BARI

Totally, 156 patients with RA who were followed-up for ≥6 months after the introduction of TOFA and 138 patients who were followed up for ≥6 months after the introduction of BARI between August 2014 and May 2020 were included. Online supplemental figure S1A shows the retention rates over 24 weeks after the introduction of JAK inhibitors in both the groups. There was no significant difference in the retention rate between the two groups (TOFA vs BARI=87.8% vs 91.3%, p=0.31). Table 1 shows the observed adverse events. Although the incidence of ≤grade 2 adverse events, as specified by the National Institutes of Health Common Terminology Criteria for Adverse Events (V.3.0), was significantly lower in the BARI group, there was no difference between the two groups in the incidence of serious infections or adverse events that could lead to discontinuation of JAK inhibitors. Laboratory data showed significant decrease in neutrophil count and significant increase in lymphocyte count, creatinine level and creatinine phosphokinase level in both the groups. A comparison of the changes in laboratory data between the two groups showed no significant differences.

Comparison of the efficacy of TOFA and BARI for 24 weeks after treatment introduction showed that the BARI group had a significantly lower CDAI (TOFA vs BARI=8.2 vs 6.2, p=0.04) and a significantly higher rate of CDAI-LDA (TOFA vs BARI=69.2% vs 81.2%, p=0.02) at week 24 after treatment introduction (online supplemental figure S1B, S1C). No difference was observed in CDAI remission.

Patient characteristics in the TOFA and BARI groups after adjustment by propensity score-based IPTW

Table 2 (left-hand side) shows the patient characteristics before adjustment. The rate of concomitant glucocorticoid (GC) use was significantly lower in the TOFA group than in the BARI group. The TOFA group also included more bDMARDs-naïve patients than the BARI group. Next, we calculated the IPTW using the propensity scores to reduce the selection bias to a minimum and adjusted the patient characteristics. The adjusted patient characteristics are shown in table 2 (right-hand side). No significant

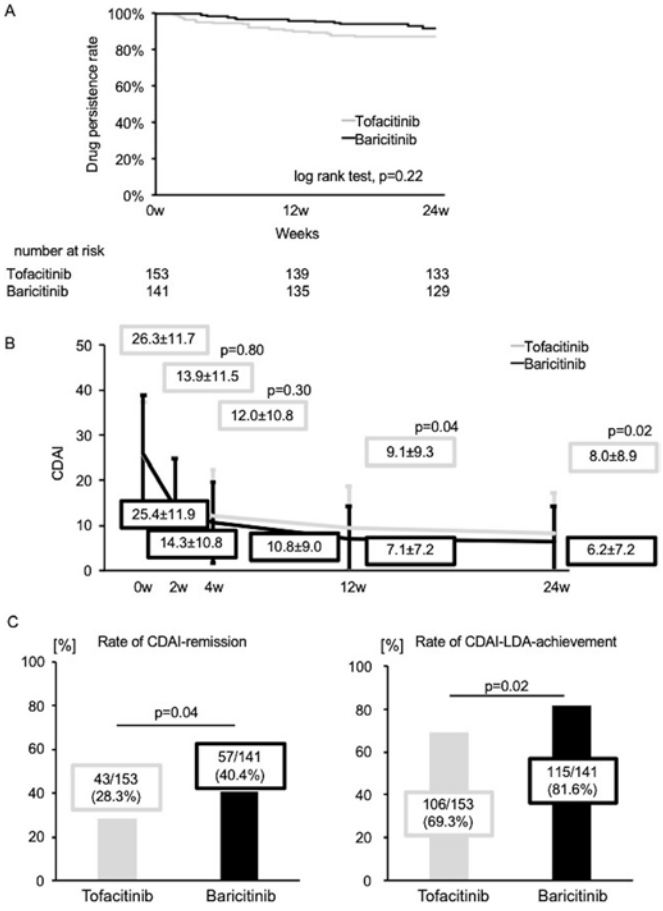

Figure 1 Changes in disease activity over 24 weeks after the introduction of JAK inhibitors after adjustment by propensity score-based IPTW. The selection bias was adjusted by propensity score-based IPTW in patients with rheumatoid arthritis treated with TOFA or BARI. (A) Retention rates over 24 weeks after the introduction of JAK inhibitors (Kaplan-Meier curves). (B) Changes in CDAI over 24 weeks after the introduction of JAK inhibitors: comparison between the TOFA and BARI groups with mean±SD and p values derived from Student's t-test. (C) Comparison of rates of CDAI remission (left) and CDAI-LDA achievement (right) between the two groups by Pearson's χ^2 test. Numbers represent percentages of all patients (%). BARI, baricitinib; CDAI, Clinical Disease Activity Index; IPTW, inverse probability of treatment weighting; JAK, Janus kinase; LDA, low disease activity; TOFA, tofacitinib.

differences were observed in any patient characteristic, and the standardised differences were <0.1 for all the characteristics. The distribution of variables was well balanced.

Comparison of efficacy and safety between the TOFA and BARI groups after adjustment by propensity score-based IPTW

Figure 1 shows the retention rate and efficacy over 24 weeks of treatment with TOFA and BARI after adjustment by IPTW. The retention rates over 24 weeks did not differ between the TOFA and BARI groups (TOFA vs BARI=86.9% vs 91.5%, p=0.22) (figure 1A). Adverse events that led to discontinuation of JAK inhibitors are shown in online supplemental table S1. No difference was observed in the incidence of adverse events leading to discontinuation of JAK inhibitors in the TOFA and BARI groups. CDAI at 24 weeks after the introduction of JAK inhibitors was 8.0±8.9 and 6.2±7.2 in the TOFA and BARI groups, respectively (figure 1B). CDAI, SDAI, HAQ-DI and C reactive protein (CRP) level were significantly improved in both the groups at week 2 after the introduction of JAK inhibitors and further improved until week 24 (table 3).

Compared with the TOFA group using a generalised linear model, the BARI group showed a significantly lower CDAI (⊿CDAI=−1.9, 95% CI: −3.7 to −0.3, p=0.02) and a significantly higher rate of CDAI remission (OR: 1.7, 95% CI: 1.1 to 2.7, p=0.04) at 24 weeks (figure 1C). Similarly, at week 24, SDAI was significantly lower in the BARI group, and the rates of SDAI remission and SDAI-LDA achievement were significantly higher in the BARI group (online supplemental figure S2). Furthermore, no differences were observed in HAQ-DI or rate of HAQ-DI-remission at week 24 (online supplemental figure S3).

Trajectories of changes in CDAI in the TOFA and BARI groups using GMM

Next, we analysed the trajectories of changes in CDAI in 294 patients receiving TOFA or BARI and the differences in changes in CDAI between the TOFA and BARI groups by using GMM. The cubic function-based linear model of trajectory showed the best fit (online supplemental table S2). As for the number of trajectory groups, the best fit was obtained when the patients were divided into the following three groups (online supplemental table S3): group 1 comprising patients with moderate disease activity (MDA) at baseline who exhibited improvement in disease activity immediately after the introduction of JAK inhibitors and achieved LDA at week 24, group 2 comprising patients with high disease activity (HDA) at baseline who exhibited improvement in disease activity immediately after the introduction of JAK inhibitors and achieved LDA at week 24 and group 3 (treatment-resistant group) comprising patients with HDA at baseline who exhibited a partial or limited response to JAK inhibitors after introduction and did not achieve LDA at week 24 (figure 2A and online supplemental table S4).

When the proportion of patients in each trajectory group was compared between the TOFA and BARI groups, the proportion of patients classified as the treatment-resistant group was lower in the BARI group than in the TOFA group (TOFA:BARI=23.7%:13.0%, p=0.02) (figure 2B). No difference was observed in the proportion of patients classified as group 1 (TOFA:BARI=50.6%:52.9%, p=0.70) and group 2 (TOFA:BARI=25.6%:34.1%, p=0.11).

The CDAI improvement rate was analysed, using GMM, and the subjects were divided into groups that followed four trajectories (online supplemental figure S4 and online supplemental table S6-S8). Group B (CDAI improvement rates increased at 12 weeks and maintained an increasing trend until 24 weeks) included a large percentage of subjects belonging to the BARI group. Moreover, Group C (CDAI had improved to approximately half at 24 weeks) included a large percentage of subjects belonging to the TOFA group. Patients who belonged to Group D, among the patients who had used TOFA, included a few bio-naïve patients and many patients who failed to respond to many bDMARDs.

Factors associated with treatment resistance in the TOFA and BARI groups

Multivariable logistic regression analysis was performed to identify factors contributing to belonging to treatment-resistance group (online supplemental table S5, table 4). The explanatory variables were age, female sex, duration of RA, concomitant

Table 3 Change in efficacy 2 weeks, 12 weeks and 24 weeks after the introduction of JAK inhibitors

	TOFA (n=153)†			BARI (n=141)†		
	Change from baseline			Change from baseline		
	Week 2	Week 12	Week 24	Week 2	Week 12	Week 24
CDAI	−12.3 (11.2)*	−17.2 (12.6)*	−18.1 (13.0)*	−11.5 (11.6)*	−18.3 (13.0)*	−19.3 (14.1)*
SDAI	−12.5 (11.6)*	−17.9 (13.5)*	−19.0 (14.1)*	−12.3 (12.5)*	−19.1 (13.8)*	−20.4 (15.0)*
HAQ-DI	−0.22 (0.41)*	−0.41 (0.53)*	−0.45 (0.61)*	−0.15 (0.38)*	−0.31 (0.55)*	−0.39 (0.65)*
CRP, mg/dL	−0.06 (-0.92–0.00)*	−0.05 (−1.30–0.02)*	−0.11 (−1.30–0.00)*	−0.07 (−0.70–0.00)*	−0.05 (−1.11–0.00)*	−0.13 (−1.55–0.02)*
ESR, mm/hour	−5.46 (14.21)*	−7.66 (21.9)*	−10.78 (26.7)*	−6.48 (14.66)*	−10.23 (22.77)*	−12.84 (33.39)*

Change from baseline data is mean (SD) and median (IQR).
*P≤0.001 from within-group mean change from baseline.
†The number of subjects changed after IPTW in the calculation; however, the actual number of subjects did not change.
BARI, baricitinib; CDAI, Clinical Disease Activity Index; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; HAQ-DI, Health Assessment Questionnaire-Disability Index; JAK, Janus kinase; SDAI, Simplified Disease Activity Index; TOFA, tofacitinib.

MTX dose, number of bDMARDs used before JAK inhibitors, TOFA use, HAQ-DI, CRP, matrix metalloproteinase 3, rheumatoid factor (RF) and anti-cyclic citrullinated peptide (CCP) antibody. EuroQol-5 Dimension was excluded from the explanatory variables because of the collinearity with HAQ-DI. Additionally, CDAI was excluded from the explanatory variables because grouping was based on the trajectories of CDAI.

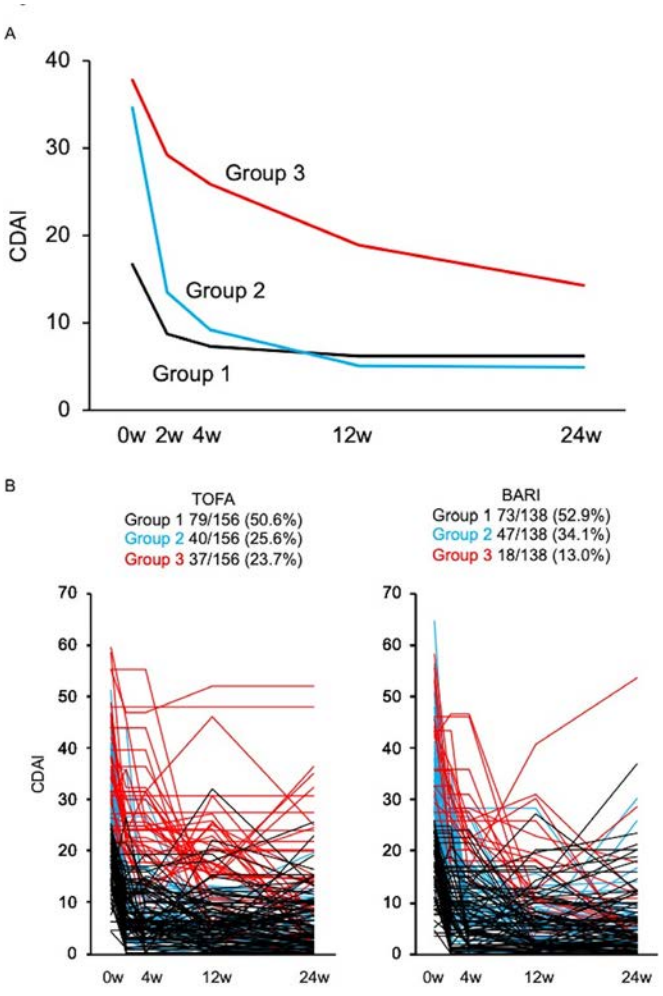

Figure 2 Patient CDAI responses and locally estimated scatterplot smoothing trajectory group modelling for patients receiving TOFA and BARI. (A) Lines are locally estimated scatterplot smoothing trajectories for the three patient trajectory groups. (B) Changes in CDAI in all patients receiving JAK inhibitors (TOFA (left) and BARI (right)) and the proportions of patients in each trajectory group. Group 1: black line, Group 2: blue line and group 3: red line. BARI, baricitinib; CDAI, Clinical Disease Activity Index; JAK, Janus kinase; TOFA, tofacitinib.

For all patients receiving JAK inhibitors, the factors contributing to belonging to treatment-resistance group were: high baseline HAQ-DI score (p=0.02) and high number of bDMARDs used before JAK inhibitors (p=0.002) and TOFA use (p=0.03).

When multivariable logistic regression analysis was separately performed for each treatment group, patients receiving more bDMARDs before the JAK inhibitor were more likely to belong to treatment-resistance group in the TOFA group (p<0.001). In the BARI group, multivariable logistic regression analysis did not identify any factors associated with belonging to treatment-resistance group.

DISCUSSION

In the present study, we compared the efficacy and safety at week 24 after the introduction of TOFA and BARI in patients with RA after reducing the selection bias to a minimum using the propensity score-based IPTW. Although the incidence of adverse events was comparable between the two groups, the BARI group showed a significantly lower CDAI and a significantly higher rate of CDAI remission at week 24. Although the CDAI numerical values displayed statistical differences, the differences in numerical values were small and may not be clinically meaningful. Although no differences were observed in HAQ-DI, even up to 24 weeks later, the duration of the analysis might have been too short for differences to be observed.

There are some reports on network meta-analysis indirectly comparing efficacy and safety of TOFA and BARI. Regarding efficacy, some reports suggested that BARI at a dose of 4 mg/day may be more effective than TOFA at a dose of 5 mg/day,[26 27] whereas another study showed that TOFA at a dose of 10 mg/day may be more effective than BARI at a dose of 4 mg/day.[28] In terms of safety, no consistent results have been reported regarding which drug is superior.[26 28 29] While these reports describe network meta-analysis by indirectly comparing results of randomised controlled trials, to the best of our knowledge, there is no study comparing efficacy and safety in real-world clinical practice. The present study is the first to compare the efficacy of TOFA and BARI in real-world clinical practice.

In this study, patients were divided into two groups: patients with MDA to HDA at baseline (group 1) and patients with HDA at baseline than group 1 (groups 2 and 3) based on the analysis of the trajectories of CDAI using GMM. In groups 1 and 2, disease activity was improved immediately after the introduction of JAK inhibitors. In group 3, disease activity was partially improved,

Table 4 Factors for belonging to treatment-resistance group identified by univariable and multivariable logistic regression analyses by treatment group

	TOFA (n=156)				BARI (n=138)			
	Univariable analysis		Multivariable analysis		Univariable analysis		Multivariable analysis	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Age	1.03 (0.99–1.06)	0.05	1.01 (0.98–1.05)	0.49	1.02 (0.98–1.06)	0.38		
Sex (female)	0.87 (0.33–2.24)	0.77			0.92 (0.28–3.04)	0.89		
RA duration	1.00 (0.99–1.01)	0.50	1.00 (0.99–1.01)	0.11	1.00 (0.99–1.00)	0.85	1.00 (0.99–1.01)	0.24
MTX dose	0.94 (0.89–0.99)	0.04	0.97 (0.90–1.04)	0.40	1.00 (0.92–1.07)	0.84		
Number of previous bDMARDs used	1.60 (1.20–2.07)	<0.001	1.77 (1.26–2.48)	<0.001	1.31 (0.97–1.78)	0.08	1.41 (0.95–2.10)	0.09
HAQ-DI	2.26 (1.36–3.77)	0.001	1.86 (1.02–3.39)	0.04	2.34 (1.19–4.59)	0.01		
CRP	1.13 (1.01–1.27)	0.04	1.10 (0.97–1.26)	0.15	1.11 (0.97–1.27)	0.13		
MMP-3	1.00 (0.99–1.00)	0.14			1.00 (0.99–1.00)	0.26		
Rheumatoid factor titre	1.00 (0.99–1.00)	0.63			1.00 (0.99–1.00)	0.31		
Anti-CCP antibody titre	1.00 (0.99–1.00)	0.96	1.00 (0.99–1.01)	0.23	1.01 (1.00–1.02)	0.03	1.00 (0.99–1.01)	0.09

BARI, baricitinib; bDMARDs, biological disease-modifying antirheumatic drugs; CCP, cyclic citrullinated peptide; CRP, C reactive protein; HAQ-DI, Health Assessment Questionnaire Disability Index; MMP-3, matrix metalloproteinase 3; MTX, methotrexate; RA, rheumatoid arthritis; TOFA, tofacitinib.

and LDA was not achieved at week 24 after the introduction of JAK inhibitors. The patients in group 3 were resistant to treatment. We also performed multivariable logistic regression analysis separately in the TOFA and BARI groups to analyse factors contributing to treatment resistance (group 3). In the TOFA group, patients who had received more bDMARDs before the JAK inhibitor were more likely to be resistant to treatment. We performed logistic regression analysis with the classification of group 3 as the dependent variable and the number of bDMARDs used before JAK inhibitors as the explanatory variable. Even though we similarly analysed the trajectory of CDAI improvement rates using GMM, we found that the subjects using TOFA belonging to the group with the lowest improvement rate included a large percentage of patients who had failed to respond to many bDMARDs. Then, we constructed receiver operating characteristic (ROC) curves to calculate the cut-off value. Results showed that patients receiving ≥4 bDMARDs were more likely to be resistant to treatment (sensitivity=0.62, specificity=0.86 and area under the curve=0.77) (data not shown). This suggested that TOFA might be partially effective in patients who received ≥4 bDMARDs. In the BARI group, high levels of HAQ-DI and anti-CCP antibody were extracted through univariable analysis as factors likely to belong to the treatment-resistance group, whereas no such factors were extracted following multivariable analysis.

Patients receiving many bDMARDs before JAK inhibitors and those receiving TOFA were more likely to be classified into the treatment resistant group in which CDAI changed, as observed in group 3. Because TOFA was partially effective in patients who did not respond to ≥4 bDMARDs, results from the present study might suggest that the efficacy of TOFA differs from that of BARI.

The present study has several limitations. First, this analysis was performed in a small number of Japanese patients, and hence, our findings may not be applicable to all patients with RA. Second, although propensity score-based IPTW was used to reduce the selection bias to a minimum and to adjust patient characteristics, not all confounding factors were adjusted. There may be unknown confounding factors. Third, there is the possibility that bias was introduced because of the use of LOCF. However, missing values were found in only seven patients, and no differences were seen in the results, even after sensitivity analyses were performed. Moreover, when NRI was used to evaluate the CDAI, SDAI and HAQ remission rates, the BARI group had significantly higher CDAI, SDAI and HAQ remission rates than the TOFA group. Fourth, because the observation period was just 24 weeks, long-term variation in efficacy of TOFA and BARI is not known; particularly, whether the difference revealed in the present study affect bone destruction was unclear. Fifth, because of the small number of patients resistant to treatment with BARI in GMM, we might have been unable to identify factors contributing to treatment resistance by performing multivariable logistic regression analysis. However, univariable analysis also showed that the number of bDMARDs used before BARI was not associated with treatment resistance. In other words, the difference in efficacy due to the number of bDMARDs used before JAK inhibitors might have contributed to the difference in efficacy between TOFA and BARI. As the treatment-resistant group was identified by the analysis using GMM, a model with better fit may be developed by conducting long-term studies. Fifth, in Japan, sales of TOFA began 4 years earlier than BARI, which might have led to a selection bias. However, even if we conducted a similar comparison of efficacy between patients to whom TOFA had been introduced after the date when the use of BARI was allowed in Japan, and patients who had used BARI, we found that the BARI group had higher efficacy (data not shown). Finally, there is no definitive basic study that supports the difference in efficacy between TOFA and BARI. Because the safety and efficacy features identified by basic analysis of signal transduction are not necessarily consistent with those observed in clinical practice, the efficacy of the drugs might have differed in the present study. Thus, further investigation is needed in this regard.

In summary, even if IPTW is used, there is a possibility that the selection bias cannot be removed entirely, and that there are confounding factors that have not been measured. TOFA may be partially effective in patients resistant to many bDMARDs. Consequently, efficacy may differ between TOFA and BARI. TOFA is likely to be less effective in patients with RA resistant to numerous bDMARDs. These results were observed in a relatively small group of patients and were obtained on hypothesis testing; accordingly, they need to be investigated in an accurately powered head-to-head trial.

Acknowledgements The authors thank all medical staff at all participating institutions for providing the data, especially Ms. Hiroko Yoshida, Ms. Youko Saitou, Ms. Machiko Mitsuiki and Ms. Ayumi Maruyama for the excellent data management in the FIRST registry. The authors thank Ms. M. Hirahara for providing excellent technical assistance. We also thank Dr Kazuyoshi Saito at Tobata General Hospital; Dr Kentaro Hanami and Dr Shunsuke Fukuyo at Wakamatsu Hospital of the University of Occupational and Environmental Health; Dr Keisuke Nakatsuka at Fukuoka Yutaka Hospital, and all staff members at Kitakyushu General Hospital and Shimonoseki Saiseikai Hospital for their engagement in data collection of the FIRST registry.

Contributors All authors were involved in the drafting and critical revision of the manuscript, and all authors approved the final version to be published. YM had full access to all of the data in the study and YT unifies the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study conception and design: YM, KN and YT. Acquisition of data: YM, KN and YI. Analysis and interpretation of data: YM, KN, SN, SK, YI, YF and YT.

Funding This work was supported in part by a Grant-In-Aid for Scientific Research from the University of Occupational and Environmental Health, Japan, through University of Occupational & Environmental Health, Japan (UOEH) for Advanced Research (19K17919).

Competing interests KN has received speaking fees from Bristol-Myers, Sanofi, AbbVie, Eisai, Eli Lilly, Chugai, Pfizer, Takeda, and Mitsubishi-Tanabe, and research grants from Mitsubishi-Tanabe and Eisai. SN has received speaking fees from Bristol-Myers, UCB, Astellas, Abbvie, Eisai, Pfizer, Takeda and has received research grants from Mitsubishi-Tanabe, Novartis and MSD. S. Kubo has received speaking fees from Bristol-Myers. YT has received consulting fees, speaking fees, and/or honoraria from Abbvie, Daiichi-Sankyo, Chugai, Takeda, Mitsubishi-Tanabe, Bristol-Myers, Astellas, Eisai, Janssen, Pfizer, Asahi-kasei, Eli Lilly, GlaxoSmithKline, UCB, Teijin, MSD, and Santen, and received research grants from Mitsubishi-Tanabe, Takeda, Chugai, Astellas, Eisai, Taisho-Toyama, Kyowa-Kirin, Abbvie, and Bristol-Myers.

Patient consent for publication Not required.

Ethics approval The FIRST registry includes RA patients who initiated treatment with molecular-targeting rheumatic drugs in a registry study conducted by the University of Occupational and Environmental Health, Japan and cooperated by its affiliated and multiple facilities. This study was approved by the ethics review board of the University of Occupational and Environmental Health, Japan (approval number #04-23), and informed consent was obtained from all patients of the FIRST registry.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. Data are available upon reasonable request.

ORCID iDs
Yusuke Miyazaki http://orcid.org/0000-0001-5692-3881
Satoshi Kubo http://orcid.org/0000-0001-9693-9263
Yoshiya Tanaka http://orcid.org/0000-0002-0807-7139

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EPIDEMIOLOGICAL SCIENCE

Associations of baseline use of biologic or targeted synthetic DMARDs with COVID-19 severity in rheumatoid arthritis: Results from the COVID-19 Global Rheumatology Alliance physician registry

Jeffrey A Sparks ,[1,2] Zachary S Wallace,[2,3] Andrea M Seet,[4] Milena A Gianfrancesco,[4] Zara Izadi,[5] Kimme L Hyrich ,[6,7] Anja Strangfeld ,[8] Laure Gossec ,[9,10] Loreto Carmona ,[11] Elsa F Mateus,[12,13] Saskia Lawson-Tovey ,[14,15] Laura Trupin,[4] Stephanie Rush,[4] Patricia Katz,[4] Gabriela Schmajuk,[4,16] Lindsay Jacobsohn,[4] Leanna Wise,[17] Emily L Gilbert,[18] Ali Duarte-García ,[19] Maria O Valenzuela-Almada,[20] Guillermo J Pons-Estel ,[21] Carolina A Isnardi,[21] Guillermo A Berbotto,[22] Tiffany Y -T Hsu ,[23] Kristin M D'Silva ,[3] Naomi J Patel,[3] Lianne Kearsley-Fleet ,[6] Martin Schäfer,[8] Sandra Lúcia Euzébio Ribeiro ,[24] Samar Al Emadi,[25] Liselotte Tidblad,[26] Carlo Alberto Scirè ,[27] Bernd Raffeiner,[28] Thierry Thomas,[29] René-Marc Flipo,[30] Jérôme Avouac,[31] Raphaèle Seror,[32] Miguel Bernardes,[33,34] Maria Margarida Cunha,[35] Rebecca Hasseli ,[36] Hendrik Schulze-Koops ,[37] Ulf Müller-Ladner,[36] Christof Specker ,[38] Viviane Angelina de Souza,[39] Licia Maria Henrique da Mota ,[40] Ana Paula Monteiro Gomides ,[41] Philippe Dieudé ,[42] Elena Nikiphorou ,[43] Vanessa L Kronzer ,[44] Namrata Singh ,[45] Manuel F Ugarte-Gil ,[46,47] Beth Wallace,[48,49] Akpabio Akpabio ,[50] Ranjeny Thomas ,[51] Suleman Bhana,[52] Wendy Costello,[53] Rebecca Grainger,[54] Jonathan S Hausmann ,[55,56] Jean W Liew,[57] Emily Sirotich ,[58,59] Paul Sufka,[60] Philip C Robinson ,[61,62] Pedro M Machado ,[63,64] Jinoos Yazdany,[4] COVID-19 Global Rheumatology Alliance

Handling editor Josef S Smolen

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2021-220418).

For numbered affiliations see end of article.

Correspondence to
Dr Jeffrey A Sparks, Department of Medicine, Division of Rheumatology, Inflammation, and Immunity, Brigham and Women's Hospital, Boston, Massachusetts, USA; jsparks@bwh.harvard.edu
Dr Zachary S Wallace; zswallace@mgh.harvard.edu

JAS and ZSW contributed equally.

JAS and ZSW are joint first authors.

Received 22 March 2021
Accepted 17 May 2021
Published Online First 28 May 2021

To cite: Sparks JA, Wallace ZS, Seet AM, *et al*. *Ann Rheum Dis* 2021;**80**:1137–1146.

ABSTRACT

Objective To investigate baseline use of biologic or targeted synthetic (b/ts) disease-modifying antirheumatic drugs (DMARDs) and COVID-19 outcomes in rheumatoid arthritis (RA).

Methods We analysed the COVID-19 Global Rheumatology Alliance physician registry (from 24 March 2020 to 12 April 2021). We investigated b/tsDMARD use for RA at the clinical onset of COVID-19 (baseline): abatacept (ABA), rituximab (RTX), Janus kinase inhibitors (JAKi), interleukin 6 inhibitors (IL-6i) or tumour necrosis factor inhibitors (TNFi, reference group). The ordinal COVID-19 severity outcome was (1) no hospitalisation, (2) hospitalisation without oxygen, (3) hospitalisation with oxygen/ventilation or (4) death. We used ordinal logistic regression to estimate the OR (odds of being one level higher on the ordinal outcome) for each drug class compared with TNFi, adjusting for potential baseline confounders.

Results Of 2869 people with RA (mean age 56.7 years, 80.8% female) on b/tsDMARD at the onset of COVID-19, there were 237 on ABA, 364 on RTX, 317 on IL-6i, 563 on JAKi and 1388 on TNFi. Overall, 613 (21%) were hospitalised and 157 (5.5%) died. RTX (OR 4.15, 95% CI 3.16 to 5.44) and JAKi (OR 2.06, 95% CI 1.60 to 2.65) were each associated with worse COVID-19 severity compared with TNFi. There were no associations between ABA or IL6i and COVID-19 severity.

Conclusions People with RA treated with RTX or JAKi had worse COVID-19 severity than those on TNFi. The strong association of RTX and JAKi use with poor COVID-19 outcomes highlights prioritisation of risk mitigation strategies for these people.

INTRODUCTION

The ongoing COVID-19 pandemic has had a significant impact on people with rheumatoid arthritis (RA), many of whom are treated with biologic or targeted synthetic disease-modifying antirheumatic drugs (b/tsDMARDs).[1] While b/tsDMARDs are important for controlling RA disease activity, their influence on COVID-19 outcomes in people with RA remains unclear. This uncertainty has led to anxiety, social isolation due to shielding practices and b/tsDMARD discontinuation, which may contribute to RA flares.[2–4] Addressing the knowledge gaps around the influence of b/tsDMARDs on COVID-19 outcomes is a priority for people with RA and their providers.

Key messages

What is already known about this subject?

- A previous international registry study of the COVID-19 Global Rheumatology Alliance (C19-GRA) suggested that people with systemic rheumatic diseases on biologic or targeted synthetic (b/ts) disease-modifying antirheumatic drugs (DMARDs) had lower odds of hospitalisation than those not using DMARDs.
- Previous studies reported that people with systemic rheumatic diseases using rituximab had higher odds of COVID-19-related mortality than those using alternative DMARDs such as methotrexate.

What does this study add?

- Using the C19-GRA, we analysed people with rheumatoid arthritis (RA) using b/tsDMARD (to limit the potential for confounding) at the time of COVID-19 onset and investigated an ordinal outcome that encompassed a range of COVID-19 outcomes.
- People with RA using rituximab or Janus kinase (JAK) inhibitors at COVID-19 onset were more likely to experience poor COVID-19 outcomes, ranging from hospitalisation to death, compared with use of tumour necrosis factor inhibitors.

How might this impact on clinical practice or future developments?

- People using rituximab or JAK inhibitors for RA are more likely to experience poor COVID-19 outcomes and should be prioritised for risk mitigation strategies.

The impact of b/tsDMARDs on COVID-19 outcomes is of particular interest since some of these medications, such as tocilizumab and baricitinib, have been studied as repurposed treatments for COVID-19. Some evidence suggests that baseline use of certain b/tsDMARDs, like tumour necrosis factor inhibitors (TNFi), for inflammatory disorders may be associated with less severe COVID-19 outcomes.[5] In addition, among patients with COVID-19, treatment with interleukin 6 inhibitors (IL-6i) and baricitinib led to improved outcomes in some clinical trials.[6–9] However, there are also concerns that baseline use of certain b/tsDMARDs, such as rituximab or abatacept, may be associated with worse COVID-19 outcomes due to impaired viral immune defences.[10 11]

Due to sample size limitations, previous studies of b/tsDMARD use and COVID-19 outcomes have combined heterogeneous rheumatic diseases and medications and/or investigated a single outcome, such as hospitalisation.[5 12] Therefore, we used the COVID-19 Global Rheumatology Alliance (C19-GRA) physician registry to evaluate the associations of different classes of b/tsDMARDs with a range of COVID-19 outcomes in people with RA.

METHODS

Data source and study sample assembly

People with rheumatic disease and COVID-19 from the C19-GRA registry and the European Alliance of Associations for Rheumatology (EULAR) COVID-19 database were included in the analyses. We included cases entered between 24 March 2020 and 12 April 2021. The C19-GRA and EULAR databases include people with rheumatic diseases diagnosed with COVID-19, as reported by rheumatology providers via two international data entry portals. The details of these registries have been previously reported.[5 12–17] We analysed people with RA on b/tsDMARD at the time of COVID-19 clinical onset. As of 12 April 2021, a total of 15 127 people with rheumatic diseases and COVID-19 have been reported. We included people with RA who were taking one of the following medication classes: Cytotoxic T lymphocyte-associated antigen immunoglobulin (CTLA4-Ig: abatacept), anti-CD20 (rituximab), IL-6i (tocilizumab, sarilumab), Janus kinase inhibitors (JAKi: tofacitinib, baricitinib or upadacitinib) or TNFi (infliximab, etanercept, adalimumab, certolizumab pegol and golimumab). The drug class of b/tsDMARD was collected, rather than individual drugs. We did not include IL-1 inhibitors since these were infrequently used for RA. Prior studies using the C19-GRA and EULAR databases have included some patients also reported in this study, but the analyses included in this study and observations reported are novel. In addition, follow-up for this study is more current than previous publications using these data.

Data quality was assessed by the University of California, San Francisco and the University of Manchester, UK, which both confirmed that there were no duplicates in the data entries.

Baseline b/tsDMARD exposures

The exposure of interest was baseline use of a b/tsDMARD at the time of COVID-19 clinical onset. As in previous C19-GRA investigations, we included confirmed and presumptive cases of COVID-19.[5 12 14] We limited this analysis to users of abatacept, rituximab, IL-6i, JAKi or TNFi to limit the cohort to people with similar RA disease severity and minimise the impact of confounding by indication. We included b/tsDMARD users regardless of whether they also used a conventional synthetic (cs) DMARD or glucocorticoids, but did not include people on csDMARDs (eg, hydroxychloroquine, methotrexate, sulfasalazine, leflunomide) monotherapy, as monotherapy may indicate less severe RA or be due to care access barriers or socioeconomic factors. TNFi users were the reference group since TNFis are the most frequently used b/tsDMARD in RA. People with RA who were reported to be on more than one b/tsDMARD were excluded from the analysis.

COVID-19 outcomes

The primary outcome of interest was a mutually exclusive ordinal COVID-19 severity outcome: (1) no hospitalisation, (2) hospitalisation with no oxygenation, (3) hospitalisation with any oxygenation or mechanical ventilation, and (4) death. We chose this primary outcome to estimate the association of b/tsDMARD exposure with general odds of worse COVID-19 severity rather than a single outcome. A similar outcome was developed by the WHO to capture the spectrum of disease and is used in clinical trials evaluating COVID-19 therapeutics.[18] If a patient met multiple levels of the outcome, they were only included at the highest level. At the time of analysis, all patients were required to have a resolved clinical course.

Covariates

Details regarding demographics, including age, race/ethnicity and continent, and patient characteristics, including obesity, smoking, comorbidities (interstitial lung disease (ILD), history of cancer, hypertension, cardiovascular disease, chronic kidney disease/end-stage kidney disease, diabetes, non-ILD pulmonary disease), RA disease activity (as judged by the reporting physician), glucocorticoid dose for RA at the time of COVID-19 onset

and use of concomitant csDMARD (methotrexate, sulfasalazine, hydroxychloroquine), were by physician report. For glucocorticoid dose, the amount of prednisone-equivalent glucocorticoid prescribed was treated as a categorical variable (none, >0–5 mg/day, 6–9 mg/day and ≥10 mg/day). Hypertension and cardiovascular disease were collapsed as a single comorbidity due to collinearity.

Statistical analysis

We reported baseline characteristics and outcomes across the exposure categories of baseline b/tsDMARD use with descriptive statistics.

Ordinal logistic regression models were used to assess the association between each b/tsDMARD compared with TNFi use and the severity of COVID-19 on an ordinal scale in unadjusted and multivariable analyses to estimate ORs and 95% CIs. The effect size of the ordinal outcome can be interpreted as the odds of being one level higher on the ordinal COVID-19 severity scale than the reference group. We assessed the proportional odds assumption for the ordinal regression model using the Brant test.[19] Models in which the proportional odds assumption was not met were refitted using the partial proportional odds model which relaxes the assumption of proportionality for offending predictors.[20] We considered potential confounders known to be associated with either b/tsDMARD use or COVID-19 severity. Covariates included in multivariable models included sociodemographic features (age, sex), obesity, smoking status (ever vs never), concomitant csDMARD use (methotrexate, hydroxychloroquine, sulfasalazine, or leflunomide), categorical glucocorticoid use/dose, categorical comorbidity count (0, 1, 2 of the following: chronic kidney insufficiency/end-stage kidney disease, diabetes, non-ILD pulmonary disease), other key comorbidities as individual variables (hypertension/cardiovascular disease, ILD and cancer), disease activity (moderate/high vs remission/low), continent (Europe, North America, South America, other) and calendar time (January–15 June 2020 vs 16 June 2020–12 April 2021).[21] These time periods were selected based on the initial publication of the RECOVERY trial, which reported a survival benefit associated with dexamethasone and influenced subsequent practice.[22] We assumed that missing data were 'missing at random'. We then performed multiple imputation five times to get pooled estimates to impute missing values for disease activity, race/ethnicity, glucocorticoid dose, smoking, hypertension/cardiovascular disease and comorbidity count. After imputation, we compared the distribution of imputed values with the distribution of variables before imputation to confirm that distributions were similar before and after imputation.

To confirm the robustness of our findings, we performed several sensitivity analyses. First, we excluded patients with ILD or cancer from the analysis since rituximab is commonly used in these patients, who may also be susceptible to poor COVID-19 outcomes. Second, given data showing a strong association between race/ethnicity and COVID-19 outcomes in the USA, we performed an analysis adjusting for this variable among US patients in the registry. The race/ethnicity variable was categorised as white, black, Hispanic, Asian or other/mixed race. However, for the model with IL-6i, there were few outcomes within the race/ethnicity variable so we were unable to perform the model. Third, we used propensity score matching to further address potential confounding by indication. We estimated propensity scores for b/tsDMARD use based on age, sex, obesity, smoking, concomitant csDMARDs, glucocorticoid use/dose, number of comorbidities, disease activity, region and calendar time. Covariate balance between each b/tsDMARD drug class and TNFi was assessed using Love plots (online supplemental figures 1–4), which showed that most of the covariates were matched with an absolute standardised mean difference less than 0.1, denoting sufficient matching performance.[23] Ordinal logistic regression was then performed after matching. Fourth, we repeated our primary analysis after excluding patients with a presumptive diagnosis of COVID-19. Presumptive cases were those that lacked one of the following: positive PCR or antigen test for SARS-CoV-2 or typical chest imaging findings. Fifth, we repeated the analysis but stratified by calendar time (before or after 15 June 2020 when RECOVERY trial's results were announced) and by continent (North America or Europe) in case calendar time and geography may have influenced the results. Sixth, we used a revised version of the ordinal COVID-19 severity outcome that considered mechanical ventilation as its own category.

We then repeated our primary analyses using dichotomised outcomes rather than the ordinal COVID-19 severity scale to investigate whether there were particular outcomes driving the associations we observed. For example, we investigated whether each b/tsDMARD was associated with hospitalisation (yes/no) compared with TNFi use.

We used the Brant test to assess whether the observed deviations from the ordinal logistic regression are larger than what could be attributed to chance alone. If the p values are greater than the alpha level of 0.05, then the covariates satisfy the proportional odds assumption. This assumption states that the estimate between each pair of outcomes across the response levels regardless of the partition that we consider. For abatacept and JAKis, both age and glucocorticoid dose violated the assumption, and for IL-6is and rituximab, age, gender and glucocorticoid dose violated the assumption. In order to address the lack of proportionality for these covariates, partial proportional odds models were run to relax this assumption for the respective covariates for each medication category (online supplemental table 1). We found that the estimates were similar when comparing the proportional odds models and the non-proportional odds model, so we reported the model without relaxing the assumption.

Results were considered statistically significant at two-sided $p<0.05$. Analyses were conducted in R V.4.0.2.

RESULTS

Study sample and baseline characteristics

From a total of 6132 RA cases reported to the registry, we identified 2869 who were on abatacept (n=237), rituximab (n=364), IL-6i (n=317), JAKi (n=563) or TNFi (n=1388) at the time of clinical COVID-19 onset. The baseline clinical characteristics are shown in table 1. The sample was predominantly female (80.8%) and the mean age was 56.7 years (SD 13.4). Most patients were from Europe (51.8%) and North America (35.0%). Overall, 354 (12.3%) were obese, 582 (20.3%) were ever smokers, 810 (28.2%) were on glucocorticoids, 1409 (49.1%) were on concomitant csDMARDs, and 510 (17.8%) had moderate/high RA disease activity. Among b/tsDMARD users, rituximab users were more likely than TNFi users to have ILD (11.0% vs 1.4%) or a history of cancer (7.4% vs 0.9%); JAKi users were slightly more likely than TNFi users to be obese (15.1% vs 10.3%).

Table 1 Baseline characteristics according to use of biologic or targeted synthetic disease-modifying antirheumatic drugs for rheumatoid arthritis at the time of COVID-19 onset

	Overall N=2869	Abatacept n=237	Rituximab n=364	IL-6 inhibitors n=317	JAK inhibitors n=563	TNF inhibitors n=1388
Demographics						
Mean age (years), SD	56.7 (13.4)	61.4 (14.0)	58.0 (12.9)	56.4 (12.0)	58.0 (12.3)	55.2 (14.0)
Female	2316 (80.8)	188 (79.3)	299 (82.1)	257 (81.3)	470 (83.5)	1102 (79.4)
Race/ethnicity						
White	1670 (69.0)	78 (69.5)	187 (64.5)	169 (67.9)	360 (73.2)	829 (69.3)
Black	113 (4.7)	5 (3.2)	14 (4.8)	11 (4.4)	22 (4.5)	60 (5.0)
Hispanic	472 (19.5)	32 (20.8)	66 (22.8)	46 (18.5)	79 (16.1)	233 (19.5)
East Asian	81 (3.3)	8 (5.2)	10 (3.4)	12 (4.8)	10 (2.0)	37 (3.1)
Other	85 (3.3)	2 (1.3)	13 (4.5)	11 (4.4)	21 (4.3)	38 (3.2)
Continent						
Europe	1486 (51.8)	103 (43.5)	218 (59.9)	183 (57.7)	283 (50.3)	699 (50.4)
North America	1005 (35.0)	105 (44.3)	111 (30.5)	83 (26.2)	208 (36.9)	498 (35.9)
South America	276 (9.6)	20 (8.4)	23 (6.3)	33 (10.4)	55 (9.8)	145 (10.4)
Other	302 (10.5)	9 (3.8)	12 (3.3)	18 (5.7)	17 (3.0)	46 (3.3)
Comorbidity count*						
0	1494 (52.1)	113 (47.7)	161 (44.2)	161 (50.8)	270 (48.0)	789 (56.8)
1	837 (29.2)	70 (29.5)	119 (32.7)	99 (31.2)	176 (31.3)	373 (26.9)
2	538 (18.8)	54 (22.8)	84 (23.1)	57 (18.0)	117 (20.8)	226 (16.3)
Individual comorbidities						
Hypertension	983 (34.3)	91 (38.4)	121 (33.2)	108 (34.1)	221 (39.3)	442 (31.8)
Cardiovascular disease	247 (8.6)	29 (12.2)	36 (9.9)	32 (10.1)	51 (9.1)	99 (7.1)
Diabetes	356 (12.5)	30 (12.8)	54 (14.9)	43 (13.6)	74 (13.2)	155 (11.3)
Chronic kidney disease	98 (3.4)	11 (4.7)	11 (3.0)	14 (4.4)	22 (3.9)	40 (2.9)
Lung disease†	432 (15.2)	41 (17.4)	87 (24.0)	44 (13.9)	92 (16.4)	168 (12.3)
Interstitial lung disease	103 (3.6)	15 (6.3)	40 (11.0)	15 (4.7)	13 (2.3)	20 (1.4)
Cancer	40 (1.5)	5 (2.5)	27 (7.4)	6 (2.2)	5 (1.0)	11 (0.9)
Obesity	354 (12.3)	31 (13.1)	52 (14.3)	43 (13.6)	85 (15.1)	143 (10.3)
Smoking status						
Ever	582 (20.3)	104 (43.9)	70 (19.2)	57 (18.0)	99 (17.6)	300 (21.6)
Never	1369 (47.7)	56 (23.6)	142 (39.0)	152 (47.9)	262 (46.5)	694 (50.)
Missing	918 (32.0)	77 (32.5)	137 (37.6)	107 (33.8)	202 (35.9)	394 (28.4)
Concomitant RA medications						
Any conventional synthetic DMARD	1409 (49.1)	118 (49.8)	194 (53.3)	102 (32.2)	228 (40.5)	767 (55.3)
Methotrexate	1188 (41.4)	92 (38.8)	146 (40.1)	91 (28.7)	188 (33.4)	671 (48.3)
Sulfasalazine	136 (4.7)	9 (3.8)	26 (7.1)	8 (2.5)	18 (3.2)	75 (5.4)
Hydroxychloroquine	260 (9.1)	25 (10.5)	58 (15.9)	18 (5.7)	43 (7.6)	116 (8.4)
Leflunomide	176 (10.5)	26 (11.0)	49 (13.5)	20 (6.3)	29 (5.2)	117 (8.4)
Glucocorticoid dose, median (IQR)	5.0 (4.0–6.0)	5.0 (4.0–5.5)	5.0 (5.0–7.5)	5.0 (4.5–7.0)	5.0 (3.0–5.0)	5.0 (5.0–7.0)
Categorical glucocorticoid use/dose						
No glucocorticoid use	1756 (61.2)	120 (56.9)	186 (51.1)	173 (54.6)	320 (63.5)	957 (76.1)
Glucocorticoid >0–5 mg/day prednisone equivalent	600 (20.9)	68 (32.2)	93 (25.5)	69 (21.8)	149 (29.6)	221 (17.6)
Glucocorticoid 6–9 mg/day prednisone equivalent	68 (2.4)	8 (3.8)	10 (2.7)	15 (4.7)	12 (2.4)	23 (1.8)
Glucocorticoid ≥10 mg/day prednisone equivalent	142 (4.9)	15 (7.1)	28 (7.7)	19 (6.0)	23 (4.6)	57 (4.5)
Missing	303 (10.6)	26 (11.0)	47 (12.9)	41 (12.9)	59 (10.5)	130 (9.4)
RA disease activity by global physician assessment						
Remission or low	1949 (67.9)	147 (74.2)	226 (76.1)	198 (77.3)	388 (78.7)	990 (81.5)
Moderate or high	510 (17.8)	51 (25.8)	71 (23.9)	58 (22.7)	105 (21.3)	225 (18.5)
Missing	410 (14.3)	39 (16.5)	67 (18.4)	61 (19.2)	70 (12.4)	173 (12.5)
Confirmed COVID-19	2333 (81.3)	201 (84.8)	304 (83.5)	244 (77.0)	475 (84.4)	1109 (79.9)

n (%) presented unless otherwise specified.
*Comorbidity count included diabetes, lung disease and chronic kidney disease.
†Interstitial lung disease, chronic obstructive pulmonary disease, asthma or other lung disease.
DMARDs, disease-modifying antirheumatic drugs; IL-6, interleukin 6; JAK, Janus kinase; RA, rheumatoid arthritis; TNF, tumour necrosis factor.

COVID-19 outcomes

Outcomes according to the COVID-19 severity scale are shown in table 2. The majority of patients (78.6%) were not hospitalised, 137 (4.8%) were hospitalised without oxygenation, 319 (11.1%) were hospitalised with any oxygen or ventilation requirement, and 157 (5.5%) died. Among rituximab users, 80 (22.0%) required hospitalisation with any oxygen or ventilation and 54 (14.8%) died compared with 103 (7.4%)

Table 2 Frequencies and proportions of outcomes in the ordinal COVID-19 severity scale according to baseline use of biologic or targeted synthetic disease-modifying antirheumatic drug for patients with rheumatoid arthritis at the time of COVID-19 onset (N=2869)

COVID-19 severity scale	Overall N=2869 n (%)	Abatacept n=237 n (%)	Rituximab n=364 n (%)	IL-6 inhibitors n=317 n (%)	JAK inhibitors n=563 n (%)	TNF inhibitors n=1388 n (%)
Not hospitalised	2256 (78.6)	181 (76.4)	210 (57.7)	271 (85.5)	409 (72.6)	1185 (85.4)
Hospitalised without oxygenation	137 (4.8)	12 (5.1)	20 (5.5)	13 (4.1)	28 (5.0)	64 (4.6)
Hospitalised with any oxygen or ventilation	319 (11.1)	26 (11.0)	80 (22.0)	24 (7.6)	86 (15.3)	103 (7.4)
Death	157 (5.5)	18 (7.6)	54 (14.8)	9 (2.8)	40 (7.1)	36 (2.6)

IL-6, interleukin 6; JAK, Janus kinase; TNF, tumour necrosis factor.

and 36 (2.6%) TNFi users, respectively. Among JAKi users, 86 (15.3%) were hospitalised with oxygen/ventilation and 40 (7.1%) died. Only 9 (2.8%) patients on baseline IL-6i died.

Associations of b/tsDMARDs with COVID-19 severity

The multivariable ordinal logistic regression model is shown in table 3. Compared with TNFi users, rituximab users had 4.15 (95% CI 3.40 to 3.80) greater odds of worse COVID-19 severity as compared with patients taking TNFi, while JAKi users had 2.06 (95% CI 1.60 to 2.65) greater odds of worse COVID-19 severity. No significant associations were found with respect to abatacept or IL-6i compared with TNFi in the primary analysis.

Sensitivity analyses

Sensitivity analyses of the drug class comparisons are shown in table 3. After excluding patients with ILD or cancer, the association between rituximab with poor COVID-19 outcomes when compared with TNFi use remained strong (OR 4.34, 95% CI 3.23 to 5.82). Among patients with RA in the USA, results were also similar when additionally adjusting for race/ethnicity. We also performed a propensity score-matched analysis instead of multivariable ordinal logistic regression. The sample for each propensity score-matched analysis is illustrated in online supplemental figure 5. Rituximab users (OR 3.36, 95% CI 2.11 to 5.34) and JAKi users (OR 1.56, 95% CI 1.01 to 2.42) had increased COVID-19 severity compared with TNFi users in this analysis. In the propensity score-matched analysis, abatacept had an OR of 1.60 (95% CI 1.02 to 2.51) for the ordinal COVID-19 severity outcome compared with TNFi. IL-6i use was not associated with COVID-19 severity in any of the analyses. Brant tests indicated that the proportional odds assumption did not hold for propensity score models; therefore, partial proportional odds models were used and confirmed that the effect estimates remained consistent (data not shown).

When stratified by calendar time (before or after 15 June 2020) and restricted to Europe or North America, the results were similar (online supplemental table 2).

Individual COVID-19 outcomes

We also performed analyses for each binary level of the COVID-19 severity scale (table 4). Rituximab and JAKi use were each associated with increased odds for each COVID-19 outcome compared with TNFi use. For example, rituximab use had increased odds for hospitalisation (OR 4.53, 95% CI 3.32 to 6.18) as well as death (OR 4.57, 95% CI 3.32 to 9.01) compared with TNFi use. JAKi use was associated with all outcomes considered, including hospitalisation requiring any oxygen or ventilation or death (OR 1.55, 95% CI 1.04 to 2.18) and death (OR 2.04, 95% CI 1.58 to 2.65) compared with TNFi. In these analyses, there were no statistically significant associations between abatacept or IL-6i use and the dichotomised outcomes when compared with TNFi use.

We considered a revised version of the ordinal outcome that included mechanical ventilation as a separate level. There were relatively few patients who survived after requiring mechanical ventilation (online supplemental table 2). Results were similar using this revised ordinal outcome (online supplemental tables 3 and 4).

DISCUSSION

Among patients with RA on b/tsDMARDs at the onset of COVID-19, rituximab and JAKi users were at increased odds for worse COVID-19 outcomes compared with TNFi users. In contrast, we did not find an association between abatacept or IL-6i use with worse COVID-19 outcomes when compared with TNFi users. These observations can inform decision making for providers and patients during the ongoing COVID-19 pandemic. Given the association between rituximab and JAKi use with poor outcomes, vaccination and public health measures such as mask wearing and social distancing for COVID-19 risk mitigation remain paramount. In addition, other specific interventions (eg, monoclonal antibody treatment) might be considered in these patients with COVID-19 exposure or early infection.[24]

Our observations, which use the largest sample of individuals with RA and COVID-19 assembled to date, regarding rituximab exposure confirm findings from prior studies suggesting an association between baseline use of B cell depleting therapies and worse COVID-19 outcomes in people with rheumatic diseases[12 25 26] and multiple sclerosis.[27] We also expand on prior observations using the C19-GRA and EULAR databases by evaluating the association of rituximab with COVID-19 severity rather than only mortality and by using an alternative reference group (TNFi rather than methotrexate) and performing propensity score analyses to further address confounding by indication. By focusing on a single disease, we also were able to identify a novel association of JAKis with COVID-19 severity. Mechanistically, the impact of B cell depletion on antibody production would be expected to impair the immune system's normal response to a viral infection. Indeed, the antibody response to COVID-19 is critical for controlling the initial infection and preventing reinfection.[28] We lacked details regarding the timing of rituximab exposure in relation to the COVID-19 infection or the duration of B cell depletion at the time of infection, which may be particularly relevant when considering the risk of a poor outcome following rituximab exposure. It is also possible that glucocorticoids given as a premedication to rituximab infusions may have contributed to the increased risk of poor COVID-19 outcomes in patients with RA on rituximab. While the results were robust to several sensitivity analyses, it is possible that the result could be confounded by factors such as unrecognised ILD.

Table 3 Results of primary and sensitivity analyses investigating the associations of baseline use of biologic or targeted synthetic disease-modifying antirheumatic drugs with COVID-19 severity (N=2869)

	Abatacept		Rituximab		IL-6i		JAKi		TNFi
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	Ref
Unadjusted	1.88 (1.35 to 2.63)	<0.01	4.63 (3.60 to 5.96)	<0.01	1.00 (0.71 to 1.41)	0.99	2.28 (1.80 to 2.88)	<0.01	Ref
Age-adjusted and sex-adjusted	1.40 (0.99 to 1.99)	0.06	4.45 (3.43 to 5.77)	<0.01	1.06 (0.68 to 1.37)	0.84	2.10 (1.64 to 2.68)	<0.01	Ref
Multivariable-adjusted (primary analysis)	1.26 (0.88 to 1.80)	0.21	4.15 (3.16 to 5.44)	<0.01	0.81 (0.56 to 1.18)	0.55	2.06 (1.60 to 2.65)	<0.01	Ref
Confirmed cases only*	1.14 (0.77 to 1.68)	0.52	4.25 (3.17 to 5.69)	<0.01	0.74 (0.49 to 1.11)	0.15	2.05 (1.57 to 2.69)	<0.01	Ref
Excluding patients with ILD or cancer†	1.18 (0.79 to 1.76)	0.43	4.34 (3.23 to 5.82)	<0.01	0.81 (0.54 to 1.21)	0.30	2.14 (1.64 to 2.79)	<0.01	Ref
Restricted to USA and additionally adjusted for race‡	1.16 (0.79 to 1.69)	0.45	4.77 (3.57 to 6.38)	<0.01†	¶	¶	2.86 (1.76 to 4.65)	<0.01†	Ref
Propensity score-matched§	1.60 (1.02 to 2.51)	0.04	4.70 (3.31 to 6.65)	<0.01	0.76 (0.46 to 1.23)	0.26	2.09 (1.50 to 2.90)	<0.01	Ref

The effect size is the odds of being one level higher on the ordinal COVID-19 severity scale than the reference group (TNFi users).
Adjusted for age, sex, region, calendar time, obesity, smoking, concomitant csDMARD use, glucocorticoid use/dose, comorbidity count, hypertension/cardiovascular disease, interstitial lung disease, cancer and rheumatoid arthritis disease activity except as otherwise indicated.
*n=2333 in the analysis analysing only confirmed COVID-19 cases.
†n=2704 in the analysis excluding ILD and cancer.
‡n=868 in the USA-only analysis.
§n for each pair of propensity score-matched analyses: abatacept: 236, TNFi: 1376; rituximab: 364, TNFi: 1382; IL-6i: 313, TNFi: 1387; JAKi: 560, TNFi: 1379.
¶Due to the small number of events in the covariate of race, the IL-6i model could not be analysed.
csDMARDs, conventional synthetic disease-modifying antirheumatic drugs; ILD, interstitial lung disease; IL6i, interleukin 6 inhibitor; JAKi, Janus kinase inhibitor; Ref, reference; TNFi, tumour necrosis factor inhibitors.

Table 4 Multivariable* OR of biologic or targeted synthetic disease-modifying antirheumatic drugs at each binary level of the COVID-19 severity scale (N=2869)

	Abatacept		Rituximab		IL-6 inhibitors		JAK inhibitors		
COVID-19 outcome	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	TNF inhibitors
Hospitalised	1.18 (0.76 to 1.82)	0.47	4.53 (3.32 to 6.18)	<0.01	0.84 (0.53 to 1.33)	0.45	2.40 (1.78 to 3.24)	<0.01	Ref
Hospitalised with oxygenation/ventilation or death	1.12 (0.70 to 1.81)	0.63	2.87 (2.03 to 4.06)	<0.01	0.72 (0.43 to 1.20)	0.20	1.55 (1.04 to 2.18)	0.01	Ref
Death	1.46 (0.72 to 2.89)	0.30	4.57 (3.32 to 9.01)	<0.01	1.13 (0.50 to 2.59)	0.77	2.04 (1.58 to 2.65)	<0.01	Ref
Mechanical ventilation (restricted to only hospitalised patients, n=613)	1.41 (0.94 to 2.10)	0.09	4.05 (3.08 to 5.33)	<0.01	0.75 (0.51 to 1.10)	0.14	2.03 (1.56 to 2.62)	<0.01	Ref
Mechanical ventilation or death	1.14 (0.78 to 1.66)	0.50	4.44 (3.39 to 5.82)	<0.01	0.74 (0.50 to 1.09)	0.12	2.02 (1.56 to 2.61)	<0.01	Ref

*Adjusted for age, sex, region, calendar time, obesity, smoking, concomitant csDMARD use, glucocorticoid use/dose, comorbidity count, hypertension/cardiovascular disease, interstitial lung disease, cancer and rheumatoid arthritis disease activity.
csDMARD, conventional synthetic disease-modifying antirheumatic drug; IL-6, interleukin 6; JAK, Janus kinase; Ref, reference; TNF, tumour necrosis factor.

Our findings are of particular interest given recent clinical trials and observational studies suggesting that IL-6i[6–8 29–32] and JAKi[9] may improve outcomes for patients in the general population with COVID-19. We found no association of baseline IL-6i use in RA with COVID-19 severity compared with TNFi use. In contrast, while baricitinib treatment may have some benefit on time to recovery for patients with more severe COVID-19,[9] we observed worse outcomes associated with baseline use of JAKi. This was also suggested in a recent population-based study investigating RA and other inflammatory joint diseases in Sweden.[25] Glucocorticoids are known to have benefits when initiated for moderate-to-severe COVID-19, but are also associated with worse outcomes among those on baseline glucocorticoids at the time of infection,[5 12] although this may be explained by residual disease activity.[33] Therefore, the timing of JAKi use relative to the COVID-19 disease course may explain our findings. Similar to glucocorticoids, baseline use of JAKi at the time of SARS-CoV-2 infection may enhance viral reproduction and dampen a healthy immune response, while JAKi initiation at clinical deterioration may dampen an aberrant systemic inflammatory response. Alternatively, there may be relevant differences in COVID-19 outcomes depending on the type of JAKi used given that JAKis like tofacitinib, baricitinib and upadacitinib target different Janus kinases. We were unable to perform analyses of each individual JAKi since these were collected as a class. While the primary analysis found no association of abatacept with COVID-19 severity, there was a statistical association in the propensity score-matched analysis. Further research is needed on the safety of abatacept for infection risk and severity since its mechanism of action may impair adaptive immune response.

Our study has a number of strengths, including the international nature of the registry and the large sample size. Additionally, we used an active comparator (TNFi), which was also a b/tsDMARD in a single rheumatic disease, as well as two different modelling approaches (multivariable logistic regression and propensity score matching) among other sensitivity analyses to account for confounding by indication and to confirm the robustness of our findings. Our observations expand on prior general population and RA cohort studies that identified older age, greater comorbidity burden and other factors associated with worse COVID-19 and must also be considered when assessing an individual's risk.

Our study also has certain limitations. First, the Global Rheumatology Alliance and EULAR registries are voluntary and require a provider to submit the details of a case, perhaps biasing our sample towards more severe cases. As such, the proportion of events reported across exposure groups may be an overestimate of that observed among all patients with RA in real-world practice and should be interpreted in that context. However, the effect size estimates do have clinical interpretation in potentially identifying patients with RA who could be susceptible to poor COVID-19 outcomes. While we designed the study to limit the potential impact of selection bias and confounding by indication by examining advanced therapies in a single rheumatic disease, it is possible that selective reporting could have varied across different b/tsDMARD classes as the exposure of interest. This potential bias may have caused an upward deflection in the effect size estimate if more severe cases of a particular b/tsDMARD class were systematically reported compared with others, and this could contribute to the findings that we report. We further mitigated this possibility by adjusting for differences in concomitant medication use, disease activity and comorbidities, as well as performing an analysis removing patients with ILD or cancer. Our findings remained when we excluded presumptive cases of COVID-19. Second, although we were able to adjust for a number of potential confounders of our observed associations, there is the potential for residual unmeasured confounding. Analysing only patients on b/tsDMARD may have helped minimise some unmeasured confounding related to access to care since all analysed patients with RA were able to receive these targeted medications. In addition, the consistent results observed in sensitivity analyses excluding patients with ILD or cancer who may be more likely to receive rituximab support the robustness of our results. However, we did not have data available on RA duration or previous RA medications (eg, previous TNFi use in patients on other classes of b/tsDMARDs), which may have affected the results. Medications were collected by DMARD class, so we were unable to compare individual medications within the same class. However, the goal of the study was to compare different biologic mechanisms of action for COVID-19 severity. Additionally, it is also possible that TNFi use may protect against severe COVID-19 outcomes. Thus, these results should be interpreted cautiously and additional studies are needed to confirm our observed associations. Third, while we leveraged the largest cohort of patients with rheumatic disease with COVID-19, a somewhat small number of outcomes of interest occurred in some subgroups, which may have limited our power to detect significant differences among abatacept users, in particular. In addition, we were unable to investigate individual JAKi or TNFi. Finally, we did not examine medication changes after COVID-19 onset since this occurred after baseline and may have mediated the relationship we report. Most of the drugs have lengthy biologic effects (especially rituximab), while JAKis have short half-lives. Some clinicians may have chosen to continue IL-6is after COVID-19 onset, as suggested by the American College of Rheumatology.[34] Future studies are needed to investigate the association of medication changes with COVID-19 outcomes.

In conclusion, use of rituximab or JAKi, but not abatacept or IL-6i, at the time of COVID-19 infection was associated with worse COVID-19 outcomes compared with TNFi among patients with RA. Additional studies are warranted to confirm these observations. Strategies are needed to improve outcomes following COVID-19 RA on rituximab or JAKis.

Author affiliations
[1]Department of Medicine, Division of Rheumatology, Inflammation, and Immunity, Brigham and Women's Hospital, Boston, Massachusetts, USA
[2]Department of Medicine, Harvard Medical School, Boston, Massachusetts, USA
[3]Clinical Epidemiology Program and Rheumatology Unit, Division of Rheumatology, Allergy, and Immunology, Massachusetts General Hospital, Boston, Massachusetts, USA
[4]Department of Medicine, Division of Rheumatology, University of California San Francisco, San Francisco, California, USA
[5]Division of Rheumatology, Department of Medicine, Department of Epidemiology and Biostatistics, University of California San Francisco, San Francisco, California, USA
[6]Centre for Epidemiology Versus Arthritis, Manchester Academic Health Science Centre, The University of Manchester, Manchester, UK
[7]National Institute for Health Research Manchester Biomedical Research Centre, Manchester University NHS Foundation Trust, Manchester Academic Health Science Centre, Manchester, UK
[8]Epidemiology and Health Care Research, German Rheumatism Research Center Berlin, Berlin, Germany
[9]INSERM, Institut Pierre Louis d'Epidémiologie et de Santé Publique, Sorbonne Universite, Paris, France
[10]APHP, Rheumatology Department, Hopital Universitaire Pitie Salpetriere, Paris, France
[11]Instituto de Salud Musculoesqueletica, Madrid, Spain
[12]Portuguese League Against Rheumatic Diseases, Lisbon, Portugal
[13]Standing Committee of People with Arthritis/Rheumatism in Europe (PARE), European League Against Rheumatism, Kilchberg, Switzerland

[14]Centre for Genetics and Genomics Versus Arthritis, The University of Manchester, Manchester, UK
[15]National Institute for Health Research Manchester Biomedical Research Centre, Manchester University NHS Foundation Trust, Manchester, UK
[16]San Francisco VA Health Care System, San Francisco, California, USA
[17]Department of Internal Medicine, Division of Rheumatology, University of Southern California, Los Angeles, California, USA
[18]Division of Rheumatology, Mayo Clinic Hospital Jacksonville, Jacksonville, Florida, USA
[19]Division of Rheumatology, Robert D and Patricia E Kern Center for the Science of Health Care Delivery, Mayo Clinic Rochester, Rochester, Minnesota, USA
[20]Division of Rheumatology, Mayo Clinic Rochester, Rochester, Minnesota, USA
[21]SAR-COVID, Argentina Society of Rheumatology, Buenos Aires, Argentina
[22]Rheumatology, Sanatorio Britanico Rosario, Buenos Aires, Argentina
[23]Division of Rheumatology, Inflammation, and Immunity, Brigham and Women's Hospital, Boston, Massachusetts, USA
[24]Hospital Universitário Getúlio Vargas, Faculty of Medicine, Federal University of Amazonas, Manaus, Amazonas, Brazil
[25]Rheumatology Department, Hamad Medical Corporation, Doha, Qatar
[26]Division of Clinical Epidemiology, Department of Medicine, Karolinska Institutet, Stockholm, Sweden
[27]Epidemiology Research Unit, Italian Society of Rheumatology, Milano, Italy
[28]Department of Rheumatology, Central Hospital of Bolzano, Padua, Italy
[29]Société Française de Rhumatologie (SFR), Department of Rheumatology, Hôpital Nord, Universite de Lyon, Lyon, France
[30]Department of Rheumatology, University of Lille, Lille, France
[31]Service de Rhumatologie, Hôpital Cochin, AP-HP.CUP, Universite de Paris, Paris, France
[32]Department of Rheumatology, Assistance Publique, Hopitaux de Paris, Universite Paris-Saclay, Saint-Aubin, France
[33]Department of Medicine, Faculty of Medicine, University of Porto, Porto, Portugal
[34]Rheumatology Department, Centro Hospitalar de Sao Joao EPE, Porto, Portugal
[35]Rheumatology Department, Garcia de Orta Hospital, Almada, Portugal
[36]Division of Rheumatology and Clinical Immunology, Campus Kerckhoff, Justus Liebig Universitat Giessen, Giessen, Germany
[37]Division of Rheumatology and Clinical Immunology, Internal Medicine IV, Ludwig-Maximilians-Universitat Munchen, Munchen, Germany
[38]Department of Rheumatology and Clinical Immunology, KEM Kliniken Essen-Mitte, Essen, Germany
[39]Universidade Federal de Juiz de Fora, Juiz de Fora, Minas Gerais, Brazil
[40]Hospital Universitário de Brasilia, Universidade de Brasilia, Brasilia, Federal District, Brazil
[41]Centro Universitario de Brasilia, Brasilia, Federal District, Brazil
[42]Rheumatology Department, Bichat-Claude Bernard Hospital, Paris University, Paris, France
[43]Centre for Rheumatic Diseases, King's College Hospital, King's College London, London, UK
[44]Division of Rheumatology, Mayo Clinic, Rochester, Minnesota, USA
[45]Division of Rheumatology, University of Washington, Seattle, Washington, USA
[46]School of Medicine, Universidad Cientifica del Sur, Lima, Peru
[47]Rheumatology Department, Level IV Hospital Guillermo Almenara Irigoyen, La Victoria, Peru
[48]Department of Internal Medicine/Rheumatology, University of Michigan, Ann Arbor, Michigan, USA
[49]VA Ann Arbor Healthcare System, Ann Arbor, Michigan, USA
[50]Rheumatology Department, Royal Hallamshire Hospital, Sheffield, UK
[51]UQ Diamantina Institute, The University of Queensland, Saint Lucia, Queensland, Australia
[52]Crystal Run Healthcare, Middletown, New York, USA
[53]Irish Children's Arthritis Network (iCan), Tipperary, Ireland
[54]Department of Medicine, University of Otago, Dunedin, New Zealand
[55]Program in Rheumatology, Boston Children's Hospital, Boston, Massachusetts, USA
[56]Division of Rheumatology and Clinical Immunology, Beth Israel Deaconess Medical Center, Boston, Massachusetts, USA
[57]Section of Rheumatology, Department of Medicine, Boston University School of Medicine, Boston, Massachusetts, USA
[58]Department of Health Research Methods, Evidence, and Impact, McMaster University, Hamilton, Ontario, Canada
[59]Canadian Arthritis Patient Alliance, Toronto, Ontario, Canada
[60]HealthPartners, St. Paul, Minnesota, USA
[61]Faculty of Medicine, The University of Queensland, Herston, Queensland, Australia
[62]Metro North Hospital and Health Service, Royal Brisbane and Women's Hospital Health Service District, Herston, Queensland, Australia
[63]Centre for Rheumatology and Department of Neuromuscular Diseases, University College London, London, UK
[64]National Institute for Health Research (NIHR) University College London Hospitals Biomedical Research Centre, University College London Hospitals NHS Foundation Trust, London, UK

Correction notice This article has been corrected since it published Online First. Collaborator names have been updated.

Twitter Jeffrey A Sparks @jeffsparks, Zachary S Wallace @zach_wallace_md, Loreto Carmona @carmona_loreto, Saskia Lawson-Tovey @saskiaamber, Guillermo J Pons-Estel @gponsestel, Kristin M D'Silva @kmdsilvaMD, Carlo Alberto Scirè @rthritis, Licia Maria Henrique da Mota @Mota_licia_reum, Philippe Dieudé @PhilippeDieude, Elena Nikiphorou @ElenaNikiUK, Manuel F Ugarte-Gil @mugartegil, Akpabio Akpabio @Akpabioakanimo, Jonathan S Hausmann @hausmannmd, Jean W Liew @rheum_cat, Emily Sirotich @emilysirotich, Philip C Robinson @philipcrobinson and Pedro M Machado @pedrommcmachado

Acknowledgements We wish to thank all rheumatology providers who entered data into the registry.

Collaborators Brahim Dahou (Association Rhumatologues Algériens Privés (ARAP), Algeria), Rosana Quintana, Gimena Gómez, Karen Roberts, Roberto Miguel Baez, Vanessa Castro Coello, María J Haye Salinas, Federico Nicolas Maldonado, Alvaro Andres Reyes Torres, Gelsomina Alle, Romina Tanten, Hernán Maldonado Ficco, Romina Nieto, Carla Gobbi, Yohana Tissera, Cecilia Pisoni, Alba Paula, Juan Alejandro Albiero, Maria Marcela Schmid, Micaela Cosatti, Maria Julieta Gamba, Carlevaris Leandro, María Alejandra Cusa, Noelia German, Veronica Bellomio, Lorena Takashima, Mariana Pera, Karina Cogo, Maria Soledad Gálvez Elkin, María Alejandra Medina, Veronica Savio, Ivana Romina Rojas Tessel, Rodolfo Perez Alamino, Marina Laura Werner, Sofía Ornella, Luciana Casalla, Maria de la Vega, María Severina, Mercedes García, Luciana Gonzalez Lucero, Cecilia Romeo, Sebastián Moyano, Tatiana Barbich, Ana Bertoli, Andrea Baños, Sandra Petruzzelli, Carla Matellan, Silvana Conti, Ma Alicia Lazaro, Gustavo Fabián Rodriguez Gil, Fabian Risueño, Maria Isabel Quaglia, Julia Scafati, Natalia Lili Cuchiaro, Jonathan Eliseo Rebak, Susana Isabel Pineda, María Elena Calvo, Eugenia Picco, Josefina Gallino Yanzi, Pablo Maid, Debora Guaglianone, Julieta Silvana Morbiducci, Sabrina Porta, Natalia Herscovich, José Luis Velasco Zamora, Boris Kisluk, Maria Sol Castaños Menescardi, Rosana Gallo, María Victoria Martire, Carla Maldini, Cecilia Goizueta, Sabrina Solange de la vega Fernandez, Carolina Aeschlimann Argentina, Gisela Subils (Argentine Society of Rheumatology, Argentina), Eva Rath (Hanusch Krankenhaus, Vienna, Austria), Yves Piette (AZ Sint-Jan Brugge, Belgium), Mieke Devinck (AZ Sint-Lucas Brugge, Belgium), Bea Maeyaert (AZ Sint-Lucas Brugge, Hospital Universitário Pedro Ernesto Universidade do, Belgium), Francinne Machado Ribeiro (Estado do Rio de Janeiro, Brazil), Sandra Lucia Euzebio Ribeiro (Federal University of Amazonas, Universidade Federal De São Paulo Escola Paulista de, Brazil), Marcelo Pinheiro (Medicina e Escola Paulista de Enfermagem, Brazil), Sebastián Ibáñez (Clínica Alemana de Santiago, Chile), Anne-Marie Chassin-Trubert (Complejo Hospitalario San José, Chile), Lingli Dong (Tongji Hospital, China), Lui Cajas (Clinica Universitaria Colombia - Centro Medico Providencia Sanitas, Colombia), Marko Barešić (University Hospital Center Zagreb, Croatia), Branimir Anić (Div Clin Immunol Rheumatol; Dept Int Med, School of Med Zagreb, University Hospital Center Zagreb, Croatia), Melanie-Ivana Čulo (University Hospital Dubrava, Zagreb, Croatia), Tea Ahel Pavelić (Clinical Hospital Center Rijeka, Croatia), Kristina Kovačević (Stranski University hospital Osijek, Croatia), Boris Karanovic (UHC Zagreb, Croatia), Jiri Vencovsky (Institute of Rheumatology, Prague, Czechia), Marta Píchová (Medipont plus s.ro., České Budějovice, Czechia), Maria Filkova (Institute of Rheumatology, Prague, Czechia), Hesham Hamoud (Al Azhar University Hospitals, Egypt), Dimitrios Vassilopoulos (Hippokration General Hospital, Athens, Greece), Gabriela Maria Guzman Melgar (Hospital del Valle, Honduras, Honduras), Ho So (Chinese University of Hong Kong, Hong Kong), Márta Király (Petz Aladár University Teaching Hospital, Győr, Hungary), Mahdi Vojdanian (Iran Rheumatology Center, Iran), Alexandra Balbir-Gurman (Rambam Rheumatology Institute, Haifa, Israel), Fatemah Abutiban (Kuwait Rheumatology Association, Kuwait), Julija Zepa, Inita Bulina (Pauls Stradins Clinical University Hospital, Riga, Latvia), Loreta Bukauskiene (Klaipeda university hospital, Lithuania), Beatriz Elena Zazueta-Montiel (Centro Medico del Angel, Mexico), Angel Alejandro Castillo-Ortiz (Centro Medico Las Americas, Mexico), Erick Zamora-Tehozol (Centro Medico Pensiones, Mexico), David Vega-Morales (Hospital General de Zona #17, Instituto Nacional de Ciencias Médicas y Nutrición, Mexico), Diana Cervántes Rosete, Eduardo Martín-Nares, Tatiana Sofia Rodriguez-Reyna (Salvador Zubirán, Instituto Nacional de Ciencias Médicas y Nutrición, Mexico), Marina Rull Gabayet (Salvador Zubirán, Mexico), Deshiré Alpízar-Rodríguez (Mexican College of Rheumatology, Mexico), Fedra Irazoque (Private Practice, Mexico), Xochitl Jimenez (Centro Medico Naval, Mexico), Lenny Geurts-van Bon (Ziekenhuisgroep Twente, The Netherlands), Theo Zijlstra (Isala Hospital, Zwolle The Netherlands), Monique Hoekstra (Isala Hospital, Zwolle The Netherlands), Nasra Al-Adhoubi (Royal Hospital, Oman), Babur Salim (Fauji Foundation Hospital, Pakistan), Enrique Giraldo (Complejo Hospitalario, Panama), Ariel Salinas (Hospital Essalud Alberto Sabogal Sologuren, Universidad Científica del Sur-Hospital Guillermo, Peru), Manuel Ugarte-Gil (Almenara Irigoyen, Peru), Jarosław Nowakowski (University Hospital, Krakow, Poland), Samar Al-Emadi (Hamad Medical Corporation, Qatar), Richard Conway (St James' Hospital, Dublin, Republic of Ireland), Rachael Flood (Tallaght University Hospital, Republic of Ireland), Geraldine McCarthy (Mater Misericordiae University Hospital, Republic of Ireland), Ioana Felea, Ileana Filipescu, Simona Rednic (County Emergency Hospital, Cluj Napoca, Romania), Laura Groseanu (Sf Maria Clinical

Hospital, Bucharest, Romania), Maria Magdelena (Tamas County Emergency Hospital, Cluj Napoca, Romania), Vanda, Mlynarikova (National Institute of Rheumatic Diseases, Piešťany, Slovak Republic), Martina Skamlova (FNSPFDR, Banská Bystrica, Slovak Republic), Martin Zlnay, Dagmar Mičeková (National Institute of Rheumatic Diseases, Piešťany, Slovak Republic), Lubica Capova (University Hospital, Bratislava, Slovak Republic), Zelmira Macejova (University Hospital, Košice, Slovak Republic), Emőke Šteňová (University Hospital Bratislava, Slovak Republic), Helena Raffayova (National Institute of Rheumatic Diseases, Piešťany, Slovak Republic), Gabriela Belakova (Medman s.r.o., Martin, Slovak Republic), Eva Strakova (Faculty hospital Prešov, Slovak Republic), Marieta Senčarová (Louis Pasteur University Hospital, Košice, Slovak Republic), Soňa Žlnayová (Poliklinika MarMedico, s.r.o., Nové Mesto nad Váhom Slovak Republic súkromná reumatologická ambulancia, Vranov nad, Slovak Republic), Anna Sabová (Topľou, Slovak Republic), Daniela Spisakova (University Hospital od L. Pasteur Kosice, Slovak Republic), Mária Oetterová (Safarik University hospital, Kosice, Slovak Republic), Olga Lukacova (National Institute of Rheumatic Diseases, Piešťany, Slovak Republic), Martina Bakosova (UNB Nemocnica Stare Mesto, Bratislava, Slovak Republic), Alojzija Hocevar (UMC Ljubljana, Slovenia), Natalia de la Torre-Rubio (Hospital Universitario Puerta de Hierro Majadahonda, Spain), Juan José Alegre Sancho (Hospital Universitari Dr Peset, Valencia, Spain), Montserrat Corteguera Coro (Complejo Asistencial Avila, Spain), Juan Carlos Cobeta Garcia (Hospital Ernest Lluch, Calatayud, Spain), Maria Carmen Torres Martin (Hospital Nuestra Senora Sonsoles, Avila, Spain), Jose Campos (Hospital Universitario Puerta de Hierro, Spain), Jose A Gomez Puerta (Hospital Clinic Barcelona, Spain), Gozd Kubra Yardımcı, Servet Akar (Hacettepe University Faculty of Medicine, Ankara, Izmir Katip Celebi University Atatürk Training and Research Hospital, Izmir, Turkey), Ozan Cemal Icacan (Bakırköy Dr. Sadi Konuk Research And Training Hospital, Istanbul, Turkey), Selda ÇELİK (Bakirkoy Dr Sadi Konuk Educational And Research Hospital, Rheumatology Department, Turkey), Viktoriia Vasylets (Multifield Medical Centre, Odessa, Ukraine), Su-Ann Yeoh (University College London Hospital, London, UK), Claire Vandevelde (Leeds Teaching Hospitals NHS Trust, UK), Sasha Dunt (Countess of Chester NHS Foundation Trust, UK), Jane Leeder (Norfolk & Norwich University Hospital, UK), Elizabeth Macphie (Lancashire and South Cumbria NHS Foundation Trust, UK), Rosaria Salerno (King's College Hospital, UK), Christine Graver (Hampshire Hospitals NHS Trust, UK), Katie Williams (York District Hospital, UK), Sheila O'Reilly (Royal Derby Hospital, UK), Kirsty Devine (York/Scarborough Hospitals, UK), Jennifer Tyler (Royal United Hospital, Bath, UK), Elizabeth Warner (Lister Hospital, UK), James Pilcher (University Hospital Lewisham, UK), Samir Patel (Queen Elizabeth hospital Woolwich, UK), Elena Nikiphorou (King's College Hospital, UK), Laura Chadwick (St Helens & Knowsley NHS Foundation Trust, UK), Caroline Mulvaney Jones (Llandudno Hospital, UK), Beverley Harrison (Salford Royal NHS FT, UK), Lucy Thornton (Bradford Royal Infirmary, UK), Diana O'Kane (RNHRD at Royal United Hospital Bath, UK), Lucia Fusi (King's College Hospital, UK), Audrey Low (Salford Royal NHS FT, UK), Sarah Horton (Minerva Health Centre, UK), Shraddha Jatwani (Albert Einstein Medical Center, PA, USA), Sara Baig, Hammad Bajwa, Vernon Berglund, Angela Dahle, Walter Dorman, Jody Hargrove, Maren Hilton, Nicholas Lebedoff, Susan Leonard, Jennifer Morgan, Emily Pfeifer, Archibald Skemp, Jeffrey Wilson, Anne Wolff (Arthritis and Rheumatology Consultants, PA, USA), Eduardo Cepeda (Austin Diagnostic Clinic, USA), Derrick Todd (Brigham and Women's Hospital, USA), Denise Hare (Capital Health Rheumatology, USA), Cassandra Calabrese (Cleveland Clinic, USA), Christopher Adams (East Alabama Medical Center, USA), Arezou Khosroshahi (Emory University, USA), Adam Kilian (George Washington University, USA), Douglas White, Melanie Winter (Gundersen Health System, USA), Theodore Fields (Hospital for Special Surgery, USA), Caroline Siegel (Hospital for Special Surgery, USA), Nicole Daver (Institute of Rheumatic and Autoimmune Diseases, USA), Melissa Harvey (Institute of Rheumatic and Autoimmune Diseases, USA), Neil Kramer (Institute of Rheumatic and Autoimmune Diseases, USA), Concetta Lamore (Institute of Rheumatic and Autoimmune Diseases, USA), Suneya Hogarty (Integrative Arthritis and Pain Consultants, USA), Karen Yeter (Kaiser Permanente, USA), Leanna Wise (Los Angeles County + USC Medical Center, USA), Faizah Siddique (Loyola University Medical Center, USA), Byung Ban (Medstar Georgetown University Hospital, USA), Tamar Tanner (Montefiore Medical Center, USA), Eric Ruderman (Northwestern Memorial, USA), William Davis, Robert Quinet, Evangeline Scopelitis, Karen Toribio Toribio, Tameka Webb-Detiege, Jerald Zakem (Ochsner Medical Center Rheumatology Department, USA), Khurram Abbass, Gilbert Kepecs (Private Practice, USA), Lilliam Miranda (Rheumatology Center INC, USA), Michael Guma, Ammar Haikal, Sushama Mody (Riverside Medical Group, USA), Daric Mueller (Shores Rheumatology PC, USA), Arundathi Jayatilleke (Temple University Hospital, USA), JoAnn Zell (University of Colorado, USA), Alison Bays (University of Washington, Seattle, USA), Kathryn Dao, Ezzati Fatemeh (UT Southwestern Medical Center, USA), Deborah Parks (Washington University Div of Rheumatology, USA), David Karp, Guillermo Quiceno (UT Southwestern Medical Center, USA).

Contributors JAS, ZSW, AS, MG and JY had access to the study data, developed the figures and tables, and vouch for the data and analyses. AS and MG performed the statistical analyses and contributed to data quality control, data analysis and interpretation of data. JAS, ZSW, AMS, MG, ZI, KLH, AS, LG, LC, EFM, SL-T, LT, SR, PK, GS, LJ, SAE, LW, ELG, AD-G, MOV-A, GJP-E, CAI, GAB, TY-TH, KMD'S, NJP, PD, EN, VLK, NS, MFU-G, BW, AA, RT, SB, WC, RG, JH, JL, ES, PS, PCR, PMM and JY contributed to data collection, data analysis and interpretation of data. JAS, ZSW and JY directed the work, designed the data collection methods, contributed to data collection, data analysis and interpretation of data, and had final responsibility for the decision to submit for publication. All authors contributed intellectual content during the drafting and revision of the work and approved the final version to be published.

Funding The study received support from the American College of Rheumatology (ACR) and European Alliance of Associations for Rheumatology (EULAR).

Disclaimer The views expressed here are those of the authors and the participating members of the COVID-19 Global Rheumatology Alliance and do not necessarily represent the views of the American College of Rheumatology (ACR), the European Alliance of Associations for Rheumatology (EULAR), the (UK) National Health Service (NHS), the National Institute for Health Research (NIHR) or the (UK) Department of Health, or any other organisation.

Competing interests JAS is supported by the National Institute of Arthritis and Musculoskeletal and Skin Diseases (grant numbers K23 AR069688, R03 AR075886, L30 AR066953, P30 AR070253 and P30 AR072577), the Rheumatology Research Foundation (K Supplement Award and R Bridge Award), the Brigham Research Institute, and the R Bruce and Joan M Mickey Research Scholar Fund. JAS has received research support from Amgen and Bristol-Myers Squibb and performed consultancy for Bristol-Myers Squibb, Gilead, Inova, Janssen and Optum, unrelated to this work. ZSW reports grant support from Bristol-Myers Squibb and Principia/Sanofi and performed consultancy for Viela Bio and MedPace, outside the submitted work. His work is supported by grants from the National Institutes of Health. MG is supported by the National Institute of Arthritis and Musculoskeletal and Skin Diseases (grant numbers K01 AR070585 and K24 AR074534; JY). KLH reports she has received speaker's fees from AbbVie and grant income from BMS, UCB and Pfizer, all unrelated to this study. KLH is also supported by the NIHR Manchester Biomedical Research Centre. LC has not received fees or personal grants from any laboratory, but her institute works by contract for laboratories such as, among other institutions, AbbVie Spain, Eisai, Gebro Pharma, Merck Sharp & Dohme España, Novartis Farmaceutica, Pfizer, Roche Farma, Sanofi Aventis, Astellas Pharma, Actelion Pharmaceuticals España, Grünenthal and UCB Pharma. LG reports research grants from Amgen, Galapagos, Janssen, Lilly, Pfizer, Sandoz and Sanofi; consulting fees from AbbVie, Amgen, BMS, Biogen, Celgene, Galapagos, Gilead, Janssen, Lilly, Novartis, Pfizer, Samsung Bioepis, Sanofi Aventis and UCB, all unrelated to this study. EFM reports that LPCDR received support for specific activities: grants from AbbVie, Novartis, Janssen-Cilag, Lilly Portugal, Sanofi, Grünenthal, MSD, Celgene, Medac, Pharma Kern and GAfPA; grants and non-financial support from Pfizer; and non-financial support from Grünenthal, outside the submitted work. AS reports grants from a consortium of 13 companies (among them AbbVie, BMS, Celltrion, Fresenius Kabi, Lilly, Mylan, Hexal, MSD, Pfizer, Roche, Samsung, Sanofi Aventis and UCB) supporting the German RABBIT register, and personal fees from lectures for AbbVie, MSD, Roche, BMS and Pfizer, outside the submitted work. AD-G has no disclosures relevant to this study. His work is supported by grants from the Centers for Disease Control and Prevention and the Rheumatology Research Foundation. KMD is supported by the National Institute of Arthritis and Musculoskeletal and Skin Diseases (T32-AR-007258) and the Rheumatology Research Foundation. NJP is supported by the National Institute of Arthritis and Musculoskeletal and Skin Diseases (T32-AR-007258). PD has received research support from Bristol-Myers Squibb, Chugai and Pfizer, and performed consultancy for Boehringer Ingelheim, Bristol-Myers Squibb, Lilly, Sanofi, Pfizer, Chugai, Roche and Janssen, unrelated to this work. NS is supported by the RRF Investigator Award and the American Heart Association. MFU-G reports grant support from Janssen and Pfizer. SB reports no competing interests related to this work. He reports non-branded consulting fees for AbbVie, Horizon, Novartis and Pfizer (all <$10 000). RG reports no competing interests related to this work. Outside of this work she reports personal and/or speaking fees from AbbVie, Janssen, Novartis, Pfizer and Cornerstones, and travel assistance from Pfizer (all <$10 000). JH reports no competing interests related to this work. He is supported by grants from the Rheumatology Research Foundation and the Childhood Arthritis and Rheumatology Research Alliance. He has performed consulting for Novartis, Sobi and Biogen, all unrelated to this work (<$10 000). JL has received research funding from Pfizer, outside the submitted work. ES is a Board Member of the Canadian Arthritis Patient Alliance, a patient-run, volunteer-based organisation whose activities are largely supported by independent grants from pharmaceutical companies. PS reports no competing interests related to this work. He reports honorarium for doing social media for American College of Rheumatology journals (<$10 000). PMM has received consulting/speaker's fees from AbbVie, BMS, Celgene, Eli Lilly, Janssen, MSD, Novartis, Pfizer, Roche and UCB, all unrelated to this study (all <$10 000). PMM is supported by the National Institute for Health Research (NIHR) University College London Hospitals (UCLH) Biomedical Research Centre (BRC). PCR reports no competing interests related to this work. Outside of this work he reports personal consulting and/or speaking fees from AbbVie, Eli Lilly, Janssen, Novartis, Pfizer and UCB, and travel assistance from Roche (all <$10 000). JY reports no competing interests related to this work. Her work is supported by grants from the National Institutes of Health, Centers for Disease Control, and the

Agency for Healthcare Research and Quality. She has performed consulting for Eli Lilly and AstraZeneca, unrelated to this project.

Patient consent for publication Not required.

Ethics approval The C19-GRA physician-reported registry was determined 'not human subjects' research' by the UK Health Research Authority and the University of Manchester, as well as under US Federal Guidelines assessed by the University of California, San Francisco Institutional Review Board. Due to the de-identified and non-interventional nature of the study, it was determined to be exempt by each institutional review board.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. Applications to access the data should be made to the C19-GRA Steering Committee.

ORCID iDs
Jeffrey A Sparks http://orcid.org/0000-0002-5556-4618
Kimme L Hyrich http://orcid.org/0000-0001-8242-9262
Anja Strangfeld http://orcid.org/0000-0002-6233-022X
Laure Gossec http://orcid.org/0000-0002-4528-310X
Loreto Carmona http://orcid.org/0000-0002-4401-2551
Saskia Lawson-Tovey http://orcid.org/0000-0002-8611-162X
Ali Duarte-García http://orcid.org/0000-0003-1749-5719
Guillermo J Pons-Estel http://orcid.org/0000-0002-0647-929X
Tiffany Y -T Hsu http://orcid.org/0000-0003-1041-8040
Kristin M D'Silva http://orcid.org/0000-0001-8370-4166
Lianne Kearsley-Fleet http://orcid.org/0000-0003-0377-1575
Sandra Lúcia Euzébio Ribeiro http://orcid.org/0000-0002-4777-8659
Carlo Alberto Scirè http://orcid.org/0000-0001-7451-0271
Rebecca Hasseli http://orcid.org/0000-0002-2982-8253
Hendrik Schulze-Koops http://orcid.org/0000-0002-1681-491X
Christof Specker http://orcid.org/0000-0003-2504-3229
Licia Maria Henrique da Mota http://orcid.org/0000-0002-8182-5121
Ana Paula Monteiro Gomides http://orcid.org/0000-0003-2884-2210
Philippe Dieudé http://orcid.org/0000-0002-4814-0307
Elena Nikiphorou http://orcid.org/0000-0001-6847-3726
Vanessa L Kronzer http://orcid.org/0000-0002-7489-3134
Namrata Singh http://orcid.org/0000-0001-7149-363X
Manuel F Ugarte-Gil http://orcid.org/0000-0003-1728-1999
Akpabio Akpabio http://orcid.org/0000-0002-4920-6494
Ranjeny Thomas http://orcid.org/0000-0002-0518-8386
Jonathan S Hausmann http://orcid.org/0000-0003-0786-8788
Emily Sirotich http://orcid.org/0000-0002-7087-8543
Philip C Robinson http://orcid.org/0000-0002-3156-3418
Pedro M Machado http://orcid.org/0000-0002-8411-7972

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CLINICAL SCIENCE

Efficacy and safety of tildrakizumab in patients with active psoriatic arthritis: results of a randomised, double-blind, placebo-controlled, multiple-dose, 52-week phase IIb study

Philip J Mease ,[1,2] Saima Chohan,[3] Ferran J Garcia Fructuoso,[4] Michael E Luggen,[5,6] Proton Rahman,[7] Siba P Raychaudhuri ,[8,9] Richard C Chou,[10] Alan M Mendelsohn,[11] Stephen J Rozzo,[11] Alice Gottlieb[12]

Handling editor Josef S Smolen

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2020-219014).

For numbered affiliations see end of article.

Correspondence to
Dr Philip J Mease, Rheumatology Research, Swedish Medical Center, Seattle, Washington, USA; pmease@philipmease.com

Received 31 August 2020
Revised 29 January 2021
Accepted 1 February 2021
Published Online First 13 May 2021

To cite: Mease PJ, Chohan S, Fructuoso FJG, *et al*. *Ann Rheum Dis* 2021;**80**:1147–1157.

ABSTRACT

Objectives To evaluate efficacy and safety of the anti-interleukin-23p19 monoclonal antibody tildrakizumab in patients with psoriatic arthritis (PsA).

Methods In this randomised, double-blind, placebo-controlled, phase IIb study, patients with active PsA were randomised 1:1:1:1:1 to tildrakizumab 200 mg every 4 weeks (Q4W); tildrakizumab 200, 100 or 20 mg Q12W; or placebo Q4W. Patients receiving tildrakizumab 20 mg or placebo switched to tildrakizumab 200 mg Q12W at W24; treatment continued to W52. The primary efficacy endpoint was proportion of patients with ACR20 response (≥20% improvement by American College of Rheumatology criteria) at W24. Secondary efficacy endpoints were assessed without adjustment for multiplicity. Safety was evaluated from treatment-emergent adverse events (TEAEs).

Results 391/500 patients screened were randomised and treated. At W24, 71.4%–79.5% of tildrakizumab-treated versus 50.6% of placebo-treated patients achieved ACR20 (all p<0.01). Patients receiving tildrakizumab versus placebo generally achieved higher rates of ACR50, Disease Activity Score in 28 joints with C reactive protein <3.2, minimal disease activity and 75%/90%/100% improvement from baseline Psoriasis Area and Severity Index responses at W24 and through W52. Improvement in dactylitis and enthesitis was not observed; results were mixed for other outcomes. Responses in patients switched to tildrakizumab 200 mg at W24 were consistent with treatment from baseline. TEAEs and serious TEAEs occurred in 64.5% and 3.3%, respectively, of all patients through W52 and were comparable among treatment arms.

Conclusions Tildrakizumab treatment significantly improved joint and skin manifestations of PsA other than dactylitis and enthesitis. Treatment was generally well tolerated through W52. Clinicaltrials.gov NCT02980692.

Key messages

What is already known about this subject?

- There is an unmet need for psoriatic arthritis (PsA) therapies that maximally address all clinical manifestations of the disease and improve patient quality of life.
- Interleukin (IL)-23 is a key regulatory cytokine in the pathogenesis of PsA, and the p19 subunit of IL-23 is an effective therapeutic target for PsA in clinical studies; tildrakizumab is a high-affinity anti-IL-23p19 monoclonal antibody approved in the USA, Europe, Australia and Japan for treatment of plaque psoriasis.

What does this study add?

- This study demonstrates that tildrakizumab was superior to placebo in achieving ACR20 (≥20% improvement by American College of Rheumatology criteria) and ACR50 responses, minimal disease activity; Disease Activity Score in 28 joints with C reactive protein <3.2 and ≥75% improvement from baseline Psoriasis Area and Severity Index (PASI 75), PASI 90 and PASI 100 responses at week 24; response rates were sustained through week 52. Improvement in dactylitis and enthesitis was not observed, and results for other outcomes were mixed among patients receiving different doses of tildrakizumab.
- Tildrakizumab was generally well tolerated with no reports of uveitis, systemic fungal infections, inflammatory bowel disease, major adverse cardiac events or deaths through week 52.

How might this impact on clinical practice or future developments?

- These findings support the efficacy and safety of tildrakizumab in patients with PsA and the planned dosing schedules in the ongoing phase III clinical programme.

INTRODUCTION

Psoriatic arthritis (PsA) is a chronic, progressive, inflammatory arthritis with estimated global prevalence of 0.2%–0.3%.[1–4] Manifestations of PsA include musculoskeletal and skin disease activity; pain; fatigue; systemic inflammation and their effects on physical function, activities of daily living and health-related quality of life (QoL).[3 5 6] Chronic joint inflammation and

potential joint damage from PsA can impose considerable economic burden.[5] There is an unmet need for therapies that address all clinical manifestations of PsA and improve patient QoL.

Treatments for PsA include non-pharmacological therapies, non-steroidal anti-inflammatory drugs, conventional systemic disease modifying antirheumatic drugs (csDMARDs; including methotrexate, sulfasalazine and leflunomide), biological DMARDs (bDMARDs) and targeted synthetic DMARDs (including Janus-associated kinase and phosphodiesterase inhibitors).[7–9] Treatment guidelines recommend csDMARDs before other therapies,[8] bDMARDs targeting tumour necrosis factor α (TNFα) before csDMARDs,[9] or either approach.[7]

Interleukin (IL)-23 is a key regulatory cytokine in PsA pathogenesis.[10 11] Targeting the IL-23/IL-12 p40 subunit with ustekinumab was effective and generally well tolerated in PsA clinical trials.[12 13] The anti-IL-23p19 subunit antibody guselkumab, which targets IL-23 alone, was also effective and is approved in the USA for treatment of signs and symptoms of PsA.[14–17] Neither agent provided incremental improvement over TNFα inhibitors. Tildrakizumab, a high-affinity anti-IL-23p19 monoclonal antibody, is approved in the USA, Europe, Australia and Japan for treatment of plaque psoriasis.[18–24] This phase IIb study evaluated tildrakizumab efficacy and safety in patients with PsA at week 24 and through week 52 (clinicaltrials.gov NCT02980692).

METHODS

Study design

This phase IIb, randomised, double-blind, multidose, placebo-controlled, multicentre study was conducted at 74 sites (including hospital dermatology units, specialty clinics, private practices and research sites) in 8 countries. All patients provided written informed consent. In part 1 (weeks 0–24), patients were randomised 1:1:1:1:1 to receive subcutaneous tildrakizumab 200 mg every 4 weeks (Q4W); tildrakizumab 200, 100 or 20 mg every 12 weeks (Q12W) or placebo Q4W (online supplemental figure S1). At week 24, patients receiving tildrakizumab 20 mg or placebo switched to tildrakizumab 200 mg Q12W. All treatments continued in part 2 (weeks 25–52, double-blind follow-up), followed by a 20-week washout period (to week 72) or rollover to the long-term extension (clinicaltrials.gov NCT03552276). This publication reports efficacy and safety outcomes for patients on treatment (through week 52).

All patients received study drug or placebo Q4W to maintain the blind through week 52; placebo was administered between tildrakizumab doses for patients receiving Q12W dosing. Randomised patients were stratified by prior anti-TNFα therapy use (yes/no; prior anti-TNFα use capped at 30% of total patients) and baseline body weight (≤90 kg/>90 kg). Randomisation was computer generated before the study; patients were allocated to treatment arms using an interactive voice recognition service (ICON Clinical Research, Dublin, Ireland). Patients without minimal response to treatment (<10% improvement from baseline swollen joint count in 66 joints (SJC66) and tender joint count in 68 joints (TJC68)) at week 16 could adjust background medications per maximum permitted dosing.

Patients

Eligible patients were ≥18 years old, with a diagnosis of PsA by the Classification Criteria for Psoriatic Arthritis for ≥6 months[25] and had TJC68 ≥3 and SJC66 ≥3 according to an independent assessor. Allowed and prohibited medications are described in online supplemental methods.

Efficacy assessments

The primary efficacy endpoint was proportion of patients with an ACR20 response (≥20% improvement by American College of Rheumatology criteria) at week 24. Prespecified secondary endpoints included proportions

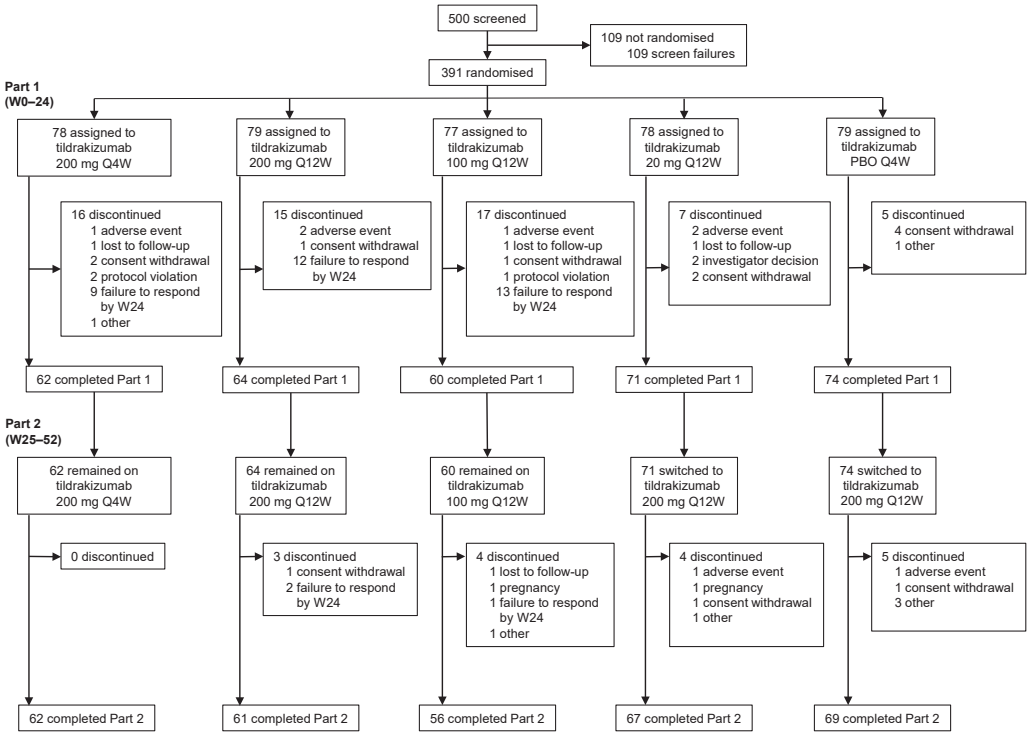

Figure 1 Patient status to week 52. PBO, placebo; Q4W, every 4 weeks; Q12W, every 12 weeks; W, week.

Table 1 Demographics and baseline clinical disease characteristics

	TIL 200 mg Q4W (n=78)	TIL 200 mg Q12W (n=79)	TIL 100 mg Q12W (n=77)	TIL 20→200 mg Q12W (n=78)	PBO Q4W→TIL 200 mg Q12W (n=79)
Demographics					
Age, years	50.1±13.3	49.3±11.2	49.2±11.9	47.2±13.4	48.1±13.3
Female, n (%)	46 (59.0)	37 (46.8)	47 (61.0)	41 (52.6)	44 (55.7)
Race, n (%)					
White	76 (97.4)	78 (98.7)	75 (97.4)	75 (96.2)	74 (93.7)
Black or African American	0	0	1 (1.3)	1 (1.3)	3 (3.8)
Other	2 (2.6)	1 (1.3)	1 (1.3)	2 (2.6)	2 (2.5)
Weight, kg	85.1±19.7	87.2±19.5	83.7±18.9	85.2±18.1	85.3±20.2
BMI, kg/m^2	30.1±6.5	30.2±6.5	29.5±6.8	29.4±5.2	29.5±6.0
Baseline disease characteristics					
Duration of PsA, years	7.5±8.5	6.2±7.2	7.0±6.6	6.6±6.7	6.3±6.1
Prior anti-TNFα therapy, n (%)*	18 (22.8)	17 (21.8)	19 (23.8)	19 (24.4)	18 (23.7)
Concomitant antirheumatic medications, n (%)					
Methotrexate†	44 (56.4)	47 (59.5)	49 (63.6)	42 (53.8)	47 (59.5)
Dose, mg	16.5±5.3	15.0±3.8	14.3±4.8	16.7±5.5	16.9±5.0
Leflunomide	2 (2.6)	3 (3.8)	2 (2.6)	5 (6.4)	3 (3.8)
Leflunomide+prednisone/prednisolone	0	0	0	0	1 (1.3)
Sulfasalazine	0	0	1 (1.3)	0	0
Prednisolone	0	1 (1.3)	0	1 (1.3)	0
Sulfasalazine+leflunomide	0	1 (1.3)	0	0	0
Swollen joint count	10.4±7.4	10.0±8.0	11.0±8.2	9.4±6.4	11.8±9.8
Tender joint count	16.6±11.9	19.5±13.9	21.3±14.8	19.0±13.0	19.7±14.7
PtGA	57.8±18.3	61.1±20.7	60.3±20.2	61.9±17.4	65.2±18.1
PGA	54.0±16.1	55.4±16.2	57.3±17.3	59.4±14.4	59.5±15.6
Patient pain assessment	55.4±19.1	59.6±23.5	59.2±22.1	60.9±19.7	64.2±20.4
HAQ-DI score	1.0±0.6	1.0±0.6	1.0±0.7	1.1±0.6	1.2±0.6
hsCRP, mg/dL	7.8±18.6	10.5±14.4	10.6±20.0	10.7±14.0	13.0±20.8
DAS28-CRP <3.2, n (%)	6 (7.7)	6 (7.6)	4 (5.2)	1 (1.3)	6 (7.6)
DAPSA	39.2±20.2	42.6±22.1	45.3±22.4	41.8±17.8	45.7±23.5
PASDAS	5.2±0.86	5.2±0.78	5.3±0.89	5.3±0.85	5.4±0.89
LEI‡	3.1±1.7	2.8±1.7	3.2±1.8	3.1±1.7	2.8±1.8
LDI‡	32.8±32.9	61.3±73.5	93.8±146.5	71.4±118.5	99.6±170.7
BSA, (%)§	11.9±16.0	9.0±12.4	13.1±16.0	10.4±14.1	8.2±12.2
BSA ≥3%, n (%)§	53 (67.9)	44 (55.7)	55 (71.4)	41 (52.6)	42 (53.2)
PASI¶	7.6±9.8	6.2±7.4	8.8±9.5	6.6±7.0	5.0±6.5
PsAID score	5.1±1.8	5.3±2.1	5.5±2.1	5.6±1.9	5.7±1.6

Shown for randomised patients who received ≥1 dose of study drug; data shown as mean±SD unless otherwise noted.
*For prior anti-TNFα therapy, total patients analysed (N)=79, 78, 80, 78 and 76 for TIL 200 mg Q4W, TIL 200 mg Q12W, TIL 100 mg, TIL 20 mg and PBO, respectively.
†Patients receiving weekly oral methotrexate at baseline. No patients received concomitant methotrexate in combination with prednisone or prednisolone.
‡For patients with baseline scores ≥1; N=48, 43, 51, 55 and 43 for LEI; N=27, 21, 21, 19 and 25 for LDI.
§Body surface area with psoriasis lesions; BSA ≥3% indicates active psoriasis.
¶For analysis of baseline PASI, all patients were analysed, regardless of % BSA involved; N=75, 79, 76, 75 and 75 for TIL 200 mg Q4W, TIL 200 mg Q12W, TIL 100 mg, TIL 20 mg and PBO.
BMI, body mass index; BSA, body surface area; DAS28-CRP, Disease Activity Score in 28 joints with C reactive protein; HAQ-DI, Health Assessment Questionnaire-Disability Index; hsCRP, high sensitivity C reactive protein; LDI, Leeds Dactylitis Index; LEI, Leeds Enthesitis Index; PASI, Psoriasis Area and Severity Index; PBO, placebo; PGA, physician global assessment of disease activity; PsA, psoriatic arthritis; PsAID, PsA impact of disease; PtGA, patient global assessment of disease activity; Q4W, every 4 weeks; Q12W, every 12 weeks; TIL, tildrakizumab; TNF, tumour necrosis factor.

of patients achieving ACR20 at week 52 and ACR50, ACR70, Disease Activity Score in 28 joints with C reactive protein (DAS28-CRP) <3.2 and minimal disease activity (MDA) at weeks 24 and 52 or requiring background medication adjustment at week 24; and change from baseline in individual ACR components, Leeds Dactylitis Index (LDI; in patients with baseline LDI ≥1), Leeds Enthesitis Index (LEI; in patients with baseline LEI ≥1) and Health Assessment Questionnaire-Disability Index (HAQ-DI) at weeks 24 and 52. Patients achieved MDA if they met 5 of 7 criteria—TJC68 ≤1, SJC66 ≤1, Psoriasis Area and Severity Index (PASI) ≤1 or body surface area (BSA) ≤3%, patient pain visual analogue scale (VAS) ≤15, patient global disease activity VAS ≤20, HAQ-DI ≤0.5 and tender entheseal points ≤1. Proportions of patients achieving 75%/90%/100% improvement from baseline PASI (PASI 75/90/100) for patients with measurable psoriasis (baseline affected BSA ≥3%) and PsA Impact of Disease (PsAID)[26] change from baseline at weeks 24 and 52 were exploratory efficacy endpoints. Post hoc analyses (online supplemental methods) included proportions of patients achieving very low disease activity (VLDA), Psoriatic Arthritis Disease Activity Score (PASDAS) <3.2, Disease Activity in Psoriatic Arthritis (DAPSA) remission (score 0–4), complete LDI/LEI resolution and minimum clinically important difference (MCID) change from baseline HAQ-DI (≥0.35) and PsAID (≥3) at weeks 24 and 52; DAPSA/PASDAS change from baseline; and median LDI/LEI.[27] The TJC, SJC, LDI/LEI and PASI assessments were performed by an independent assessor. Other assessment details are summarised in online supplemental methods.

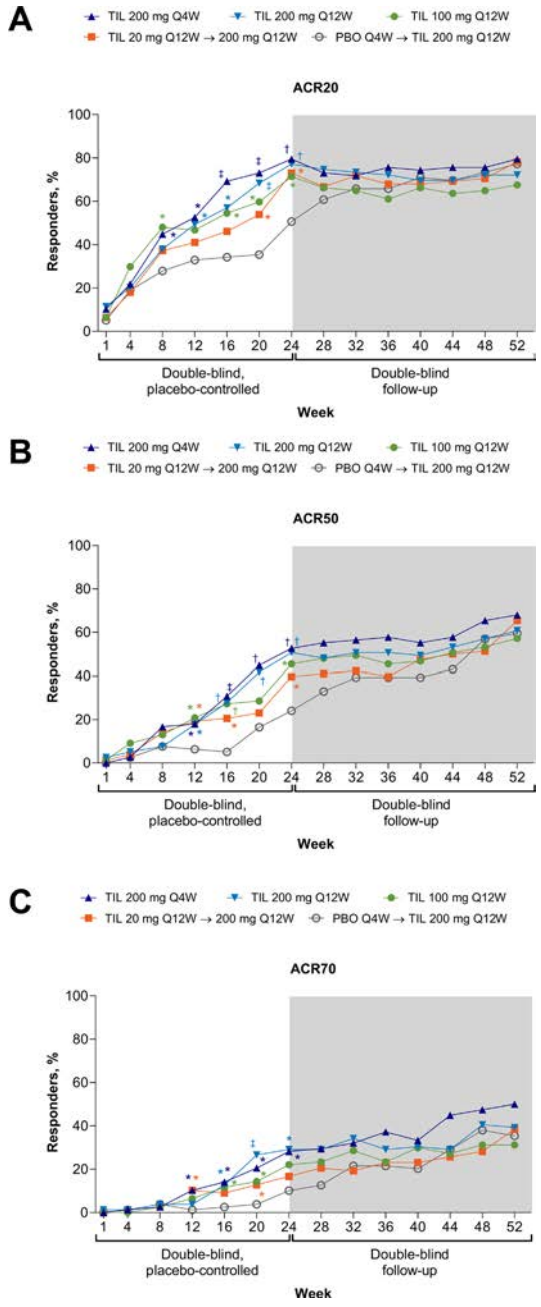

Figure 2 Response rates for (A) ACR20, (B) ACR50 and (C) ACR70 through week 52. Supporting values shown in online supplemental table S3. Missing responses were imputed as non-responses. Shown for randomised patients who received ≥1 dose of study drug. TIL 200 mg Q4W, n=78; TIL 200 mg Q12W, n=79; TIL 100 mg Q12W, n=77; TIL 20 mg Q12W→200 mg Q12W, n=78; PBO Q4W→TIL 200 mg Q12W, n=79. *p<0.05; †p<0.001; ‡p<0.0001 versus PBO; not adjusted for multiplicity, except ACR20 at week 24. P values were not analysed beyond week 24. ACR, American College of Rheumatology; PBO, placebo; Q4W, every 4 weeks; Q12W, every 12 weeks; TIL, tildrakizumab.

Safety assessments

Safety endpoints included treatment-emergent adverse events (TEAEs), serious TEAEs and TEAEs of special and clinical interest. TEAEs were coded by Medical Dictionary of Regulatory Activities V.20.1 and defined as any AE occurring or worsening on/after the day of first dose of study drug up to week 52 or on/before last dosing date if the patient discontinued treatment. TEAEs of special interest were severe infection, malignancy (including non-melanoma and melanoma skin cancer), confirmed major adverse cardiovascular event (MACE) or drug-related hypersensitivity reaction (details in online supplemental methods). TEAEs of clinical interest included any non-serious TEAE considered of special interest and reported to the sponsor similarly to a serious TEAE (details in online supplemental methods). Routine laboratory investigations and physical examinations were performed, and vital signs were monitored at screening and throughout the study. To ensure patient safety, an independent data safety monitoring board routinely reviewed data and provided the sponsor with recommendations.

Statistical analyses

The number of patients enrolled was based on assumed ACR20 response rates of 30%, 35% and 50% for placebo, tildrakizumab 20 mg, and higher doses of tildrakizumab, respectively; a one-sided alpha=0.05; 80.7% power and a 5% dropout rate. All analyses were performed using SAS V.9.4 or later.[28] Efficacy and safety analyses included all randomised patients who received ≥1 dose of study drug or placebo (full analysis set). Statistical comparison of ACR20/50/70, PASI 75/90/100 and MDA response rates between tildrakizumab arms versus placebo used the Cochran-Mantel-Haenszel test, stratified by prior anti-TNFα therapy use and baseline body weight. Two-sided 95% CIs and p values were calculated for each tildrakizumab treatment arm versus placebo. Non-response imputation (NRI) was used for patients who withdrew from the study or had incomplete data at week 52 unless otherwise specified. Patients who required adjustments to background medications were counted as non-responders for the primary analysis. Continuous endpoints were analysed by mixed-model repeated measure analysis with fixed effects of treatment, visit, treatment by visit interaction, prior anti-TNFα therapy use (yes/no), baseline bodyweight (≤90 kg/>90 kg) and baseline value; missing data were imputed using NRI. For the primary endpoint of ACR20 response at week 24, Type I error was controlled using the Simes testing procedure (online supplemental methods); there was no multiplicity adjustment for secondary endpoints. No formal hypothesis testing was performed for post hoc analyses.

Patient and public involvement

Patients and the public were not involved in study design, recruitment or dissemination of results, and patients were not asked to assess the burden of study participation.

RESULTS

Patients

From 19 April 2017, to 25 April 2018, 500 patients were screened, of whom 391 were randomised to tildrakizumab 200 mg Q4W (n=78), tildrakizumab 200 mg Q12W (n=79), tildrakizumab 100 mg Q12W (n=77), tildrakizumab 20→200 mg Q12W (n=78) and placebo Q4W→tildrakizumab 200 mg Q12W (n=79) (figure 1). Overall, 331 (84.7%) patients completed part 1 and 315 (80.6%) completed part 2. By week 52, 76 (19.4%) patients discontinued, most commonly due to lack of efficacy (9.5%) or withdrawn consent (3.3%). The last follow-up was on 5 October 2019.

Demographics were comparable between treatment arms (table 1). Of patients analysed, 91 (23.3%) were anti-TNFα therapy-experienced. Across treatment arms, mean duration of PsA was 6–7.5 years, 53%–71% of patients had moderate-to-severe psoriasis (BSA ≥3%) and >60% of patients were

Table 2 Efficacy outcomes at week 24

	TIL 200 mg Q4W (n=78)	TIL 200 mg Q12W (n=79)	TIL 100 mg Q12W (n=77)	TIL 20 mg Q12W (n=78)	PBO Q4W (n=79)
Primary efficacy endpoint					
ACR20	79.5±4.6 (0.0001)*	77.2±4.7 (0.0006)*	71.4±5.2 (0.0088)*	73.1±5.0 (0.0041)*	50.6±5.6
Secondary efficacy endpoints and related analyses					
ACR50	52.6±5.7 (0.0002)	50.6±5.6 (0.0006)	45.5±5.7 (0.0059)	39.7±5.5 (0.0364)	24.1±4.8
ACR70	28.2±5.1 (0.0040)	29.1±5.1 (0.0033)	22.1±4.7 (0.0550)	16.7±4.2 (0.2495)	10.1±3.4
ACR components					
TJC68, LSM CFB±SE	−10.8±1.1 (0.1704)	−11.8±1.1 (0.0448)	−12.4±1.1 (0.0174)	−10.7±1.1 (0.2037)	−8.8±1.1
SJC66, LSM CFB±SE	−7.6±0.56 (0.0476)	−7.2±0.56 (0.1149)	−7.9±0.57 (0.0153)	−6.8±0.56 (0.2916)	−6.0±0.56
PtGA, LSM CFB±SE	−31.3±2.3 (0.0005)	−30.9±2.3 (0.0007)	−31.1±2.4 (0.0006)	−26.9±2.3 (0.0321)	−20.0±2.3
PGA, LSM CFB±SE	−32.7±2.1 (0.0002)	−36.2±2.0 (<0.0001)	−35.4±2.1 (<0.0001)	−32.5±2.1 (0.0002)	−21.9±2.1
Patient pain assessment, LSM CFB±SE	−31.7±2.7 (0.0029)	−30.4±2.6 (0.0080)	−30.3±2.7 (0.0091)	−25.7±2.7 (0.1672)	−20.6±2.6
HAQ-DI, LSM CFB±SE	−0.3±0.05 (0.1829)	−0.3±0.05 (0.0420)	−0.3±0.05 (0.0467)	−0.2±0.05 (0.7267)	−0.2±0.05
Improvement ≥0.35†‡	5.9±2.9	5.9±2.9	1.7±1.7	7.4±3.2	5.6±2.7
hsCRP, mg/L, LSM CFB±SE§	−4.4±1.1 (0.0003)	−2.8±1.0 (0.0098)	−3.6±1.0 (0.0019)	−2.4±1.1 (0.0245)	0.79±1.0
DAS28-CRP <3.2	59.0±5.6 (0.0003)	64.6±5.4 (<0.0001)	58.4±5.6 (0.0005)	53.9±5.6 (0.0034)	30.4±5.2
MDA	33.3±5.3 (<0.0001)	34.2±5.3 (<0.0001)	28.6±5.2 (0.0004)	19.2±4.5 (0.0172)	6.3±2.7
Tender joint count ≤1	30.8±5.2 (0.0107)	30.4±5.2 (0.0152)	18.2±4.4 (0.4556)	20.5±4.6 (0.2939)	13.9±3.9
Swollen joint count ≤1	53.9±5.6 (0.0006)	55.7±5.6 (0.0002)	57.1±5.6 (0.0002)	50.0±5.7 (0.0030)	26.6±5.0
VLDA†	15.4±4.1	16.5±4.2	6.5±2.8	6.4±2.8	1.3±1.3
LDI, LSM CFB±SE¶	−46.7±6.5 (0.1983)	−45.4±7.3 (0.1750)	−45.2±7.2 (0.1692)	−45.6±7.7 (0.1950)	−58.5±6.8
LDI, median (Q1, Q3)†, ¶	16.6 (3.1, 28.6)	21.5 (0, 28.3)	19.4 (6.0, 32.1)	10.5 (0.03, 33.8)	3.6 (0, 26.3)
LEI, LSM CFB±SE**	−1.8±0.23 (0.1196)	−1.6±0.25 (0.3496)	−1.8±0.23 (0.1541)	−1.6±0.22 (0.4778)	−1.3±0.25
LEI, median (Q1, Q3)†, **	0 (0, 2.0)	0 (0, 2.0)	1.0 (0, 2.0)	1.0 (0, 3.0)	1.0 (0, 2.0)
LDI/LEI=0†, ††	–	11.1±10.5	14.3±9.4	12.5±8.3	17.7±9.3
Background medication adjustment required, n (%)	1 (1.3)	0	0	0	1 (1.3)
Other exploratory and post hoc analyses					
DAPSA, LSM CFB±SE†	−25.1±1.8	−25.5±1.8	−27.0±1.8	−23.1±1.8	−19.3±1.8
PASDAS, LSM CFB±SE†	−1.5±0.1	−1.5±0.1	−1.5±0.1	−1.4±0.1	−1.0±0.1
PsAID, LSM CFB±SE	−2.1±0.2 (0.0048)	−2.3±0.2 (0.0002)	−2.2±0.2 (0.0010)	−2.0±0.2 (0.0131)	−1.3±0.2
Decrease by ≥3†	30.8±5.2	31.7±5.2	32.5±5.3	37.2±5.5	29.1±5.1

Data are shown as response rate (%)±SE unless otherwise noted; numbers in parentheses indicate p values unless otherwise noted. Missing responses were imputed as non-responses.
Some post hoc analyses are grouped with the related secondary endpoint for ease of reading.
*Statistically significant. P values for other comparisons are not multiplicity-controlled and are presented for informational purposes only.
†Post hoc analysis; no formal hypothesis testing was performed.
‡Improvement in HAQ-DI scores was assessed in patients with baseline HAQ-DI score ≥0.35; tildrakizumab 200 mg Q4W, n=68; tildrakizumab 200 mg Q12W, n=68; tildrakizumab 100 mg Q12W, n=58; tildrakizumab 20 mg Q12W, n=68; placebo Q4W, n=72.
§hsCRP change from baseline reported for tildrakizumab 200 mg Q4W, n=71; tildrakizumab 200 mg Q12W, n=76; tildrakizumab 100 mg Q12W, n=73; tildrakizumab 20 mg Q12W, n=71; placebo Q4W, n=74.
¶LDI change from baseline is reported in patients with baseline LDI ≥1; tildrakizumab 200 mg Q4W, n=27; tildrakizumab 200 mg Q12W, n=21; tildrakizumab 100 mg Q12W, n=21; tildrakizumab 20 mg Q12W, n=19; placebo Q4W, n=25.
**LEI change from baseline is reported in patients with baseline LEI ≥1; tildrakizumab 200 mg Q4W, n=48; tildrakizumab 200 mg Q12W, n=43; tildrakizumab 100 mg Q12W, n=51; tildrakizumab 20 mg Q12W, n=55; placebo Q4W, n=43.
††Complete resolution for both LDI and LEI is reported in patients with both LDI and LEI ≥1 at baseline; tildrakizumab 200 mg Q4W, n=0; tildrakizumab 200 mg Q12W, n=9; tildrakizumab 100 mg Q12W, n=14; tildrakizumab 20 mg Q12W, n=16; placebo Q4W, n=17.
ACR, American College of Rheumatology response criteria; DAPSA, disease activity in psoriatic arthritis; DAS28-CRP, Disease Activity Score in 28 joints with C reactive protein; HAQ-DI, Health Assessment Questionnaire-Disability Index; LDI, Leeds Dactylitis Index; LEI, Leeds Enthesitis Index; LSM, least squares mean; MDA, minimal disease activity; PASDAS, psoriatic arthritis disease activity score; PBO, placebo; PGA, physician's global assessment; PsAID, psoriatic arthritis impact of disease; PtGA, patient's general assessment; Q1, 25th percentile; Q3, 75th percentile; Q4W, every 4 weeks; Q12W, every 12 weeks; SJC66, swollen joint count in 66 joints; TIL, tildrakizumab; TJC68, tender joint count in 68 joints; VLDA, very low disease activity.

receiving csDMARDs with or without corticosteroids at baseline (table 1). Baseline TJC68, patient global assessment (PtGA), physician global assessment (PGA) and patient pain assessment were lower among patients receiving tildrakizumab 200 mg Q4W versus other treatments. Less than half of patients had measurable dactylitis at baseline, and baseline LDI was higher among patients receiving tildrakizumab 100 mg Q12W and placebo Q4W→tildrakizumab 200 mg Q12W, and lower among patients receiving tildrakizumab 200 mg Q4W, compared with other treatments.

Efficacy

At week 24, a significantly higher proportion of patients receiving any dose of tildrakizumab achieved ACR20 (71.4%–79.5%) relative to placebo-treated patients (50.6%) (figure 2A, table 2, all p≤0.0125), with more responders to tildrakizumab 200 mg Q4W and 100 mg Q12W by week 8—after one dose of study medication.

The secondary endpoints, subgroup analyses and exploratory efficacy endpoints were not multiplicity controlled; nominal p values are provided for information only. At week 24, patients receiving tildrakizumab 200 mg (Q4W or Q12W) achieved higher rates of ACR50/70, DAS28-CRP <3.2 and MDA and greater improvement in PtGA, PGA, patient pain assessment and high-sensitivity CRP (hsCRP) level relative to placebo-treated patients (nominal p<0.05; table 2; figure 2B,C; figure 3A,B; proportions of patients with ≥20% improvement in ACR components over time online supplemental figure S2; change in patient pain assessment over time online supplemental figure S3). Improvement in SJC66 was greater for patients receiving tildrakizumab 200 mg Q4W but not Q12W, and improvements in TJC68

and HAQ-DI were greater for patients receiving tildrakizumab 200 mg Q12W but not Q4W, relative to placebo-treated patients (nominal p<0.05). Patients receiving tildrakizumab 100 mg Q12W versus placebo achieved higher rates or greater improvement in the same outcomes except ACR70; patients receiving tildrakizumab 20 mg Q12W achieved higher rates of ACR50, DAS28-CRP <3.2 and MDA and greater improvement in PtGA, PGA and hsCRP level, but not other measures, relative to placebo-treated patients (nominal p<0.05). Responses were maintained through week 52, and patients who switched from placebo or tildrakizumab 20 mg to tildrakizumab 200 mg Q12W at week 24 had similar responses as patients treated from baseline (figures 2–3, online supplemental table S1, online supplemental figure S3). Improvement in LDI and LEI at week 24 was not observed following any dose of tildrakizumab versus placebo (table 2, online supplemental figure S4). Only two patients required adjustment of background medication (table 2).

Subgroup analysis by prior anti-TNFα therapy experience was performed for ACR20/50/70 response rates. Week 24 response rates were numerically lower in anti-TNFα-experienced versus anti-TNFα-naïve patients within each treatment arm, but the ACR20/50/70 treatment response pattern was generally similar regardless of prior anti-TNFα therapy (online supplemental figure S5). ACR20 response rates by country are shown in online supplemental table S2; lower proportions of patients receiving tildrakizumab 200 mg or placebo achieved ACR20 response in the USA and Spain relative to other countries.

In exploratory and post hoc analyses, greater proportions of patients with measurable psoriasis at baseline (BSA ≥3%) achieved PASI 75/90/100 at week 24 following treatment with tildrakizumab (any dose) versus placebo, with sustained response through week 52 (figure 4). Impact of PsA on patients' lives, assessed via PsAID, decreased for patients receiving tildrakizumab (all doses) versus placebo (table 2); improvement was sustained through week 52 (online supplemental table S1). The proportion of patients with VLDA was numerically greater for tildrakizumab 200 mg Q4W and Q12W versus placebo by week 24 (figure 3C, table 2); no hypothesis testing was performed. Tildrakizumab treatment did not increase combined LDI/LEI resolution (table 2) relative to placebo at week 24. At week 52, ≥50% of patients with baseline LEI ≥1 had LEI resolution (online supplemental table S1). DAPSA and PASDAS scores numerically decreased and proportions of patients achieving DAPSA remission and PASDAS <3.2 were numerically larger following treatment with tildrakizumab versus placebo at week 24 and through week 52 (table 2, figure 5, online supplemental table S1); no hypothesis testing was performed. Proportions of patients achieving MCID from baseline HAQ-DI and PsAID at week 24 appeared similar among treatment arms (table 2).

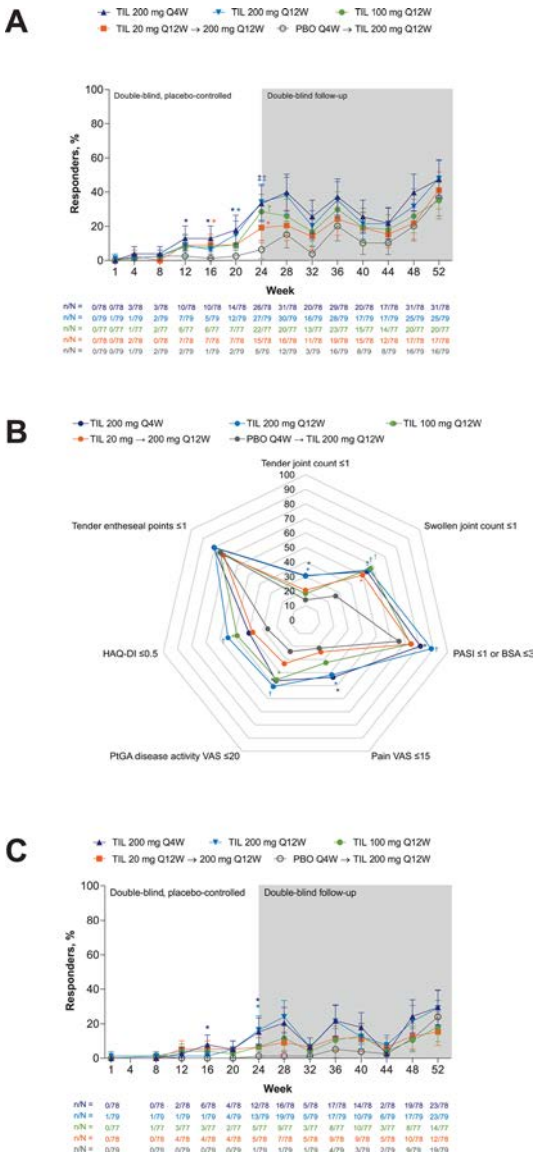

Figure 3 MDA responders (A) over time, (B) responders for each MDA subcomponent at week 24 and (C) VLDA responders by treatment and time point. Supporting values shown in online supplemental table S4. Shown for randomised patients who received ≥1 dose of study drug. Error bars represent 95% CI. Missing responses were imputed as non-responses. Proportion of responders shown as % in (B). TIL 200 mg Q4W, n=78; TIL 200 mg Q12W, n=79; TIL 100 mg Q12W, n=77; TIL 20 mg Q12W→200 mg Q12W, n=78; PBO Q4W→TIL 200 mg Q12W, n=79 except for tender entheseal points ≤1 in (B) (TIL 200 mg Q4W, n=76; TIL 100 mg Q12W, n=76; PBO Q4W→TIL 200 mg Q12W, n=78). *p<0.05; †p<0.001; ‡p<0.0001 versus PBO; not adjusted for multiplicity. P values were not analysed beyond week 24. BSA, body surface area; HAQ-DI, Health Assessment Questionnaire-Disability Index; MDA, Minimum Disease Activity; PASI, Psoriasis Area and Severity Index; PBO, placebo; PtGA, patients global assessment; Q4W, every 4 weeks; Q12W, every 12 weeks; TIL, tildrakizumab; VAS, visual analogue scale; VLDA, very low disease activity.

Safety

There were no deaths through study week 52. Of 391 patients analysed, 1 (0.3%) patient discontinued due to a TEAE (hypertension, tildrakizumab 200 mg Q12W). Across all treatment arms, 252 (64.5%) patients had a TEAE, most frequently nasopharyngitis (8.4%) and upper respiratory tract infection (6.4%) (table 3). Two (0.5%) patients had a fungal skin infection (candida, both tildrakizumab 200 mg Q4W). Most TEAEs, including infections, were mild and comparable among treatment arms. During weeks 25–52, one patient (0.3%) was diagnosed with malignancy (intraductal proliferative breast lesion, tildrakizumab 20→200 mg Q12W).

Serious TEAEs were observed in nine (3.3%) patients. One serious infection (chronic tonsillitis) was reported during the first 24 weeks (tildrakizumab 20 mg Q12W). One case each

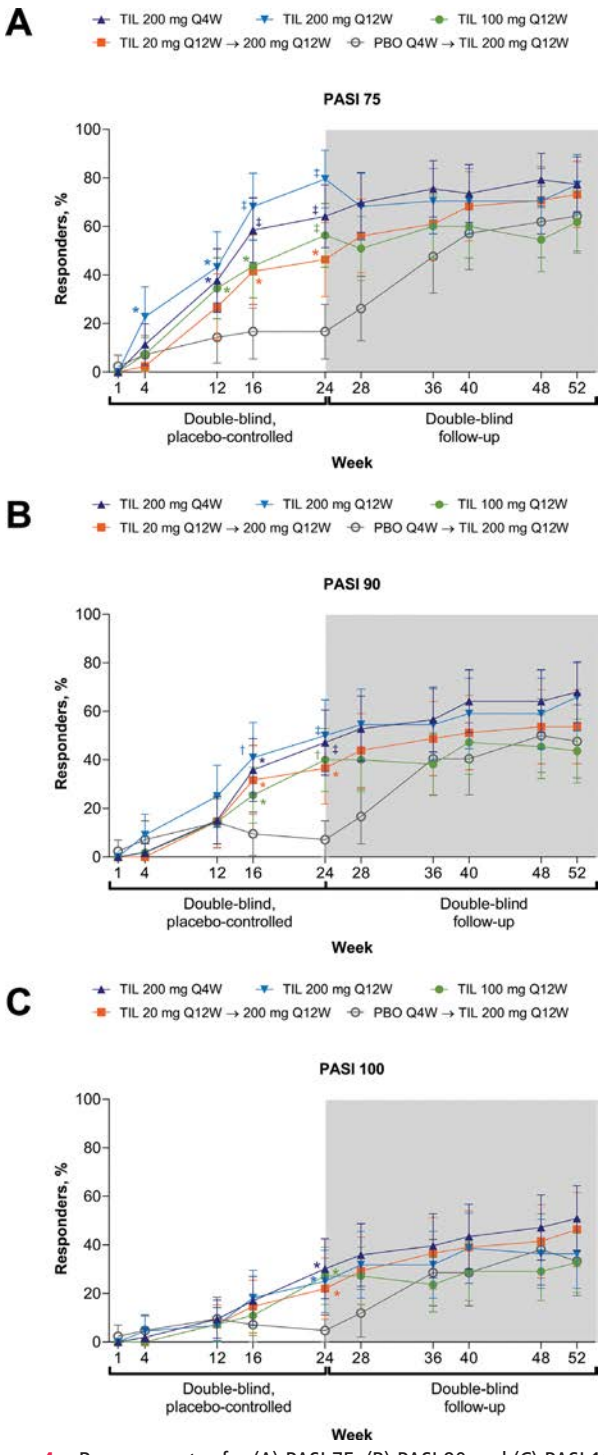

Figure 4 Response rates for (A) PASI 75, (B) PASI 90 and (C) PASI 100 through week 52 across treatment and time point. Supporting values shown in online supplemental table S6. Response rates were calculated in randomised patients who received ≥1 dose of study drug with BSA ≥3% at baseline. Error bars represent 95% CI. Missing responses were imputed as non-responses. TIL 200 mg Q4W, n=53; TIL 200 mg Q12W, n=44; TIL 100 mg Q12W, n=55; TIL 20 mg Q12W→200 mg Q12W, n=41; PBO Q4W→TIL 200 mg Q12W, n=42. P values are based on Cochran-Mantel-Haenszel test (with prior anti-TNF use and baseline weight as stratification factors). *p<0.05; †p<0.001; ‡p<0.0001 versus PBO; not adjusted for multiplicity. P values were not analysed beyond week 24. BSA, body surface area; PASI, Psoriasis Area and Severity Index; PBO, placebo; Q4W, every 4 weeks; Q12W, every 12 weeks; TIL, tildrakizumab; TNF, tumour necrosis factor.

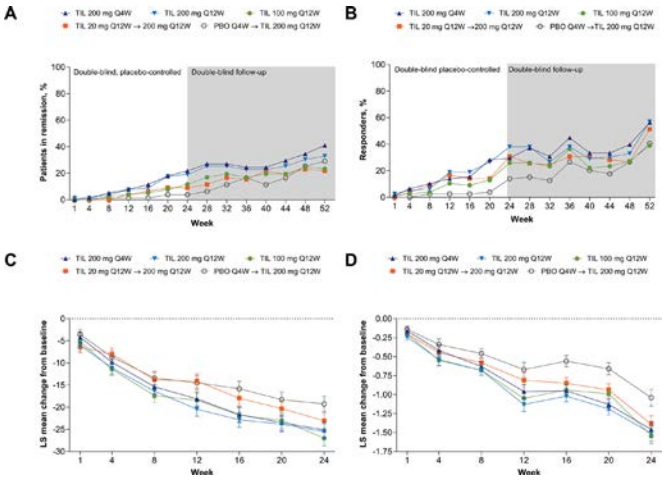

Figure 5 Proportion of patients in remission based on (A) DAPSA, proportion of patients with PASDAS <3.2 (B), and change from baseline DAPSA (C) and PASDAS (D). Supporting values shown in online supplemental table S5. Missing responses were imputed as non-responses. DAPSA remission was defined as a score between 0 and 4. TIL 200 mg Q4W, n=78; TIL 200 mg Q12W, n=79; TIL 100 mg Q12W, n=77; TIL 20 mg Q12W→200 mg Q12W, n=78; PBO Q4W→TIL 200 mg Q12W, n=79. P values not analysed. DAPSA, Disease Activity in Psoriatic Arthritis; LS, least squares; PASDAS, Psoriatic Arthritis Disease Activity Score; PBO, placebo; Q4W, every 4 weeks; Q12W, every 12 weeks; TIL, tildrakizumab.

of pyelonephritis and urinary tract infection were reported as TEAEs of special interest in the same patient (tildrakizumab 100 mg Q12W) (table 3). There were no reports of systemic candidiasis, uveitis, inflammatory bowel disease, MACE, suicidality or deaths, and no changes in laboratory parameters considered serious TEAEs, from baseline through week 24 or week 25 through 52.

DISCUSSION

Significantly greater proportions of patients receiving all tildrakizumab doses versus placebo achieved the primary endpoint of ACR20 at week 24. Among patients with prior anti-TNFα therapy, ACR20/50/70 treatment difference versus placebo was not apparent for all tildrakizumab dose arms, although there were relatively few such patients (n=17–19 per treatment arm). At week 24, PASI 75, 90 and 100 response rates were higher following treatment with all tildrakizumab doses versus placebo.

Definitive comparisons between the present results and other clinical studies cannot be made due to differences in trial design, study population and placebo response rates. However, proportions of ACR20/50/70 responders among patients receiving tildrakizumab 200 mg (Q4W and Q12W) were also numerically higher compared with previous trials of biologicals for PsA treatment.[12–16 29–33] PASI 75/90/100 response rates were consistent with those reported in previous trials for tildrakizumab in psoriasis.[22]

MDA responses assess efficacy across the spectrum of PsA manifestations and are strongly associated with significant improvements in health-related QoL and productivity, making MDA an increasingly important treatment target in randomised PsA trials.[34] In this study, significantly more patients receiving tildrakizumab versus placebo achieved MDA by week 24. PASDAS and DAPSA—additional established composite indices for measuring PsA disease activity[27 35 36]—were added as post hoc efficacy measures based on increasing recognition of their

Table 3 Summary of safety findings

	Through week 24					Through week 52				
	TIL 200 mg Q4W (n=78)	TIL 200 mg Q12W (n=79)	TIL 100 mg Q12W (n=77)	TIL 20 mg Q12W (n=78)	PBO Q4W (n=79)	TIL 200 mg Q4W (n=78)	TIL 200 mg Q12W (n=79)	TIL 100 mg Q12W (n=77)	TIL 20 mg → 200 mg Q12W (n=78)	PBO → TIL 200 mg Q12W (n=79)
Any TEAE	39 (50.0)	39 (49.4)	44 (57.1)	34 (43.6)	34 (43.0)	51 (65.4)	50 (63.3)	53 (68.8)	51 (65.4)	47 (59.5)
Serious TEAEs	2 (2.6)	2 (2.5)	2 (2.6)	1 (1.3)	2 (2.5)	2 (2.6)	2 (2.5)	2 (2.6)	4 (5.1)	3 (3.8)
Discontinuations due to TEAEs	0	0	0	0	0	0	1 (1.3)	0	0	0
Deaths due to TEAEs	0	0	0	0	0	0	0	0	0	0
Any TEAE of special interest*	0	0	1 (1.3)	0	0	0	0	1 (1.3)	1 (1.3)	0
Any TEAE of clinical interest	0	0	1 (1.3)	0	0	0	0	1 (1.3)	2 (2.6)	1 (1.3)
Serious TEAEs (≥1)										
Hypertension	0	2 (2.5)	0	0	0	0	2 (2.5)	0	0	0
Osteoarthritis	0	0	1 (1.3)	0	1 (1.3)	0	0	1 (1.3)	0	1 (1.3)
Parathyroid tumour benign	0	0	0	0	1 (1.3)	0	0	0	0	1 (1.3)
Hypokalaemia	0	0	1 (1.3)	0	0	0	0	1 (1.3)	0	0
Ovarian cyst	1 (1.3)	0	0	0	0	1 (1.3)	0	0	0	0
Ovarian cyst ruptured	1 (1.3)	0	0	0	0	1 (1.3)	0	0	0	0
Syncope	0	0	1 (1.3)	0	0	0	0	1 (1.3)	0	0
Chronic tonsillitis	0	0	0	1 (1.3)	0	0	0	0	1 (1.3)	0
Angina pectoris	0	0	0	0	0	0	0	0	1 (1.3)	0
Intraductal proliferative breast lesion	0	0	0	0	0	0	0	0	1 (1.3)	0
Lumbar radiculopathy	0	0	0	0	0	0	0	0	1 (1.3)	0
Chronic obstructive pulmonary disease	0	0	0	0	0	0	0	0	0	1 (1.3)
TEAEs of special or clinical interest (≥1)*										
Pyelonephritis	0	0	1 (1.3)	0	0	0	0	1 (1.3)	0	0
Urinary tract infection	0	0	1 (1.3)	0	0	0	0	1 (1.3)	0	0
Depression	0	0	0	1 (1.3)	0	0	0	0	1 (1.3)	1 (1.3)
AST increased	0	0	0	0	0	0	0	0	1 (1.3)	0
Blood bilirubin increased	0	0	0	0	0	0	0	0	1 (1.3)	0
Intraductal proliferative breast lesion	0	0	0	0	0	0	0	0	1 (1.3)	0
Most frequent TEAEs (≥5% through week 52)										
Nasopharyngitis	7 (9.0)	1 (1.3)	4 (5.2)	5 (6.4)	5 (6.3)	9 (11.5)	3 (3.8)	6 (7.8)	8 (10.3)	7 (8.9)
Headache	4 (5.1)	1 (1.3)	5 (6.5)	5 (6.4)	2 (2.5)	5 (6.4)	4 (5.1)	7 (9.1)	5 (6.4)	3 (3.8)
Hypertension	3 (3.8)	5 (6.3)	1 (1.3)	2 (2.6)	4 (5.1)	5 (6.4)	6 (7.6)	1 (1.3)	2 (2.6)	5 (6.3)
Upper respiratory tract infection	2 (2.6)	4 (5.1)	3 (3.9)	2 (2.6)	1 (1.3)	4 (5.1)	8 (10.1)	5 (6.5)	7 (9.0)	1 (1.3)
Anxiety	1 (1.3)	4 (5.1)	0	1 (1.3)	1 (1.3)	1 (1.3)	5 (6.3)	1 (1.3)	2 (2.6)	2 (2.5)
Sleep disorder	1 (1.3)	5 (6.3)	0	1 (1.3)	1 (1.3)	1 (1.3)	5 (6.3)	0	1 (1.3)	1 (1.3)
Diarrhoea	0	0	0	4 (5.1)	0	0	1 (1.3)	2 (2.6)	6 (7.7)	1 (1.3)
Nausea	0	0	2 (2.6)	1 (1.3)	1 (1.3)	0	0	4 (5.2)	2 (2.6)	1 (1.3)
Pharyngitis	1 (1.3)	0	2 (2.6)	0	2 (2.5)	1 (1.3)	1 (1.3)	3 (3.9)	4 (5.1)	2 (2.5)
Sinusitis	0	1 (1.3)	1 (1.3)	0	2 (2.5)	1 (1.3)	2 (2.5)	4 (5.2)	1 (1.3)	2 (2.5)
Urinary tract infection	0	1 (1.3)	3 (3.9)	1 (1.3)	3 (3.8)	1 (1.3)	1 (1.3)	3 (3.9)	4 (5.1)	4 (5.1)
ALT increased	0	1 (1.3)	1 (1.3)	0	3 (3.8)	2 (2.6)	1 (1.3)	1 (1.3)	1 (1.3)	5 (6.3)

Continued

Table 3 Continued

	Through week 24					Through week 52				
	TIL 200 mg Q4W (n=78)	TIL 200 mg Q12W (n=79)	TIL 100 mg Q12W (n=77)	TIL 20 mg Q12W (n=78)	PBO Q4W (n=79)	TIL 200 mg Q4W (n=78)	TIL 200 mg Q12W (n=79)	TIL 100 mg Q12W (n=77)	TIL 20 mg → 200 mg Q12W (n=78)	PBO→ TIL 200 mg Q12W (n=79)
AST increased	0	1 (1.3)	1 (1.3)	0	1 (1.3)	2 (2.6)	1 (1.3)	1 (1.3)	2 (2.6)	5 (6.3)
Blood pressure increased	1 (1.3)	0	2 (2.6)	0	0	2 (2.6)	0	4 (5.2)	0	0
Gamma-glutamyl transferase increased	1 (1.3)	1 (1.3)	3 (3.9)	0	1 (1.3)	1 (1.3)	1 (1.3)	4 (5.2)	1 (1.3)	2 (2.5)
Arthralgia	2 (2.6)	0	0	3 (3.8)	0	4 (5.1)	0	0	4 (5.1)	0
Back pain	2 (2.6)	0	2 (2.6)	1 (1.3)	2 (2.5)	4 (5.1)	0	2 (2.6)	1 (1.3)	2 (2.5)

Data shown are n (%) for randomised patients who received ≥1 dose of study drug.
*AEs of special interest were major adverse cardiac events, malignancies and severe infections.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; PBO, placebo; Q4W, every 4 weeks; Q12W, every 12 weeks; TEAE, treatment-emergent adverse event; TIL, tildrakizumab.

utility. Although statistical significance was not assessed for post hoc analyses, proportions of tildrakizumab-treated patients achieving DAPSA remission and PASDAS <3.2 at week 24 were higher relative to placebo-treated patients.

Tildrakizumab was generally well tolerated through week 52. Overall, safety findings were similar to the safety profile in phase III trials of tildrakizumab for treatment of plaque psoriasis (reSURFACE 1 and reSURFACE 2).[22] There were no deaths or reports of systemic candidiasis, inflammatory bowel disease, MACE, or significantly increased liver enzymes through week 52.

Aberrant activation of the IL-23/IL-17 cytokine system is critical in the pathogenesis of PsA.[37] IL-23 is thought to promote joint degeneration by inducing osteoclastogenesis and osteoclast-mediated activation of nuclear factor of activated T cells that regulate expression of genes facilitating pathological bone resorption (eg, matrix metallopeptidase 9).[38 39] The efficacy and safety findings reported here may be attributed to selective antagonism of IL-23 by tildrakizumab. A plausible mechanism of action of tildrakizumab is inhibition of the IL-23-induced kinase signalling system resulting in reduced Th17 cell proliferation and downregulation of the Th17 cell-secreted inflammatory cytokines such as IL-17 and IL-22.[10 37 39–41] By selectively targeting IL-23p19, tildrakizumab blocks IL-23-mediated signalling[10 11 40] without targeting the p40 subunit common to both IL-23 and IL-12 (eg, ustekinumab). Tildrakizumab may thus circumvent potential adverse effects on cell immunity by sparing IL-12 function.[22 42 43]

Study limitations included high placebo response rates, confounding interpretation of results. Due to small numbers of patients in each subgroup, post hoc analyses detected no meaningful relationship between ACR20 placebo response at week 24 and patient baseline characteristics, background medication use, or country; however, placebo response rates were numerically lower in the USA and Spain relative to other countries. Per recent analyses, placebo response rates have increased over time in clinical trials across several disease states including rheumatoid arthritis, with no definitive explanation[44–46]; speculated causes in the rheumatoid arthritis study included expectation bias; therapeutic improvements resulting in a limited pool of eligible patients, possibly leading to recruitment during transient disease flares; and greater recruitment in resource-poor countries.[44] Although placebo response was higher than expected, treatment difference and, therefore, statistical power were preserved. Relatively few patients with dactylitis or enthesitis were included, and baseline dactylitis in particular was not balanced among treatment arms, so the study was not powered to detect statistically significant differences in related endpoints. This is planned for attention in the phase III programme. The study was also not powered to differentiate tildrakizumab 100 and 200 mg doses. Mixed dose effects were observed. Patients treated with tildrakizumab 20 mg Q12W achieved greater improvement relative to placebo-treated patients for some efficacy measures, although response rates and improvement were smaller compared with patients receiving higher doses. Among patients receiving tildrakizumab 200 mg, Q4W dosing was not consistently superior to Q12W dosing. Patients receiving tildrakizumab 100 mg had numerically lower rates of ACR responses but numerically greater improvement in some component measures relative to those treated with tildrakizumab 200 mg. These findings were generally consistent

with the small numbers of patients and the expected tildrakizumab dose–response relationship. Optimal dosing of tildrakizumab in patients with PsA is planned for investigation in the phase III programme.

CONCLUSION

These findings demonstrate that treatment with tildrakizumab 200 or 100 mg was more effective than placebo for rates of ACR20/50, DAS28-CRP, MDA and PASI 75/90/100 responses as well as improvement in physical function; effects were smaller for tildrakizumab 20 mg relative to higher doses. Tildrakizumab was well tolerated through 52 weeks of treatment. These results support tildrakizumab phase III clinical development in PsA.

Author affiliations
[1]Rheumatology Research, Swedish Medical Center, Seattle, Washington, USA
[2]Providence St. Joseph Health and University of Washington, Seattle, Washington, USA
[3]Arizona Arthritis and Rheumatology Research, PLLC, Phoenix, Arizona, USA
[4]Hospital CIMA Sanitas, Barcelona, Spain
[5]Cincinnati Rheumatic Disease Study Group, Cincinnati, Ohio, USA
[6]Division of Immunology, Allergy, and Rheumatology, University of Cincinnati College of Medicine, Cincinnati, Ohio, USA
[7]Medicine, Eastern Health and Memorial University, St. Johns, Newfoundland, Canada
[8]Division of Rheumatology, Allergy and Clinical Immunology, University of California School of Medicine, Davis, California, USA
[9]Rheumatology, VA Medical Center Sacramento, Sacramento, California, USA
[10]Division of Allergy, Immunology and Rheumatology, University at Buffalo School of Medicine and Biomedical Sciences, Buffalo, New York, USA
[11]Sun Pharmaceutical Industries, Princeton, New Jersey, USA
[12]Department of Dermatology, Icahn School of Medicine at Mount Sinai, New York, New York, USA

Acknowledgements We thank the patients for their participation and Dr Siu-Long Yao of Sun Pharmaceutical Industries for study design and oversight. Some data were previously presented at the EULAR 2020 Annual European Congress of Rheumatology E-Congress beginning 3 June 2020 and published in the following congress abstracts: Mease PJ et al. *Ann Rheum Dis* 2020;79(Suppl 1):145; Gottlieb AB et al. *Ann Rheum Dis* 2020;79(suppl 1):1157; Nash P et al. *Ann Rheum Dis* 2020;79(Suppl 1):1167. Editorial support was provided by Puneet Dang, PhD; Claire Daniele, PhD, CMPP; and Judy Phillips, DVM, PhD, of AlphaBioCom, LLC, and funded by Sun Pharmaceutical Industries.

Contributors All authors contributed to data interpretation and manuscript development, critically reviewed each draft for intellectual content and approved the final version for submission.

Funding The study was funded by Sun Pharma Global FZE. Analyses were funded by Sun Pharmaceutical Industries, Princeton, NJ, USA.

Competing interests PJM has received research grants from AbbVie; Amgen; Bristol Myers Squibb; Celgene; Janssen; Eli Lilly; Novartis; Pfizer; Sun Pharmaceutical Industries, Inc.; and UCB; consulting fees from AbbVie; Amgen; Bristol Myers Squibb; Boehringer Ingelheim; Galapagos; Gilead; GlaxoSmithKline; Janssen; Eli Lilly; Merck; Novartis; Pfizer; Sun Pharmaceutical Industries, Inc.; and UCB; and speaker fees from AbbVie, Amgen, Bristol Myers Squibb, Genentech, Janssen, Eli Lilly, Novartis, Pfizer and UCB. SC is a partner/physician at Arizona Arthritis and Rheumatology Associates. FJGF has received research grants, consulting fees and/or speaker fees from AbbVie, Eli Lilly, Gedeon Richter, MedImmune, Nichi-Iko, Pfizer, Sanofi-Aventis, Takeda and UCB. MEL has received research grants, consulting fees and/or speaker fees from AbbVie; Amgen; Eli Lilly; Genentech; Nichi-Iko; Novartis; Pfizer; R-Pharm; and Sun Pharmaceutical Industries, Inc. PR has received research grants from Janssen and Novartis, consulting fees from AbbVie, Amgen, Bristol Myers Squibb, Celgene, Janssen, Eli Lilly, Novartis, Pfizer, Roche and UCB; and speaker fees from AbbVie, Janssen, Eli Lilly, Novartis, Pfizer and UCB. SPR has received grants/research support from AbbVie; Janssen; Novartis; Pfizer; and Sun Pharmaceutical Industries, Inc.; and consulting fees from Amgen, Eli Lilly, Janssen, Novartis and Pfizer. RCC receives consultation fees from Sun Pharmaceutical Industries, Inc. AMM is a former employee of Sun Pharmaceutical Industries, Inc.; and has individual shares in Johnson and Johnson, and as part of retirement account/mutual funds. SJR is an employee of Sun Pharmaceutical Industries, Inc. ABG has received honoraria as an advisory board member and consultant for Avotres Therapeutics; Beiersdorf; Boehringer Ingelheim; Bristol-Myers Squibb Co.; Janssen; LEO Pharma; Eli Lilly; Novartis; Sun Pharmaceutical Industries, Inc.; UCB; and Xbiotech (stock options); and has received research/educational grants from Boehringer Ingelheim; Incyte; Janssen; Novartis; Sun Pharmaceutical Industries, Inc.; UCB; and Xbiotech (all paid to Mount Sinai School of Medicine).

Patient consent for publication Not required.

Ethics approval The study protocol (online supplemental file) was approved by independent ethics committees or institutional review boards at each site. The trial was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonisation Good Clinical Practice guidelines.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. Data and other documents will be made available after publication, with no end date, to anyone who submits a reasonable request to the study sponsor.

ORCID iDs
Philip J Mease http://orcid.org/0000-0002-6620-0457
Siba P Raychaudhuri http://orcid.org/0000-0001-7594-6528

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CLINICAL SCIENCE

Safety and efficacy of faecal microbiota transplantation for active peripheral psoriatic arthritis: an exploratory randomised placebo-controlled trial

Maja Skov Kragsnaes,[1,2] Jens Kjeldsen ,[3] Hans Christian Horn ,[1] Heidi Lausten Munk ,[1] Jens Kristian Pedersen ,[4] Søren Andreas Just,[4] Palle Ahlquist,[5] Finn Moeller Pedersen,[3] Maarten de Wit ,[6] Sören Möller ,[2,7] Vibeke Andersen ,[8,9] Karsten Kristiansen ,[10,11] Dorte Kinggaard Holm ,[12] Hanne Marie Holt,[13] Robin Christensen ,[2,14] Torkell Ellingsen [1,2]

Handling editor Josef S Smolen

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2020-219511).

For numbered affiliations see end of article.

Correspondence to
Professor Torkell Ellingsen, Rheumatology Research Unit, Department of Rheumatology, Odense University Hospital, Odense, Denmark; torkell.ellingsen@rsyd.dk

Received 13 November 2020
Revised 12 April 2021
Accepted 14 April 2021
Published Online First 29 April 2021

To cite: Kragsnaes MS, Kjeldsen J, Horn HC, *et al*. *Ann Rheum Dis* 2021;**80**:1158–1167.

ABSTRACT

Objectives Although causality remains to be established, targeting dysbiosis of the intestinal microbiota by faecal microbiota transplantation (FMT) has been proposed as a novel treatment for inflammatory diseases. In this exploratory, proof-of-concept study, we evaluated the safety and efficacy of FMT in psoriatic arthritis (PsA).

Methods In this double-blind, parallel-group, placebo-controlled, superiority trial, we randomly allocated (1:1) adults with active peripheral PsA (≥3 swollen joints) despite ongoing treatment with methotrexate to one gastroscopic-guided FMT or sham transplantation into the duodenum. Safety was monitored throughout the trial. The primary efficacy endpoint was the proportion of participants experiencing treatment failure (ie, needing treatment intensification) through 26 weeks. Key secondary endpoints were change in Health Assessment Questionnaire Disability Index (HAQ-DI) and American College of Rheumatology (ACR20) response at week 26.

Results Of 97 screened, 31 (32%) underwent randomisation (15 allocated to FMT) and 30 (97%) completed the 26-week clinical evaluation. No serious adverse events were observed. Treatment failure occurred more frequently in the FMT group than in the sham group (9 (60%) vs 3 (19%); risk ratio, 3.20; 95% CI 1.06 to 9.62; p=0.018). Improvement in HAQ-DI differed between groups (0.07 vs 0.30) by 0.23 points (95% CI 0.02 to 0.44; p=0.031) in favour of sham. There was no difference in the proportion of ACR20 responders between groups (7 of 15 (47%) vs 8 of 16 (50%)).

Conclusions In this first preliminary, interventional randomised controlled trial of FMT in immune-mediated arthritis, we did not observe any serious adverse events. Overall, FMT appeared to be inferior to sham in treating active peripheral PsA.

Trial registration number NCT03058900.

Key messages

What is already known about this subject?

- Psoriatic arthritis (PsA) is a systemic immune-mediated disease associated with subclinical gut inflammation and dysbiosis of the intestinal microbiota.
- Faecal microbiota transplantation (FMT) has demonstrated local therapeutic immune-modulating abilities in patients with chronic inflammatory bowel disease.

What does this study add?

- In this first preliminary, randomised controlled trial of FMT in immune-mediated arthritis, transfer of donor microbiota was safe, but appeared inferior to sham in reducing disease activity in patients with active peripheral PsA concomitantly treated with methotrexate.

How might this impact on clinical practice or future developments?

- Whether microbial dysbiosis or specific bacteria are common or decisive mediators of disease activity in PsA and whether this proposed relation can be modified without exacerbating the disease will be crucial to clarify to determine the future role of microbiota-targeted interventions in the management of PsA.

INTRODUCTION

For a century, the link between enteric infections and reactive arthritis[1] has motivated investigation into the proposed gut–joint axis implicating intestinal micro-organisms in the aetiology of immune-mediated arthritic disease.[2] Recently, this theory has gained renewed interest due to accumulating evidence of disease-related imbalance (dysbiosis) in the composition and function of the intestinal microbiota in chronic disorders.[3–5] Among these, psoriatic arthritis (PsA)[6] has been associated with decreased intestinal bacterial diversity, displaying both disease-specific patterns[7] and microbial abnormalities similar to those seen in other subtypes of spondyloarthritis, rheumatoid arthritis and inflammatory bowel disease (IBD).[8] These findings have encouraged research into the host–microbiota interplay in the dysregulated

immunological cascade underlying immune-mediated arthritis and the prospects of microbiota-targeted therapies.[9]

Faecal microbiota transplantation (FMT) is currently considered the most efficient method to restore a healthy diversity of the gastrointestinal microbiota.[10 11] Indeed, the transfer of faeces containing minimally manipulated communities of micro-organisms from a donor to a recipient has revolutionised the treatment of *Clostridioides difficile* infection.[12] FMT may also induce beneficial responses in patients with IBD, thereby demonstrating local therapeutic immune-modulating abilities.[13] However, whether manipulation of the intestinal microbiota can treat extraintestinal, immune-mediated disorders remains to be established.[14] This is the first exploratory, randomised trial to assess the safety and efficacy of FMT in patients with active, peripheral PsA.

METHODS

Trial design

This is a proof-of-concept, 26-week, 1:1 randomised, parallel-group, double-blind, placebo-controlled, single-centre superiority trial. In 2015, the Regional Committees on Health Research Ethics for Southern Denmark (DK-S-20150080) and the Danish Data Protection Agency (15/41684) approved the trial protocol (see online supplemental appendices S1 and S1A). Although not required by the Danish Health and Medicines Authority, we fulfilled the requirements of documentation, monitoring and reporting according to the principles of Good Clinical Practice. We registered the trial with ClinicalTrials.gov. Our trial protocol paper was published in 2018.[15] The study was conducted at one Danish tertiary referral hospital with nationwide inclusion. All participants gave written informed consent. The trial was temporarily suspended from March to May 2020 due to the COVID-19 pandemic (see online supplemental appendix S4). A statistical analysis plan (see online supplemental appendix S2) was developed with subsequent closure (2 April 2020) before unmasking and analysis (10 June 2020).

Participants

We included adults who were between 18 and 75 years of age, fulfilled the Classification for Psoriatic Arthritis criteria,[16] and had active peripheral disease, defined as three or more swollen joints, despite ongoing treatment with methotrexate at the maximal tolerable dose (≥15 mg/week) for at least 3 months prior to study inclusion. A washout period of 12 weeks (26 weeks for biologic agents) was required in patients previously treated with intra-articular or systemic glucocorticoids, and non-methotrexate conventional synthetic and biologic disease-modifying antirheumatic drugs. Key exclusion criteria were immune-mediated arthritis other than PsA, IBD, cancer, severe chronic infection, and history of food allergy, severe food intolerance or coeliac disease.[15]

Donor selection and stool preparation

The transplants were obtained from four healthy stool donors recruited from a non-profit, public stool bank located at the local blood and tissue transplant service.[17] Every step of the donation process and the laboratory handling were in agreement with the requirements of the European Union's regulative directives on human cells and tissues (2004/23/EC). Donors did not receive any compensation and had to pass an extensive screening programme (see online supplemental table S3) before and after the 30-day donation cycle.[15] Stool donations were transported to the stool bank facility within 1 hour after defaecation in an airtight container placed in a cooling bag. The donation was processed at normal room temperature within 2 hours of delivery under aerobic conditions, including 10–15 s of blending, before storage at −80°C (median storage time: 20 months; range: 2.5–30).[18]

Interventions

The transplant consisted of a single stool donation (50 g) mixed with saline (0.9%) and glycerol (10%) to a total volume of 250 mL. Before transplantation, we thawed the material to 36°C. The sham transplant consisted of 250 mL saline (0.9%) mixed with three drops of food colouring (E150c). We performed the allocated treatment within 14 days of the baseline visit. Treatment preparation included a 6-hour fast and one dose of oral proton-pump inhibitor. The transplant suspension was transferred into the third part of the duodenum via a closed system of tubes under gastroscopic guidance.

Outcomes

Safety was monitored by open assessment of adverse events (AEs) and evaluated before unmasking. The National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0 was used to grade the severity.[19] We evaluated causality for expected AEs of grade 2 and above and for all unexpected AEs regardless of severity. The primary efficacy endpoint was a composite outcome on the proportion of participants who experienced treatment failure through 26 weeks, defined as need for at least one of the following: more than one intra-articular glucocorticoid injection, and non-methotrexate conventional synthetic and/or biologic disease-modifying antirheumatic drugs. This endpoint covered disease activity and shared decision-making between the patient and the rheumatologist in accordance with the European PsA recommendations.[20] Three key secondary endpoints were evaluated at week 26: change from baseline in Health Assessment Questionnaire Disability Index (HAQ-DI),[21] proportion of participants fulfilling the American College of Rheumatology (ACR20) response[22] and change from baseline in the Spondyloarthritis Research Consortium of Canada (SPARCC) Enthesitis Index.[23] Additional secondary outcomes addressed all essential domains in the PsA core outcome set[24] (see table 1).

Randomisation and masking

We assigned participants to FMT or sham transplantation using permuted blocks with varying sizes of four and six, according to computer-generated random numbers. These lists were generated by the trial statistician and uploaded to a concealed area of a password-protected trial database (REDCap[25]) by an independent and otherwise trial agnostic data manager.[15] The trial coordinator implemented the randomisation. The randomisation record and the signed transfusion journals were stored in the database with restricted access separate from the patient record and other study data so the participants and the treating rheumatologists (ie, care providers and outcome assessors) remained unaware of treatment allocation and treatment.

Sample size and power considerations

Conceptually guided by the idea that at least twice as many participants in the sham group would be treatment failures, compared with the FMT group if the procedure should potentially be considered clinically relevant, we wanted to randomly assign 80 patients with PsA to two groups (40 patients to each), providing a good statistical power (90%) to detect a difference

Table 1 Comparison of efficacy endpoints at week 26

Endpoint	FMT (n=15)	Sham (n=16)	Difference between groups (95% CI)
Primary efficacy endpoint			
Treatment failure (primary endpoint), n (%)†‡	9 (60)*	3 (19)	3.20 (1.06 to 9.62)
Components of failure			
Total patients receiving >1 IA glucocorticoid injection, n (%)‡	2 (13)	1 (6)	2.13 (0.22 to 21.17)
Total patients starting non-methotrexate conventional synthetic DMARD(s), n (%)‡	0 (0)	1 (6)	0 (NA)
Total patients starting biologic DMARD(s), n (%)‡	8 (53)	3 (19)	2.84 (0.92 to 8.76)
Key secondary efficacy endpoints			
Change from baseline in HAQ-DI score§¶	−0.07 (−0.22 to 0.09)*	−0.30 (−0.44 to −0.15)	0.23 (0.02 to 0.44)
ACR20 response, n (%)‡**	7 (47)	8 (50)	0.93 (0.45 to 1.94)
Change from baseline in SPARCC Enthesitis Index score†††‡	−1.9 (−3.5 to −0.3)	−4.3 (−5.8 to −2.8)	2.3 (0.1 to 4.5)
Other secondary efficacy endpoints			
Modified PsARC response n (%)‡§§	11 (73)	13 (81)	0.90 (0.61 to 1.33)
Change from baseline in DLQI score¶,¶¶***	−1.5 (−3.5 to 0.5)	0.8 (−0.9 to 2.4)	−2.2 (−4.9 to 0.4)
Change from baseline in PASI score‡‡***†††	0.1 (−3.7 to 3.9)	0.3 (−2.4 to 2.9)	−0.2 (−4.8 to 4.5)
Change from baseline in the number of digits affected with dactylitis‡‡***	−1.2 (−2.1 to −0.2)	−1.5 (−2.7 to −0.4)	0.3 (−1.2 to 1.8)
ACR50 response, n (%)‡**	3 (20)	8 (50)	0.40 (0.13 to 1.23)
ACR70 response, n (%)‡**	1 (7)	6 (38)	0.18 (0.02 to 1.31)
Change from baseline in patient's global assessment, VAS¶‡‡‡	−4.3 (−14.8 to 6.3)	−25.6 (−35.4 to −15.7)	21.3 (6.9 to 35.7)
Change from baseline in arthritis pain, VAS¶‡‡‡	−8.8 (−19.1 to 1.6)	−24.8 (−34.6 to −15.0)	16.0 (1.8 to 30.3)
Change from baseline in fatigue, VAS¶‡‡‡	−0.0 (−11.5 to 11.5)	−18.1 (−29.0 to −7.2)	18.0 (2.2 to 33.9)
Change from baseline in tender joint count‡‡	−5.2 (−9.8 to −0.6)	−9.9 (−14.2 to −5.5)	4.72 (−1.6 to 11.0)
Change from baseline in C reactive protein§§§	0.4 (−1.0 to 1.7)	−1.0 (−2.3 to 0.2)	1.4 (−0.4 to 3.2)

All analyses were performed according to the intention-to-treat principle.
*P<0.05 for comparison with sham. Statistical testing was stopped following ACR20 in accordance with the predefined testing hierarchy.
†Treatment failure was defined as need for at least one of the following: more than one intra-articular glucocorticoid injection, and non-methotrexate conventional synthetic and/or biologic disease-modifying antirheumatic drugs.
‡Comparison was calculated as risk ratios based on crude relative risk.
§HAQ-DI score ranges from 0 to 3, with higher scores indicating greater disability.
¶Comparison was calculated as least squares means based on repeated measures linear mixed model across time points (baseline and weeks 1, 2, 3, 4, 12 and 26).
**ACR20/50/70 response (≥20%/50%/70% improvement from baseline in the number of tender and swollen joints and in at least 3 of 5 other specified domains).
††SPARCC Enthesitis Index score ranges from 0 to 16, with higher scores indicating more severe disease.
‡‡Comparison was calculated as least squares means based on repeated measures linear mixed model across time points (baseline and weeks 12 and 26).
§§Modified PsARC response (two of the following, one of which has to be a tender (68) and swollen (66) joint count, and no worsening of any measure: tender or swollen joint count improvement of 30% and/or patient global or physician global improvement of at least 1 point on the 5-point Likert scale).
¶¶DLQI score ranges from 0 to 30, with higher scores indicating more severe disease.
***PASI score, DLQI score and dactylitis count only in patients with baseline value >0.
†††PASI score ranges from 0 to 72, with higher scores indicating more severe disease.
‡‡‡VAS of 0–100, with higher scores indicating greater disease activity or pain.
§§§Comparison was calculated as least squares means based on repeated measures linear mixed model across time points (baseline and weeks 4, 12 and 26).
ACR, American College of Rheumatology; DLQI, Dermatology Life Quality Index; DMARD, disease-modifying antirheumatic drug; FMT, faecal microbiota transplantation; HAQ-DI, Health Assessment Questionnaire Disability Index; IA, intra-articular; NA, not available; PASI, Psoriasis Area Severity Index; PsARC, Psoriatic Arthritis Response Criteria; SPARCC, Spondyloarthritis Research Consortium of Canada; VAS, Visual Analogue Scale.

between two proportions (35% vs 70%) with a significance level of 5% (see further details in online supplemental appendices S1 and S2). In April 2019, we decided to stop recruitment by 31 December 2019, thereby adhering to the original planned trial completion date of 1 July 2020 (see online supplemental appendix S3). The main reason for this was that essential funding would stop following this date. Due to a slower than expected recruitment rate, only 31 participants were enrolled.

Patient involvement

Patients were directly involved in the design, funding, recruitment, conduct, reporting and dissemination of the study (see description at the end of the manuscript).

Statistical analysis

Analyses were based on the intention-to-treat (ITT) population including all randomised individuals, independent of subsequent adherence to the trial protocol. To assess the effect of FMT on the risk of treatment failure during the 26-week trial, we compared groups using risk ratios with 95% CIs based on an unadjusted (crude) model. Time-to-treatment failure was analysed based on a Kaplan-Meier plot from baseline to week 26 using Cox regression to estimate the HR with 95% CI and p value. Primary analyses in the ITT population at week 26 were based on a conservative treatment failure imputation default option for binary outcomes and a mixed-effects repeated measures model for continuous variables. For continuous outcome measures, we modelled between-group differences in outcomes at 0, 1, 2, 3, 4, 12 and 26 weeks with mixed-effects models, using time and group as categorical fixed-effect factors, interactions between time and group, random intercepts, and an unstructured covariance matrix; these models are reported as least squares means (and the difference between them) with 95% CIs.

All p values and 95% CIs were two-sided. We did not apply adjustments for multiplicity, rather we analysed the three key secondary outcomes in a prioritised order with a gatekeeping rule for serial testing. The other secondary outcomes are presented without conducting any formal statistical testing. Safety data are summarised descriptively in the full analysis set. In addition, we conducted a per-protocol analysis (see online supplemental table S1 and online supplemental figure S1) which included participants in whom the assigned transplant was successfully transferred into the third part of the duodenum. In addition, participants were excluded (censored) from the analysis following the day where they were categorised as treatment failures. Results of additional sensitivity analyses (including analyses based on the ITT population with missing data handled with multiple imputation) are presented in online supplemental table S2. We performed analyses using StataSE-64 V.16.1.

RESULTS

Participants

We enrolled participants to the trial between 16 May 2017 and 11 December 2019 at one Danish tertiary referral hospital (nationwide recruitment) with 26-week follow-up until 2 June 2020. Of 97 patients screened, 31 (32%) fulfilled the study requirements and were randomised to FMT or sham transplantation. All received the assigned intervention and 30 (97%) completed the clinical evaluation at week 26 (figure 1). Demographics and clinical characteristics of the two groups were comparable at baseline (table 2), except for some imbalance in sex and disease duration. Of the 31 participants, 27 (87%) had a personal history of skin psoriasis. A complete list of medication is presented in online supplemental table 4.

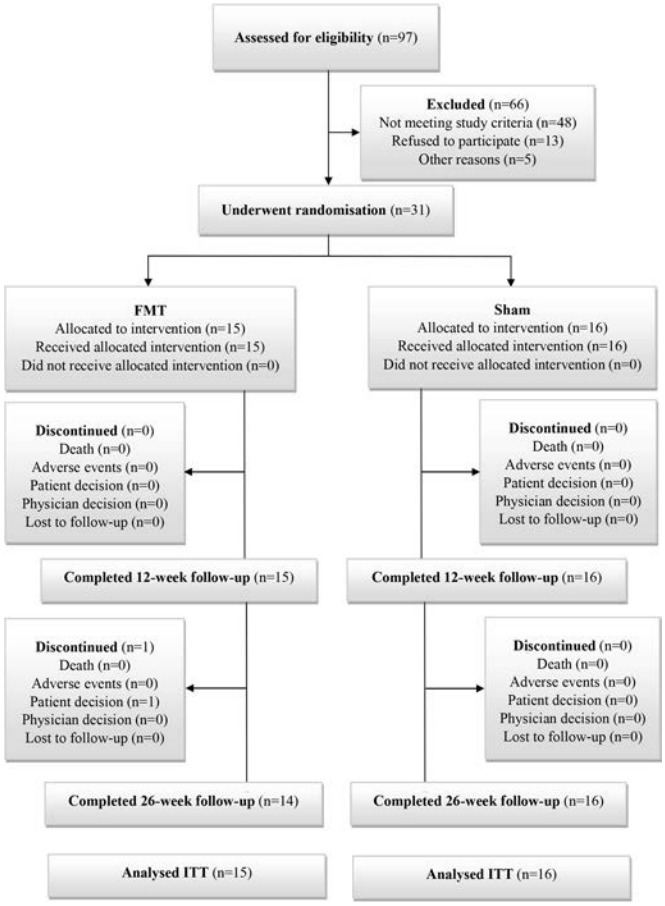

Figure 1 Patient disposition. Reasons for not meeting study criteria (n=48): not diagnosed with psoriatic arthritis (n=5), not ≥3 swollen joints (n=11), treated with other csDMARD (n=2) or bDMARD (n=5), methotrexate (≥15 mg/week) toxicity (n=6), age below or above limit (n=3), systemic inflammatory comorbidity (n=1), living abroad (n=14) and closed inclusion 2 days after initial contact (n=1). bDMARD, biologic disease-modifying antirheumatic drug; csDMARD, conventional synthetic disease-modifying antirheumatic drug; FMT, faecal microbiota transplantation; ITT, intention-to-treat.

Safety

We observed no serious AEs or deaths in any of the groups. Forty-seven AEs occurred in 14 participants (93%) receiving FMT, and 53 AEs occurred in 14 participants (88%) receiving sham. No participants withdrew from the trial due to AEs. In the FMT group, AEs were mainly related to the gastrointestinal tract and included abdominal discomfort, flatulence, nausea and vomiting. One case of diverticulitis was deemed unrelated to FMT because the participant had a history of diverticulitis before trial enrolment and the episode occurred 24 weeks after FMT.

Three AEs were deemed related to the gastroscopic procedure. Of those, facial capillary rupture and uncontrolled defaecation both occurred within an hour of the procedure. The third procedure-related AE was exacerbation of known asthmatic disease (grade 2) 3 days after the procedure. This participant vomited during the gastroscopy. We did not suspect pulmonary aspiration, and the clinical evaluation (including measures of C reactive protein) performed by the participant's general practitioner 4 and 7 days after the procedure did not suggest an underlying bacterial aetiology. A complete list of AEs, routine laboratory findings and metabolic changes is presented in table 3.

Efficacy

During the entire 26 weeks of observation, the rate of the primary outcome (treatment failure) was significantly higher in the FMT than in the sham group (HR, 4.87 (95% CI 1.31 to 18.18); p=0.018) (see figure 2). After 26 weeks, treatment had failed in more FMT-treated participants (9 of 15, 60%) than sham-treated participants (3 of 16, 19%) (crude relative risk, 3.20 (95% CI 1.06 to 9.62); p=0.018) (see table 1). Starting biologic disease-modifying antirheumatic drug was the main reason for being categorised as treatment failure (8 of 15 (53%) vs 3 of 16 (19%)). In the FMT group, eight patients (53%) had initiated biologic therapy at the 12-week visit compared with two (13%) in the sham group. The median time from trial intervention to starting biologics was 32 days (IQR 23–64) in the FMT group and 99 days (IQR 60–175) in the sham group. The median time from starting biologics to evaluation at week 26 was 155 days (IQR 118–173; total group exposure time, 995 days) in the FMT group and 70 days (IQR 0–126; total group exposure time, 196 days) in the sham group.

The HAQ-DI decreased more (indicating better physical function) in the sham group than in the FMT group (least squares means, −0.30 (95% CI −0.44 to −0.15) vs −0.07 (95% CI −0.22 to 0.09), difference 0.23 (0.02 to 0.44); p=0.031). The trajectories for HAQ-DI by treatment group from baseline to week 26 are presented in figure 3. Hierarchical statistical testing failed with regard to the proportion of ACR20 responders when comparing the FMT group with sham (7 of 15 (47%) vs 8 of 16 (50%); crude relative risk, 0.93 (0.45 to 1.94)).

Table 2 Baseline demographics and disease characteristics

Characteristics	FMT (n=15)	Sham (n=16)	Total (N=31)
Female sex, n (%)	8 (53)	12 (75)	20 (65)
Age, years	48.9 (16.1)	52.4 (11.0)	50.7 (13.6)
Height, cm	175.2 (7.0)	169.8 (8.6)	172.4 (8.2)
Weight, kg	93.6 (15.4)	92.4 (24.8)	93.0 (20.5)
Time since diagnosis, years*	2.6 (0.3–5.8)	5.6 (0.5–8.8)	3.7 (0.5–8.3)
Rheumatoid factor IgM negative, n (%)†	13 (93)	15 (94)	28 (93)
Anticitrullinated peptide antibody negative, n (%)†	14 (100)	16 (100)	30 (100)
HLA-B27 negative, n (%)	15 (100)	13 (81)	28 (90)
C reactive protein, mg/L	4.98 (7.18)	5.54 (5.87)	5.27 (6.43)
HAQ-DI‡	0.89 (0.51)	0.78 (0.50)	0.83 (0.50)
Swollen joint 66 count	7.5 (3.0)	6.7 (2.7)	7.1 (2.8)
Tender joint 68 count	14.9 (8.9)	17.3 (8.8)	16.1 (8.8)
SPARCC Enthesitis Index§			
Score ≥1, n (%)	13 (87)	15 (94)	28 (90)
Score in patients with a score ≥1	8.1 (4.3)	7.2 (3.3)	7.6 (3.8)
Dactylitis			
Affected digit(s) ≥1, n (%)	6 (40)	4 (25)	10 (32)
Digits affected in patients with affected digit(s) ≥1	1.5 (0.5)	1.5 (0.6)	1.5 (0.5)
Physician's global assessment of disease activity, VAS¶	35.1 (15.8)	31.4 (13.0)	33.2 (14.3)
Patient's global assessment of disease activity, VAS¶	56.1 (22.3)	56.0 (23.7)	56.0 (22.7)
Arthritis pain, VAS¶	54.1 (23.3)	48.8 (19.9)	51.9 (21.3)
Fatigue, VAS¶	56.5 (26.2)	57.4 (26.4)	57.0 (25.9)
Presence of nail disease, n (%)	12 (80)	10 (63)	22 (71)
PASI**			
Score >0, n (%)	4 (27)	6 (38)	10 (32)
Score in patients with a score of more than 0	1.07 (2.09)	1.39 (2.17)	1.24 (2.11)
DLQI score††			
Score >0, n (%)	9 (60)	12 (75)	21 (68)
Score in patients with a score of more than 0	2.1 (3.9)	1.8 (1.7)	1.9 (2.9)
Methotrexate			
Oral administration route, n (%)	2 (13)	4 (25)	6 (19)
Oral dose, mg/week	15.0 (0.0)	20.0 (4.1)	18.3 (4.1)
Subcutaneous administration route, n (%)	13 (87)	12 (75)	25 (81)
Subcutaneous dose, mg/week	20.0 (4.1)	20.0 (3.7)	20.0 (3.8)
Previous use of biologic DMARD, n (%)	2 (13)	3 (19)	5 (16)
Previous use of non-methotrexate conventional synthetic DMARD, n (%)	4 (27)	3 (19)	7 (23)
Antibiotics within 1 year of inclusion, n (%)	4 (27)	6 (38)	10 (32)
Positive IgG serology‡‡			
Yersinia, n (%)	0 (0)	1 (6)	1 (3)
Campylobacter, n (%)	0 (0)	0 (0)	0 (0)
Salmonella, n (%)	0 (0)	1 (6)	1 (3)
Faecal calprotectin, mg/kg§§	29 (17–63)	27 (15–72)	28 (15–68)
Stool frequency per week	10.4 (3.7)	10.7 (4.3)	10.6 (4.0)
Bristol Stool Scale score¶¶	4.3 (1.3)	3.6 (1.3)	4.0 (1.3)
Presence of gastrointestinal symptoms within the last week			
Abdominal pain¶	14.1 (14.7)	23.9 (23.4)	19.2 (20.0)
Pyrosis, n (%)	2 (13)	2 (13)	4 (13)
Nausea, n (%)	6 (40)	3 (19)	9 (29)
Vomiting, n (%)	1 (7)	0 (0)	1 (3)
Mucus in the stool, n (%)	3 (20)	1 (6)	4 (13)
Blood in the stool, n (%)	1 (7)	0 (0)	1 (3)
Smoking status			
Current, n (%)	5 (33)	4 (25)	9 (29)

Continued

Table 2 Continued

Characteristics	FMT (n=15)	Sham (n=16)	Total (N=31)
Previous, n (%)	4 (27)	8 (50)	12 (39)
Never, n (%)	6 (40)	4 (25)	10 (32)
Alcohol consumption, units per week	0.9 (0.9)	0.8 (0.6)	0.8 (0.7)

Data are mean (SD) or n (%) unless otherwise stated.
*Time since diagnosis of psoriatic arthritis is presented as median and IQR.
†Presence of rheumatoid factor (IgM) and anticitrullinated peptide antibody was not accessed in one patient from the FMT group.
‡HAQ-DI scores range from 0 to 3, with higher scores indicating greater disability.
§SPARCC Enthesitis Index scores range from 0 to 16, with higher scores indicating more severe disease.
¶This evaluation is based on a VAS of 0–100, with higher scores indicating greater disease activity or pain.
**PASI scores range from 0 to 72, with higher scores indicating more severe disease.
††DLQI scores range from 0 to 30, with higher scores indicating more severe disease.
‡‡Serology specification: *Salmonella enteritidis* and *S. typhimurium*, *Yersinia enterocolitica*, and *Campylobacter coli* and *C. jejuni*.
§§Median (IQR). Lower detectable limit of faecal calprotectin is 15 mg/g.
¶¶Bristol Stool Scale scores range from 1 to 7, with types 1 and 2 indicating constipation, types 3 and 4 normal, and types 5–7 tending towards diarrhoea (loose to watery stool).
DLQI, Dermatology Life Quality Index; DMARD, disease-modifying antirheumatic drug; FMT, faecal microbiota transplantation; HAQ-DI, Health Assessment Questionnaire Disability Index; HLA-B27, human leukocyte antigen-B27; PASI, Psoriasis Area Severity Index; SPARCC, Spondyloarthritis Research Consortium of Canada; VAS, Visual Analogue Scale.

DISCUSSION

Modification of the immunogenic, intestinal microbial communities and their metabolites associated with immune-mediated diseases has been highlighted as a possible way to either directly or indirectly modulate a dysregulated immune response in the recipient. In this first FMT trial in PsA, we performed one upper, single-donor FMT to evaluate safety and efficacy in patients with active, peripheral PsA treated with steady state dose methotrexate (≥15 mg/week). Most importantly, one FMT appeared to be safe in this patient population. Contrary to our hypothesis, the rate of treatment failure (ie, patients needing treatment intensification) was significantly higher in the FMT group than in the sham group. Likewise, the HAQ-DI improved more in sham-treated (indicating better physical function) than in FMT-treated patients.[26] The event curve (figure 2) demonstrates that treatment failure occurred very quickly after the procedure in patients receiving FMT. Because of the comparable disease activity between groups at baseline, our findings suggest that FMT from selected donors can worsen the symptoms of PsA. This contrasts a case report of a patient with PsA where FMT seemed to assert beneficial effects on the arthritic disease.[27]

FMT for other immune-mediated conditions such as IBD has demonstrated local therapeutic immune-modulating abilities, and disease flares following FMT seem to occur in similar rates among control group patients.[13] Although transient increase in C reactive protein and self-limiting fever are well-known side effects associated with an immunological response in patients receiving FMT for *C. difficile* infection and/or IBD,[28] based on the existing evidence, thoroughly screened stool for FMT is in general considered safe and has not been related to severe immune responses. Hence, our findings add to the growing body of evidence suggesting a gut–joint axis in the pathogenesis of PsA.[29 30]

A strength of our study is that we designed the trial to provide results relevant to clinical practice. This included the use of FMT products from routine treatment of recurrent *C. difficile* infections, the timing of the intervention, the long follow-up, the allowance of antibiotics and other medication during follow-up (except for non-methotrexate disease-modifying antirheumatic drugs), and the lack of diet restrictions. Due to the randomised design, allocation concealment, masking of patients and treating rheumatologists/outcome assessors, high treatment adherence, low attrition, no missing data on the primary outcome, and only few cases of protocol violations (see online supplemental text S1), we deem the risk of bias to be low. Furthermore, because our decision to stop the trial before reaching 80 patients was made independently of the trial findings, we do not expect that this decision has biased the results.[31] Nevertheless, the small study population did affect the precision of the trial estimates, making the conclusions less robust.

Limitations include the initiation of additional disease-modifying antirheumatic drugs in patients experiencing treatment failure, which may likely have exerted positive effects on the secondary outcomes (HAQ-DI, ACR20 and SPARCC Enthesitis Index) evaluated at week 26. Although this may especially be true for the FMT group, where the majority of participants received biologics within the first 12 weeks of the trial, this explanation is not valid in the sham group, where less than one in five received additional treatment. Hence, the significant clinical improvement observed in this group suggests the presence of trial participation effects.[32] Findings from a qualitative study nested within the trial support this notion.[33] Nevertheless, based on the double-blind, randomised design and the fairly comparable demographics and disease characteristics of FMT-treated and sham-treated patients at baseline, we have no reason to believe that exposure, intensity and susceptibility of these effects differed between groups.[34]

The compositional nature of the primary endpoint combining both the patient's values, preferences and needs in relation to the outcome domains that were important to him/her (eg, pain, physical function, fatigue and social participation) with the physician's assessment of disease (eg, musculoskeletal disease activity and systemic inflammation), in addition to the very high ACR50/70 response in the sham group, complicated the interpretation of the trial results. For example, we cannot rule out that parts of patients' perceptions of the disease, and the resulting motivation for receiving additional treatment, could have been affected by elements of the disease not related to active inflammation (which was our hypothesised target of the FMT) such as central sensitisation and structural damage. In addition, while measures of disease activity appeared comparable between groups at baseline, the (random) imbalance in sex and disease duration could hypothetically have had an effect on the between-group differences in the primary and secondary outcomes.

Table 3 Adverse events through 26 weeks

AE events*	FMT (n=15) PYRS=7.4	Sham (n=16) PYRS=8.5
Total AEs, n	57	53
Total patients with AE, n (%)	14 (93)	14 (88)
Total SAEs, n	0	0
Total patients with SAE, n (%)	0	0
Withdrawal due to any AE, n (%)	0	0
Withdrawal due to any SAE, n (%)	0	0
Death, n (%)	0	0
AEs of special interest†		
Change from baseline in abdominal pain, VAS‡§	8.8 (1.9 to 15.7)	−18.0 (−24.5 to −11.4)
Change from baseline in Bristol Stool Scale score, types 1–7§¶	−0.2 (−0.7 to 0.3)	0.0 (−0.5 to 0.5)
Change from baseline in faecal calprotectin, mg/kg**	86 (−60 to 324)	16 (−36 to 69)
Total episodes of patient-reported fever, n	13	12
Patients treated with antibiotics, n (%)	2 (13)	5 (31)
Nausea, n (%)	9 (60)	7 (44)
Reflux, n (%)	8 (53)	8 (50)
Vomiting, n (%)	6 (40)	1 (6)
Blood in the stool, n (%)	0 (0)	1 (6)
Mucus in the stool, n (%)	4 (27)	0 (0)
Elevated plasma alanine aminotransferase (men >70 U/L; women >45 U/L), n (%)	2 (13)	5 (31)
Abnormal white cell count		
Low (<3.50×10^9/L), n (%)	0 (0)	0 (0)
High (>8.80×10^9/L), n (%)	5 (33)	6 (38)
Abnormal platelet count		
Low (men <145×10^9/L; women <165×10^9/L), n (%)	0 (0)	1 (6)
High (men >350×10^9/L; women >400×10^9/L), n (%)	1 (7)	2 (13)
Low level of haemoglobin (men < 134 g/L; women < 118 g/L), n (%)	3 (20)	1 (6)
Change in weight, kg††	0.9 (−0.6 to 2.3)	−0.1 (−1.5 to 1.2)
Change in haemoglobin A1c level, mmol/mol††	1.43 (0.35 to 2.52)	0.31 (−0.70 to 1.33)
Change in plasma cholesterol level, mmol/L††	0.23 (−0.03 to 0.49)	−0.08 (−0.31 to 0.16)
Change in plasma low-density lipoprotein cholesterol level, mmol/L††	0.13 (−0.08 to 0.34)	−0.14 (−0.33 to 0.05)
Change in plasma high-density lipoprotein cholesterol level, mmol/L††	0.05 (−0.05 to 0.15)	−0.01 (−0.10 to 0.08)
Change in plasma triglyceride level, mmol/L††	−0.01 (−0.37 to 0.34)	0.15 (−0.17 to 0.47)
Other reported AEs, n (%)†		
Pneumonia	1 (7)	1 (6)
Gastroenteritis	0 (0)	1 (6)
Sinusitis	0 (0)	1 (6)
Wound infection	0 (0)	1 (6)
Cystitis	1 (7)	1 (6)
Conjunctivitis	0 (0)	1 (6)
Diverticulitis	1 (7)	0 (0)
Influenza symptoms	0 (0)	1 (6)
Bronchial asthma exacerbation	1 (7)	0 (0)
Benign paroxysmal positional vertigo	0 (0)	1 (6)
Flatulence	1 (7)	0 (0)
Uncontrolled defaecation	1 (7)	0 (0)
Facial capillary rupture	0 (0)	1 (6)

Full analysis set: all patients who were randomly assigned to a study group and had exposure to the intervention (FMT or sham) independent of group.
*Any AEs: data are number of events or number of patients (%). Treatment with antibiotics was not counted as a separate AE.
†AEs of special interest and other reported AEs: dichotomous data are number of patients with at least one episode (%) unless otherwise specified.
‡VAS of 0–100, with higher scores indicating greater disease activity or pain.
§Abdominal pain and Bristol Stool Scale score are reported as the main effect of group based on a repeated measures linear mixed model across time points (baseline, week 1, week 2, week 3, week 4, week 12 and week 26).
¶Bristol Stool Scale score ranges from 1 to 7, with types 1 and 2 indicating constipation, types 3 and 4 normal, and types 5–7 tending towards diarrhoea (loose to watery stool).
**Faecal calprotectin is reported as the main effect of group based on a repeated measures linear mixed model (bootstrap SE) across time points (baseline, week 4, week 12 and week 26).
††The metabolic markers (weight, haemoglobin A1c, cholesterol and triglyceride) are evaluated as change from baseline to week 26. Positive values signify an increase, whereas negative values signify a decrease.
AE, adverse event; FMT, faecal microbiota transplantation; PYRS, person years at risk; SAE, serious adverse event; VAS, Visual Analogue Scale.

Furthermore, the participants of our trial constituted primarily adults with active, polyarticular PsA, which is a relatively rare condition in clinical practice.[20] Consequently, although the spectrum of patients with PsA that are enrolled in pharmacological trials is skewed towards this study population, the ability to generalise our findings to the majority of patients with PsA is limited. Moreover, because only 10 participants (32%) had active skin psoriasis at the time of inclusion, this trial was not suited for assessment of the potential of microbiota modulation in cutaneous inflammation. Finally, our study was neither large enough nor long enough to evaluate uncommon serious AEs and long-term risks.[35]

Previous findings in patients with ulcerative colitis indicate that one FMT regimen performed within 1 week is insufficient to maintain long-lasting (12 months) local anti-inflammatory response in the majority of patients with a beneficial response after 8 weeks.[36] Hence, we could have missed early clinical significant changes in the secondary outcomes that abated before the 26-week evaluation. In addition, pooled donor batches and high-intensity induction of FMT followed by frequent administration of donor transplant seem to enhance the chances for clinical remission in ulcerative colitis.[36–38] Perhaps as importantly, previous successes of FMT in IBD appear to have been driven by 'super-donors' characterised by the presence or absence of specific bacteria species.[36 39] To further complicate this picture, matching of donor and recipient could be another important factor to consider.

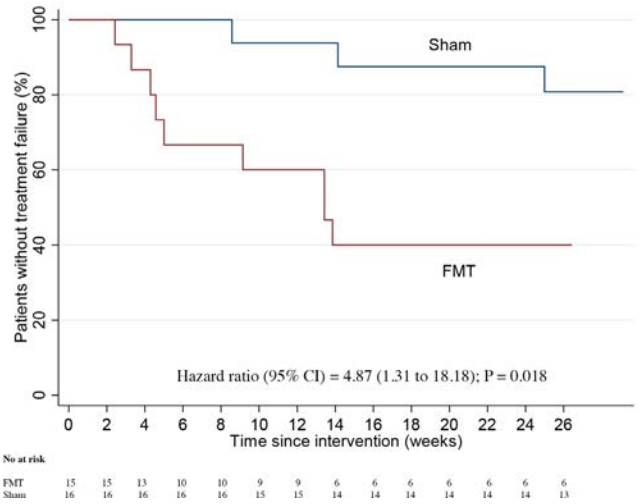

Figure 2 Event curves of treatment failure by intervention group from baseline to week 26. Time-to-treatment failure was analysed using survival analysis as Kaplan-Meier curves from baseline to week 26. Cox regression was used to provide an estimate of the HR with 95% CI and p value. The number of participants remaining at risk is displayed below the horizontal axis. Treatment failure was defined as need for at least one of the following: more than one intra-articular glucocorticoid injection, and non-methotrexate conventional synthetic and/or biologic disease-modifying antirheumatic drugs. FMT, faecal microbiota transplantation.

In conclusion, further investigation is needed to explore whether extrinsic factors related to the FMT method, such as single versus multiple donor batches, fresh versus frozen products, aerobic versus anaerobic environment, type of stool preparation protocol, storage time, freeze–thaw cycles, pretreatment preparation such as bowel lavage and antibiotics, delivery form, and overall treatment strategy (dose and frequency), may influence the outcome of FMT in PsA.[40] Future FMT trials could pursue an approach that is similar to the ones used in IBD studies.[41] Moreover, evaluation of dysbiosis in patients (or donors) prior to trial entry could hypothetically

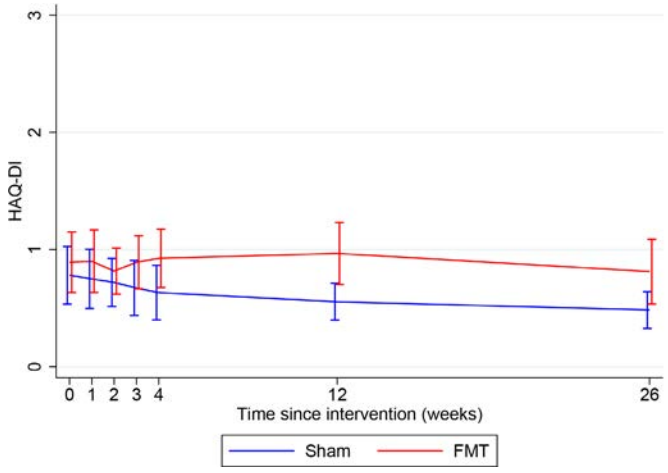

Figure 3 HAQ-DI scores by treatment group from baseline to week 26. Least squares means calculated from the repeated measures linear mixed model: intention-to-treat population. Bars indicate 95% CI. HAQ-DI scores range from 0 to 3, with higher scores indicating greater disability. FMT, faecal microbiota transplantation; HAQ-DI, Health Assessment Questionnaire Disability Index.

enhance FMT efficiency. Other mechanisms that should be thoroughly investigated in future studies include the degree and durability of donor microbiota engraftment, changes in patients' microbiota following FMT and comparator intervention, and characterisation of 'good' and 'bad' donations. Indeed, the lack of comprehensive microbiota analyses is a limitation of the current study.

In this preliminary randomised controlled trial with focus on safety, we did not observe any serious AEs. Although no firm conclusions can be drawn from this small trial and despite the similar proportions of ACR20 responders between groups at 26 weeks, our findings indicate that FMT may lead to worsening of PsA, suggesting a role of the intestinal microbiota in downstream immune effects of this disease. Larger, randomised trials of FMT where a sufficient amount of participants will be included combined with exploration of immunological effects and indepth analyses of the composition and functional potential of the microbiota in donor and recipients should be undertaken to further investigate the safety and potential benefits of therapeutic targeting of the gut–joint axis in immune-mediated arthritis.

Patient and public involvement

Patients and the public played an important part in all elements of the research process beyond the conception of the trial. In the design phase, we asked patients who attended the outpatient clinic to provide input on ethical challenges and trial logistics, especially regarding donor selection and method of FMT administration. Patients also gave feedback to the wording and design of patient information material and recruitment flyers. During the undertaking of the trial, we asked patient organisation and the public to assist the refinement of our recruitment strategy and to help with publicity and funding. Following the last trial visit and before unmasking, we interviewed 10 participants about their trial experiences, which provided insight into their motivation for participation, impact on everyday life, FMT acceptability, and factors related to the trial that may have promoted trial participation effects (the results of this qualitative study will be presented elsewhere). In the dissemination phase of the trial, we invited a patient research partner (MW), who did not participate in the trial, to become coauthor. He made valuable suggestions for improving the reporting of the study and helped us clarify the main findings as seen from a patient's perspective. Finally, we disseminated the results of the trial in a letter to all study participants and invited them to attend an online meeting, where we further explained and discussed the findings of the trial.

Author affiliations
[1]Rheumatology Research Unit, Department of Rheumatology, Odense University Hospital, Odense, Denmark
[2]Department of Clinical Research, University of Southern Denmark, Odense, Denmark
[3]Department of Medical Gastroenterology, Odense University Hospital, Odense, Denmark
[4]Section of Rheumatology, Department of Medicine, Svendborg Hospital, Svendborg, Denmark
[5]Reumaklinik Fyn, Odense, Denmark
[6]Patient Research Partner, Amsterdam, The Netherlands
[7]OPEN – Open Patient data Explorative Network, Odense University Hospital, Odense, Denmark
[8]IRS-Center Sønderjylland, University Hospital of Southern Denmark, Aabenraa, Denmark
[9]Institute of Molecular Medicine, University of Southern Denmark, Odense, Denmark
[10]Laboratory of Genomics and Molecular Biomedicine, Department of Biology, University of Copenhagen, Copenhagen, Denmark
[11]Institute of Metagenomics, BGI-Shenzhen, Shenzhen, China
[12]Department of Clinical Immunology, Odense University Hospital, Odense, Denmark

[13]Department of Clinical Microbiology, Odense University Hospital, Odense, Denmark
[14]Section for Biostatistics and Evidence-Based Research, the Parker Institute, Bispebjerg and Frederiksberg Hospital, Copenhagen, Denmark

Twitter Søren Andreas Just @JustSoren

Acknowledgements We thank all participants for their contribution. We thank CS Klinkby, trial nurse, for assistance in relation to the conduct of the trial visits. We also thank L Albjerg (biomedical laboratory technologist), AC Nilsson (consultant), KF Rasmussen (consultant) and J Georgsen (consultant) at the Department of Clinical Immunology, Odense University Hospital, Denmark, for assisting in the implementation of the FMT stool bank.

Contributors TE, MSK, RC and JK designed the study. MSK and TE were responsible for funding. MSK, DKH and HMH were responsible for donor recruitment, screening and FMT product manufacture. JK and FMP were responsible for the FMT procedure. MSK, TE, HCH, HLM, JKP, PA and SAJ were responsible for patient recruitment. TE, HCH and HLM acquired the clinical data. MSK, SM and RC analysed the clinical data. TE, MSK, RC, JK, HLM, HCH, KK and MW interpreted the results. MSK, TE and RC drafted the report. All authors critically reviewed the report and approved the final version. MSK and TE are guarantors. The corresponding author (TE) attests that all listed authors meet the authorship criteria and that no others meeting the criteria have been omitted.

Funding This study was supported by the Danish Rheumatism Association, the Danish Psoriasis Research Foundation, the University of Southern Denmark Research Fund, the Research Fund of Odense University Hospital, the Danish Regions (Medicinpuljen), the Region of Southern Denmark Research Fund and Novartis Healthcare (unrestricted grant).

Competing interests VA declares personal fees from Merck (MSD) and personal fees from Janssen, outside the submitted work. RC declares a core grant to his institution (Parker Institute, Bispebjerg and Frederiksberg Hospital) from the Oak Foundation (OCAY-18-774-OFIL) and honorariums paid to his institution in relation to the following activities: lecture, research methods (Pfizer, DK; 2017); lecture, GRADE lecture (Celgene, DK; 2017); ad board lecture, CAM (Orkla Health, DK; 2017); project grant: 'GreenWhistle' (Mundipharma, 2019); lecture: diet in RMD (Novartis, DK; 2019); consultancy report, Network MA's (Biogen, DK; 2017); ad board lecture, GRADE (Lilly, DK; 2017); consultancy report, GRADE (Celgene, 2018); and lecture, Network MA's (LEO; 2020).

Patient consent for publication Not required.

Ethics approval The study was approved by the Regional Committees on Health Research Ethics for Southern Denmark (DK-S-20150080).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. Requests on data sharing can be made by contacting the corresponding author. Data will be shared after review and approval by the trial scientific board, and terms of collaboration will be reached together with a signed data access agreement.

ORCID iDs
Jens Kjeldsen http://orcid.org/0000-0001-8148-6572
Hans Christian Horn http://orcid.org/0000-0002-0792-0366
Heidi Lausten Munk http://orcid.org/0000-0002-2212-6283
Jens Kristian Pedersen http://orcid.org/0000-0003-2531-9547
Maarten de Wit http://orcid.org/0000-0002-8428-6354
Sören Möller http://orcid.org/0000-0003-0858-4269
Vibeke Andersen http://orcid.org/0000-0002-0127-2863
Karsten Kristiansen http://orcid.org/0000-0002-6024-0917
Dorte Kinggaard Holm http://orcid.org/0000-0002-8156-7177
Robin Christensen http://orcid.org/0000-0002-6600-0631
Torkell Ellingsen http://orcid.org/0000-0003-0426-4962

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TRANSLATIONAL SCIENCE

Polygenic Risk Scores have high diagnostic capacity in ankylosing spondylitis

Zhixiu Li [1], Xin Wu,[2] Paul J Leo,[1] Erika De Guzman,[3] Nurullah Akkoc [4], Maxime Breban [5,6,7], Gary J Macfarlane [8,9], Mahdi Mahmoudi [10], Helena Marzo-Ortega [11,12], Lisa K Anderson,[3] Lawrie Wheeler,[3] Chung-Tei Chou,[13,14] Andrew A Harrison [15], Simon Stebbings [16], Gareth T Jones [8,9], So-Young Bang,[17] Geng Wang,[18] Ahmadreza Jamshidi,[10] Elham Farhadi,[10] Jing Song,[2] Li Lin,[2] Mengmeng Li,[2] James Cheng-Chung Wei [19,20,21], Nicholas G Martin,[22] Margaret J Wright,[23] MinJae Lee [24], Yuqin Wang,[25] Jian Zhan,[26] Jin-San Zhang,[27,28] Xiaobing Wang [29], Zi-Bing Jin [30], Michael H Weisman,[31] Lianne S Gensler,[32] Michael M Ward [33], Mohammad Hossein Rahbar,[34] Laura Diekman,[35] Tae-Hwan Kim [17], John D Reveille [35], Bryan Paul Wordsworth [36], Huji Xu [2,37,38], Matthew A Brown [27,39], TCRI AS Group

Handling editor Josef S Smolen

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2020-219446).

For numbered affiliations see end of article.

Correspondence to
Professor Matthew A Brown, NIHR Biomedical Research Centre at Guy's and St Thomas' NHS Foundation Trust and King's College London, London, SE1 9RT, United Kingdom; matt.brown@kcl.ac.uk and Professor Huji Xu, Department of Rheumatology and Immunology, Changzheng Hospital, The Second Military Medical University, Shanghai, China; xuhuji@smmu.edu.cn

ZL and XW contributed equally. HX and MAB contributed equally.

Received 4 November 2020
Revised 23 March 2021
Accepted 29 March 2021
Published Online First 20 April 2021

To cite: Li Z, Wu X, Leo PJ, *et al*. *Ann Rheum Dis* 2021;**80**:1168–1174.

ABSTRACT

Objective We sought to test the hypothesis that Polygenic Risk Scores (PRSs) have strong capacity to discriminate cases of ankylosing spondylitis (AS) from healthy controls and individuals in the community with chronic back pain.

Methods PRSs were developed and validated in individuals of European and East Asian ethnicity, using data from genome-wide association studies in 15 585 AS cases and 20 452 controls. The discriminatory values of PRSs in these populations were compared with other widely used diagnostic tests, including C-reactive protein (CRP), *HLA-B27* and sacroiliac MRI.

Results In people of European descent, PRS had high discriminatory capacity with area under the curve (AUC) in receiver operator characteristic analysis of 0.924. This was significantly better than for *HLA-B27* testing alone (AUC=0.869), MRI (AUC=0.885) or C-reactive protein (AUC=0.700). PRS developed and validated in individuals of East Asian descent performed similarly (AUC=0.948). Assuming a prior probability of AS of 10% such as in patients with chronic back pain under 45 years of age, compared with *HLA-B27* testing alone, PRS provides higher positive values for 35% of patients and negative predictive values for 67.5% of patients. For PRS, in people of European descent, the maximum positive predictive value was 78.2% and negative predictive value was 100%, whereas for *HLA-B27,* these values were 51.9% and 97.9%, respectively.

Conclusions PRS have higher discriminatory capacity for AS than CRP, sacroiliac MRI or *HLA-B27* status alone. For optimal performance, PRS should be developed for use in the specific ethnic groups to which they are to be applied.

Key messages

What is already known about this subject?

► *HLA-B27* testing is widely used in the diagnostic pathway in ankylosing spondylitis (AS), but only captures a moderate proportion (~20%) of the overall genetic risk for the disease.

What does this study add?

► Polygenic Risk Scores (PRSs) for AS perform better than *HLA-B27* testing and other standard diagnostic tests employed in AS including C-reactive protein measurement and MRI scanning.

How might this impact on clinical practice or future developments?

► PRS for AS should be used to assist diagnosing AS among patients with chronic back pain.

INTRODUCTION

Ankylosing spondylitis (AS) affects approximately 0.2%–0.6% of individuals of European descent and Chinese.[1 2] Early treatment with biologic therapies in those with more severe forms of the disease achieves more effective clinical responses[3] and probably reduces the rate joint fusion in the long term.[4] However, other causes of chronic back pain are common in the community, and AS is responsible for only a minority of these cases. It can be difficult to distinguish AS from other causes of back pain, particularly early in the disease with the consequence that the diagnosis of AS is often significantly delayed; many surveys undertaken in a variety of different health systems suggest an average delay of 6–10 years.[5–7] A recent North American survey reported that fewer than half (37.1%) of patients with AS reported that they were correctly diagnosed within 1 year of seeking medical attention, and 32.8% waited more than a decade to receive the diagnosis.[7] Population surveys suggest that as many as 80% of cases in the community remain

undiagnosed[8] and therefore may not receive appropriate effective treatment. There is thus a great need for improved testing to improve early accurate diagnosis.

Currently, the most widely used tests for AS in those with chronic back pain are measurements of acute phase reactants, such as erythrocyte sedimentation rate and C-reactive protein (CRP), genetic testing for *HLA-B27* and imaging—either plain radiographs or MRI of the sacroiliac joints.[9] However, each of these tests has limitations. In brief, acute phase reactants and MRI are only positive after disease develops and are therefore not useful for predicting disease risk. Acute phase reactants have only moderate sensitivity and specificity, particularly in early disease. MRI is expensive and is not universally available. Genetic factors are the major determinants of the risk of developing AS, with heritability assessed in twins of >90%.[10 11] Although *HLA-B27* alone contributes 20% of the variation in disease risk,[12] the remainder of the genetic risk is determined by thousands of common genetic variants, each of which has only a very small effect. Polygenic Risk Scores (PRS) use combinations of hundreds to thousands of genetic variants to quantify an individual's genetic risk of disease. Unlike *HLA-B27* testing which is categorical or dichotomous in outcome, PRS are continuous measures. They are of particularly strong predictive value for low-frequency diseases with high heritability,[13] such as AS. Here, we describe the development and validation of PRS for AS in two different ethnic groups and compare its performance to standard screening or diagnostic tests.

METHODS

Study population

AS was defined according to the modified New York criteria.[14] Following genotyping quality control, there were 8244 cases and 14 274 controls of western European descent; 6001 cases and 4493 controls of East Asian (Chinese) descent; and 1340 cases and 1685 controls of Turkish and Iranian origin, respectively. Written informed consent was obtained from all cases, with approval from the relevant research ethics authorities at each participating centre. Cohort details are provided in online supplemental table S1.

Genetic data

Samples were genotyped using the Illumina Core-Exome SNP genotyping microarray, according to the manufacturer's recommendations (chip versions used per cohort are provided in online supplemental table S1). Bead intensity data were processed and normalised for each sample, and genotypes called, using Genome Studio V.2.0 software (GenomeStudio Software Downloads (illumina.com)). Standard quality control measures as outlined in the Supplementary Methods were applied including identification and exclusion of cryptic-related samples, exclusion of samples with an outlying heterozygosity rate (3 SD from the mean in each cohort) or excess missingness (>5%). Single nucleotide polymorphisms (SNPs) with genotyping missing rate >2%, p value of Hardy-Weinberg equilibrium test $<1\times10^{-6}$, or with allele frequency <1% were removed. Population stratification was accessed using Shellfish (http://www.stats.ox.ac.uk/~davison/software/shellfish/shellfish.php). PRS analyses were performed with and without inclusion of principal components and gender as covariates. Results including principal components and gender as covariates are reported in online supplemental table S2 and are very similar to the results not including these covariates.

HLA-B27 imputation was performed using SNP2HLA, using a deep sequencing Chinese reference panel (n=10 689)[15] for East Asian samples and Type 1 Diabetes Genetics Consortium (n=5225) panel of combined HLA types and MHC SNP genotypes for all other subjects.[16]

PRS were calculated for each individual using the adaptive MultiBLUP algorithm (implemented in the software LDAK V.5.0).[17] LDAK first divides the genetic data into chunks of size 75 000 bp and then performs association test for all the chunks and thinned out SNPs in strong linkage disequilibrium. The significant chunks with p value $<1\times10^{-5}$ and all adjacent chunks with p value <0.01 are merged into regions. Then the variance components and effect size of SNPs are estimated, and the effect size of the SNPs used to calculate the PRS. A 10-fold cross-validation analysis was performed as internal validation; a separate external validation was performed in the British and North American subjects, as well as through comparison of performance of PRS trained in either European descent or East Asian subjects, then validated in a separate ethnic group. In regard to cross-validation studies, the case–control cohort being studied is divided into 10 equal folds randomly with same case–control ratio. Nine folds of samples were used as a training set and the remaining fold of samples was retained as the validation data for testing the model generated by the training set. The process was repeated 10 times, with each of the 10-folds used only once as the validation data. The out-of-fold predictions based on the effect sizes of the selected SNPs were obtained for the test fold. All the predictions of 10 test folds were merged, after which statistical analysis was performed using all out-of-fold test set predictions to maximise sample size for internal testing. The resulting weighted predictors were then applied to the test cohort to obtain per sample scores from which the area under the curve (AUC) was obtained using receiver operator characteristic (ROC) analysis. R package pROC was used to calculate the 95% CI of the AUC and also compare AUCs from two models.[18] Positive (PPV) and negative predictive values (NPV) were then calculated for PRS centiles, assuming different prior probabilities of AS. The continuous net reclassification improvement (NRI),[19] a statistic that aims to quantify differences in classification performance of different models, was calculated using the R package PredictABEL[20] and used to compare accuracy of diagnostic assignment by *HLA-B27* testing and PRS.

RESULTS

ROC analyses of test discriminatory capacity are summarised in table 1. In 10-fold cross-validation in this case–control cohort, the PRS had AUC of 0.924 (95% CI 0.920 to 0.928) (figure 1). The AUC of *HLA-B27* testing alone was 0.869 (95% CI 0.865 to 0.874), which was statistically significantly less discriminatory than the PRS ($p<2.2\times10^{-16}$). Additionally, the NRI was positive (0.717, 95% CI 0.692 to 0.743), confirming that the PRS is an improvement on *HLA-B27* alone. A PRS including only non-MHC SNPs performed less well (AUC 0.782), as did a PRS including only 103 (genotyped or imputed) loci previously reported to have achieved genome-wide significance in AS (AUC=0.659).[21] MRI has a reported sensitivity of 85% and specificity of 92% in AS,[22] which correlates with an AUC of 0.885. CRP has a reported sensitivity of 50% and specificity of 80% for the disease (AUC=0.7).[23]

To test the performance of the PRS using external validation, the European descent cases were divided into British and North American cohorts, and controls divided in the same proportion as the two case cohorts. PRS was then

Table 1 ROC analysis findings (AUC) of genetic risk scores in different populations

	Population tested in			
Predictors	**European**	**East Asian**	**Iranian**	**Turkish**
HLA-B27 alone	0.869 (0.865–0.874)	0.901 (0.895–0.906)	0.831 (0.807–0.854)	0.821 (0.804–0.838)
European non-MHC PRS	0.782 (0.776–0.788)*	0.594 (0.539–0.560)	0.534 (0.500–0.569)	0.568 (0.542–0.595)
European overall PRS	0.924 (0.920–0.928)*	0.788 (0.779–0.796)	0.852 (0.826–0.879)	0.854 (0.836–0.872)
East Asian non-MHC PRS	0.555 (0.547–0.563)	0.731 (0.722–0.741)*	0.565 (0.531–0.598)	0.554 (0.528–0.581)
East Asian overall PRS	0.880 (0.875–0.887)	0.948 (0.943–0.952)*	0.872 (0.848–0.895)	0.840 (0.821–0.860)
MRI EUR	0.885			
MRI CH[41]	0.62			
CRP	0.7			

*10-fold cross-validation. All other PRS AUC values are external validation statistics.
AUC, area under the curve; CRP, C-reactive protein; PRS, Polygenic Risk Score; ROC, receiver operator characteristic .

developed in the British training set (n=6499 cases, 12 163 controls) and externally validated in the North American case–control cohort (n=1128 cases, 2111 controls). The PRS in the North American cohort had AUC of 0.928 (95% CI 0.918 to 0.939), significantly higher than *HLA-B27* alone (0.895, 95% CI 0.883 to 0.906, $p=1.73\times10^{-5}$) (online supplemental figure S1). These findings are very similar to the cross-validation analysis of the overall dataset reported above.

The PRS developed in all the European descent subjects, with 3994 SNPs (including 2244 major histocompatibility complex (MHC) SNPs), had moderate discriminatory capacity in East Asian, Iranian and Turkish cases and controls (AUC=0.788, 0.852 and 0.854, respectively), better than the performance of HLA-B27 alone in the Iranian and Turkish cohorts, but not in East Asians. In contrast, the PRS developed in East Asian subjects, then tested by cross-validation (i.e. also in East Asian subjects), had much better discriminatory capacity (AUC=0.948, 95% CI 0.943 to 0.952) than did the PRS developed in European descent subjects when tested in East Asian subjects. The PRS involving 8659 SNPs (including 2417 MHC SNPs) developed with all the East Asian subjects also performed well in European descent subjects (AUC=0.880, online supplemental figure S2), better than the discriminatory performance of HLA-B27 in each of the other three populations tested.

In clinical practice, the utility of all such tests depends on the prior probability of the disease concerned. The PPV and NPV of the PRS and *HLA-B27* in European subjects are presented in figure 2 in the setting of a patient under 45 years of age, attending a physician with a history of back pain for 3 months or more. Published studies report that in this setting the prior probability of AS is ~30%,[24–26] but as this may vary according to referral patterns, we have additionally provided findings for prior probabilities of 10% and 20% (online supplemental figures S5 and S6; East Asian specific findings are presented in online supplemental figures S7-S9). Assuming a prior probability for AS of 30%,

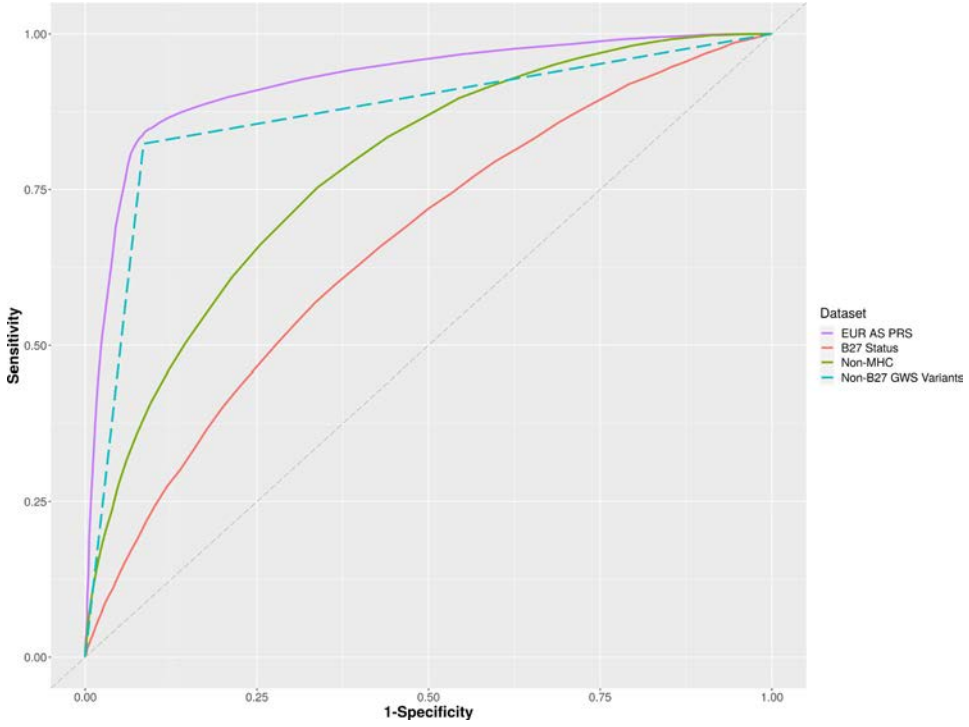

Figure 1 Receiver operating characteristic curve plot of performance of Polygenic Risk Scores (PRS) (purple dashes, area under the curve (AUC)=0.924), HLA-B27 (aqua dashes, AUC=0.869), PRS less major histocompatibility complex (MHC) (green line, AUC=0.782) and genome-wide significant loci only (red line, AUC=0.659).

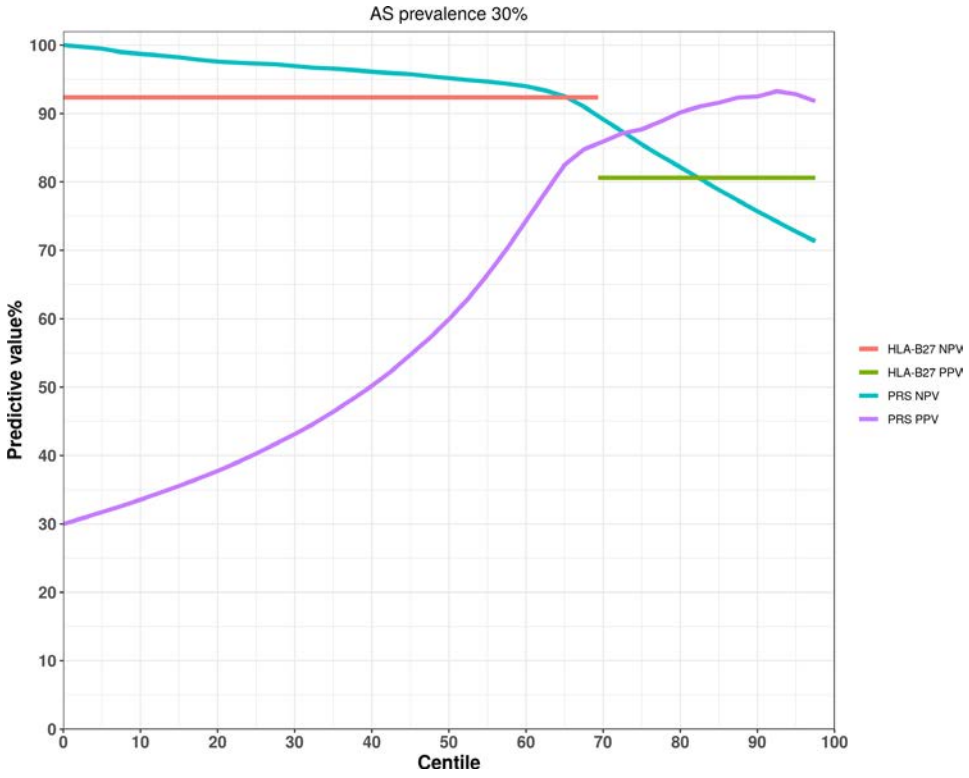

Figure 2 Positive (PPV) and negative predictive values (NPV) of Polygenic Risk Scores (PRS) and HLA-B27 for ankylosing spondylitis (AS), assuming prior probability of AS of 30%, among Europeans. Centiles refer to the population distribution of the PRS.

an *HLA-B27* test will be positive in 31% of those tested with a PPV of 80.6%, and in the 69% of those with a negative test, the NPV is 92.4%. Using the PRS, the PPV is >80.6% for top 35% of those screened, and achieves a higher maximum value (93.3%) than does *HLA-B27* (80.6%) (figure 2). The PRS NPV will be >92.4% for 65% of those screened, and also achieves a higher maximum value (99.6%) than does *HLA-B27* (92.4%). Considering the situation where only 10% of screened patients have AS, then *HLA-B27* will be positive in 16% of those tested. In this group, *HLA-B27* positivity has a PPV of 51.9%, and a negative result (seen in 84% of screened patients) has an NPV of 97.9%. Using the PRS, the PPV is >51.9% for 35% of patients and has a much higher maximum value (78.2% vs 51.9%). The NPV for the PRS is >97.9% for 65% of patients and achieves a slightly higher maximum value than *HLA-B27* testing (100% vs 97.9%).

Considering general population screening, at least 8% of the European population carry *HLA-B27*,[27] yet only 5% of carriers of this allele will develop AS[28]; as such, no higher PPV can be achieved using *HLA-B27* testing alone. In contrast, for the PRS, the PPV for the top 8% of the population is three times higher (15.1%), and it is higher than 5% for the top 35% of the population. The NPV for *HLA-B27*-negative status is 99.9%, which is exceeded by the PRS for 62.5% of the population.

DISCUSSION

Distinguishing AS from other causes of chronic back pain remains an important issue in rheumatology. *HLA-B27* testing can have a valuable PPV for AS, particularly in clinical settings where the pretest probability of the disease is relatively high compared with the general population. It is therefore included in the Assessment of Spondyloarthritis International Study Group (ASAS) axial spondyloarthritis (axSpA) classification criteria and is an essential criterion for those with no available imaging evidence of disease. *HLA-B27* testing has also been recommended for screening patients with chronic back pain to identify those at higher risk of AS or the related group of diseases axSpA, for referral to specialist services.[23 25] However, *HLA-B27* only contributes ~20% of the overall heritability of AS, which is estimated to be ≥90% overall, indicating a substantial non-MHC component.[29] This suggests that PRS, which capture the common-variant component of heritability, are likely to be much more informative than *HLA-B27* tests alone. Our study confirms this, with the PRS performing better than *HLA-B27* testing in both AUC and continuous NRI analyses, irrespective of the prevalence of AS among those being tested. We confirm these findings both by internal cross-validation and by external validation. For 35% of the population, the PPV is higher for the PRS than for *HLA-B27* testing, and the NPV is higher for >65%. In particular, the peak PPV is substantially higher for the PRS than for *HLA-B27* and is informative for a far higher proportion of patients, as it is a continuous variable whereas HLA-B27 is dichotomous. PRS testing also has higher discriminatory capacity for AS than MRI, and far higher than CRP. Accurate interpretation of MRI scans is known to be dependent on training and experience, and particularly in inexperienced, untrained hands may perform worse than the average reported performance, in which setting PRS may be particularly valuable.

Chronic back pain of >3 months' duration has previously been shown to have very low heritability attributable to common genetic variants (minor allele frequency >0.01) such as those included in our AS PRS (common variant heritability=6.43%[30]–7.6%[31]) and not to be genetically correlated with AS. Therefore, it is unlikely that the AS

PRS will prove less discriminatory in practice in the clinical setting of patients presenting with chronic back pain than the estimates presented here. A limitation of this study is that the performance of the PRS has not been formally tested in this setting, where it will require further evaluation.

axSpA refers to a spectrum of diseases. Patients with radiographic sacroiliitis are classified as having AS, whereas those without X-ray changes are classified as having non-radiographic (nr)-axSpA. The current PRS may have prognostic value in distinguishing the 16%–24% of nr-axSpA cases that are likely to go on to develop AS.[32 33] Whether the PRS we report here will prove more informative than *HLA-B27* testing alone in patients with nr-axSpA itself is unknown. The ASAS have previously demonstrated that patients meeting the ASAS classification criteria for axSpA who do not yet have AS have a much lower average genetic risk score than patients with AS, using only genome-wide significant AS loci.[34] Whether this is because nr-axSpA is actually genetically distinct from AS, or reflects the greater clinical and likely aetiopathogenic heterogeneity of nr-axSpA,[35] will require further study.

As with the use of PRS in the screening of individuals with chronic back pain, its performance in nr-axSpA will also require further study. Similarly, the performance of the PRS in males compared with females, in subjects with environmental risk factors for the disease such as cigarette smoking,[36] and in subsets of patients such as those with extraskeletal manifestations of AS requires further study. In that regard, the excellent performance of a PRS in patients with acute anterior uveitis complicating AS (AUC=0.96; 95% CI 0.955 to 0.966) suggests that at least in some AS subsets the performance of the PRS will be even better than reported here.[37]

PRS testing can be performed using data from any dense SNP microarray. Indeed, the performance of the PRS reported here was high despite our use of a relatively low density SNP microarray—the Illumina Core-Exome chip (>520 000 variants, including many rare and non-polymorphic variants that do not contribute to the PRS). The performance of PRS testing would be likely to improve further with use of microarrays with better SNP coverage, or with whole genome sequencing. It has been estimated that up to 12 million Americans have had SNP microarray testing performed by commercial services such as 23andMe and Ancestry.[38] At little additional cost, these data would probably prove suitable for the calculation of the AS PRS we report, as well as enabling PRS for many other diseases in which they have been shown to be informative. The cost-effectiveness of the PRS we report here needs to be confirmed in further studies. As the genetic profile of AS becomes better understood, the discriminatory capacity of these tests is also likely to increase. For example, it is likely that many of the SNPs included in the PRS at present are not truly associated with AS, but just add noise to the test.

As there is no preventive therapy yet for AS, general population screening to identify patients at high risk of the disease is not recommended except, perhaps, for those at increased risk, such as the relatives of those with AS (given the high sibling recurrence risk of 8.2%).[39] PRS performs significantly better than *HLA-B27* testing alone in the general population, with the PPV of the ~8% of the general population who carry *HLA-B27* being 5%, compared with the peak PPV of the PRS of 15.1%. Similarly, the NPV for the PRS exceeds that of *HLA-B27* testing for most of the population. Although the PPV for PRS testing for general population screening is modest, the test performs well compared with other widely used screening tests. For example, the PPVs for 10-year risk of coronary heart disease of a high total cholesterol (≥240 mg/dL)—a threshold above which many patients will be prescribed cholesterol-lowering therapy—are 10.3% in women and 18.6% in men,[40] similar to the top 20% of PPVs of PRS for AS in general population screening. Among those who have already had SNP microarray testing performed, knowledge of a high AS-PRS even in the absence of symptoms may heighten clinician awareness of the possible diagnosis, reduce delay and assist with earlier appropriate and effective treatment, given the current long diagnostic delays.

Our study shows that the performance of the PRS varies between ethnic groups, although it remains moderately high even when a PRS developed in subjects of (western) European descent is tested in eastern European/west Asian subjects such as Turks and Iranians. The PRS developed specifically for East Asians performed far better in that population than did the European PRS, indicating that at least for populations that are remotely related, ethnic-specific PRSs are preferable.

We conclude that PRS testing for AS has greater discriminatory capacity than *HLA-B27* testing, MRI scanning or CRP testing, either alone or in combination. PRS could be used to screen patients with chronic back pain to identify subjects at increased risk of the disease for referral to secondary care and to assist in diagnosing the condition.

Author affiliations
[1]Queensland University of Technology, Centre for Genomics and Personalised Health, School of Biomedical Sciences, Faculty of Health, Translational Research Institute, Woolloongabba, Queensland, Australia
[2]Department of Rheumatology and Immunology, Shanghai Changzheng Hospital, Second Military Medical University, Shanghai, Shanghai, China
[3]Australian Translational Genomics Centre, Queensland University of Technology (QUT), Translational Research Institute, Woolloongabba, Queensland, Australia
[4]Department of Internal Medicine, Division of Rheumatology, School of Medicine, Manisa Celal Bayar University, Manisa, Turkey
[5]UMR 1173, Inserm, University of Versailles Saint-Quentin, Montigny-le-Bretonneux, France
[6]Service de Rhumatologie, Hôpital Ambroise Paré, Assistance Publique-Hôpitaux de Paris, Boulogne-Billancourt, France
[7]Laboratoire d'Excellence Inflamex, Université Paris Diderot, Sorbonne Paris Cité, Paris, France
[8]Epidemiology Group, Institute of Applied Health Sciences, School of Medicine, Medical Sciences and Nutrition, University of Aberdeen, Foresterhill, Aberdeen, UK
[9]Aberdeen Centre for Arthritis and Musculoskeletal Health, University of Aberdeen, Foresterhill, Aberdeen, UK
[10]Rheumatology Research Center, Tehran University of Medical Sciences, Tehran, Tehran, Iran (the Islamic Republic of)
[11]NIHR Leeds Biomedical Research Centre, Leeds Teaching Hospitals NHS Trust, Leeds, UK
[12]Leeds Institute of Rheumatic and Musculoskeletal Medicine, University of Leeds, Leeds, UK
[13]Division of Allergy, Immunology, Rheumatology, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan
[14]School of Medicine, National Yang-Ming University, Taipei, Taiwan
[15]Department of Medicine, University of Otago Wellington, Wellington, New Zealand
[16]Department of Medicine, Dunedin School of Medicine, University of Otago, Dunedin, New Zealand
[17]Hanyang University Hospital for Rheumatic Diseases, Hanyang University, Seoul, Korea (the Republic of)
[18]University of Queensland Diamantina Institute, University of Queensland, Brisbane, Queensland, Australia
[19]Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan
[20]Department of Medicine, Chung Shan Medical University, Taichung, Taiwan
[21]Graduate Institute of Integrated Medicine, China Medical University, Taichung, Taiwan
[22]QIMR Berghofer Medical Research Institute, Herston, Queensland, Australia
[23]Queensland Brain Institute, University of Queensland, Brisbane, Queensland, Australia
[24]Population & Data Sciences, University of Texas Southwestern Medical Center, Dallas, Texas, USA
[25]State Key Laboratory of Optometry, Ophthalmology, and Vision Science, Affiliated Eye Hospital, Wenzhou Medical University, Wenzhou, Zhejiang, China
[26]Institute for Glycomics, Griffith University, Nathan, Queensland, Australia
[27]Center for Precision Medicine, First Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, China

[28]Institute of Life Sciences, Wenzhou University, Wenzhou, Zhejiang, China
[29]Rheumatology Department, First Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, China
[30]Beijing Institute of Ophthalmology, Beijing Tongren Eye Center, Beijing Tongren Hospital, Capital Medical University, Beijing Ophthalmology & Visual Sciences Key Lab, Beijing, Beijing, China
[31]Department of Medicine/Rheumatology, Cedars-Sinai Medical Center, Los Angeles, California, USA
[32]Division of Medicine/Rheumatology, University of California San Francisco, San Francisco, California, USA
[33]Intramural Research Program, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, Maryland, USA
[34]Internal Medicine, The University of Texas Health Science Center at Houston John P and Katherine G McGovern Medical School, Houston, Texas, USA
[35]Department of Internal Medicine, Division of Rheumatology, McGovern Medical School at The University of Texas Health Science Center, Houston, Texas, USA
[36]NIHR Oxford Musculoskeletal Biomedical Research Unit, Botnar Research Centre, University of Oxford, Oxford, UK
[37]School of Clinical Medicine, Tsinghua University, Beijing, Beijing, China
[38]Peking-Tsinghua Center for Life Sciences, Tsinghua University, Beijing, China
[39]NIHR Biomedical Research Centre at Guy's and Saint Thomas' NHS Foundation Trust and King's College London, London, UK

Twitter Nurullah Akkoc @nurullahakkoc, Gary J Macfarlane @UAberdeenEpi and Gareth T Jones @hteraG_senoJ

Acknowledgements We would like to thank all participating subjects with ankylosing spondylitis and healthy individuals who provided the DNA and clinical information necessary for this study. The TASC study was funded by the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) grants P01-052915 and R01-AR046208. Funding was also received from the University of Texas Health Science Center at Houston CTSA grant UL1RR02418, Cedars-Sinai GCRC grant MO1-RR00425, Intramural Research Program, NIAMS/NIH and Rebecca Cooper Foundation (Australia).

Collaborators TCRI AS Group Jian Yin[1], Lei Jiang[1], Lin Zhou[1], Ting Li[1], Qingwen Wang[2], Tianwang Li[3], Guanmin Gao[4], Shengqian Xu[5], Weiguo Xiao[6], Hui Shen[6], Jingguo Zhou[7], Yuquan You[8], Dongbao Zhao[9], Qing Cai[9], Shengming Dai[10], Lan He[11], Ping Zhu[12], Zhenyu Jiang[13], Jian Xu[14], Huaxiang Wu[15], Lie Dai[16], Yang Li[17], Feng Ding[18], Xiaochun Zhu[19], Chongyang Liu[20], Dongyi He[21], Liyun Zhang[22], Zhijun Li[23], Futao Zhao[24], Hanshi Xu[25], Niansong Wang[10], Youlian Wang[26], Lindi Jiang[27], Yu Zhang[28], Jinwei Chen[29], Fang Cheng[24], Zhiyi Zhang[30], Yifang Mei[30] , Liangjing Lv[31], Lingli Dong[32], Jing Yang[33], Yinong Li[34], Xiaodong Wang[35], Xiaofeng Li[36], Hongsheng Sun[37], Xianming Long[38], Xiao Zhang[39], Qinghong Yu[40], Xiaodan Kong[41], Yi Zheng[42], Miaojia Zhang[43], Yi Tao[44], Yisha Li[45], Xinwang Duan[46], Qianghua Wei[47], Xiaofei Wang[48], Jie Han[49], Rong Mu[50], Yiping Lin[51], Jian Zhu[52], Xiaoyuan Chen[53]. 1. Department of Rheumatology and Immunology, Shanghai Changzheng Hospital, The Second Military Medical University; 2. Peking University Shenzhen Hospital; 3. Guangdong Second Provincial General Hospital; 4. The First Affiliated Hospital of Zhengzhou University; 5. The First Affiliated Hospital of Anhui Medical University; 6. The First Affiliated Hospital of China Medical University; 7. The Affiliated Hospital of North Sichuan Medical College; 8. Quanzhou Orthopedic-Traumatological Hospital of Fujian Traditional Chinese Medical University; 9. Shanghai Changhai hospital; 10. Shanghai Sixth People's Hospital; 11. The First Affiliated Hospital of Xi'an Jiaotong University; 12. Xijing Hospital; 13. Bethune First Hospital of Jilin University; 14. The First Affiliated Hospital of Kunming Medical University; 15. The Second Affiliated Hospital of Zhejiang University School of Medicine; 16. The Second Affiliated Hospital of Sun Yat-sen University; 17. The Second Affiliated Hospital of Harbin Medical University; 18. Qilu Hospital of Shandong University; 19. The First Affiliated Hospital of Wenzhou Medical University; 20. The Third Affiliated Hospital of Chongqing Medical University; 21. Shanghai GuangHua Hospital of Integrated Traditiongnal Chinese and Western Medicine; 22. Shanxi Dayi Hospital; 23. The First Affiliated Hospital of Bengbu Medical College; 24. Shanghai Ninth People's Hospital; 25. The First Affiliated Hospital of Sun Yat-sen University; 26. Jiangxi Provincial People's Hospital; 27. Shanghai Zhongshan Hospital; 28. Northern Jiangsu People's Hospital; 29. The Second Xiangya Hospital of Central South University; 30. The First Affiliated Hospital of Harbin Medical University; 31. Shanghai Renji Hospital; 32. Tongji Hospital of Huazhong University of Science & Technology; 33. Mianyang Hospital of Traditional Chinese Medicine; 34. Fujian armed police hospital; 35. Affiliated Hopital of Weifang Medical University; 36. The Second Affiliated Hospital of Shanxi Medical University; 37. Shandong Provincial Hospital; 38. The First Affiliated Hospital to Soochow University; 39. Guangdong General Hospital; 40. Zhujiang Hospital of Southern Medical University; 41. The Second Affiliated Hospital of Dalian Medical University; 42. Beijing Chao-Yang Hospital; 43. Jiangsu Province Hospital; 44. The Second Affiliated Hospital of Guangzhou Medical University; 45. The First Xiangya Hospital of Central South University; 46. The Second Affiliated Hospital of Nanchang University; 47. Shanghai General Hospital; 48. Shengjing Hospital of China Medical University; 49. Shanghai Dongfang Hospital; 50. Peking University People's Hospital; 51. The People's Liberation Army's 202 Hospital; 52. Beijing Tsinghua Changgung Hospital, School of Clinical Medicine, Tsinghua University; 53. Tsinghua Clinical Research Center, School of Medicine, Tsinghua University. Ann Morgan, NIHR Leeds Biomedical Research Centre, Leeds Teaching Hospitals NHS Trust, Leeds, UK, and Leeds Institute of Rheumatic and Musculoskeletal Medicine, University of Leeds, Leeds, UK.

Contributors Study design was performed by ZL, XW, PL, MHW, LG, MMW, MHR, JDR, BPW, HX and MAB. Case recruitment was performed by XW, NA, MA, GM, MM, HMO, CTC, AAH, SS, GTJ, SYB, GW, AJ, EF, JS, LL, ML, JCCW, NM, MJW, MJL, YW, JZ, JSZ, XW, JZB, MHW, LG, MMW, MHR, LD, THK, JDR, BPW, HX and MAB. Genotyping and/or analysis were performed by ZL,XW, ML PL, GW, EDG, LA, LW, XH and MAB. The manuscript was prepared by ZL, JDR, BPW, XH and MAB. All authors were involved in completion of, and approved, the final manuscript.

Funding This study was funded, in part, by Arthritis Research UK (Grants 19536 and 18797), by the Wellcome Trust (grant number 076113) and by the Oxford Comprehensive Biomedical Research Centre ankylosing spondylitis chronic disease cohort (Theme Code: A91202). XH is supported by the National Natural Science Foundation of China (Grant No. 31821003), National Key Research and Development Project (Grant No. 2018AAA0100302), Shanghai Municipal Key Clinical Specialty (shslczdzk02602), and Shanghai Science and Technology Development Funds (2020-SH-XY-2). ZBJ was funded by a grant from the Zhejiang Provincial Natural Science Foundation of China (LD18H120001LD). The New Zealand data were derived from participants in the Spondyloarthritis Genetics and the Environment Study (SAGE) and was funded by The Health Research Council, New Zealand. We acknowledge the Understanding Society: The UK Household Longitudinal Study. This is led by the Institute for Social and Economic Research at the University of Essex and funded by the Economic and Social Research Council. The survey was conducted by NatCen and the genome-wide scan data were analysed and deposited by the Wellcome Trust Sanger Institute. Information on how to access the data can be found on the Understanding Society website https: www.understandingsociety.ac.uk/. HMO is supported by the National Institute for Health Research (NIHR) Leeds Biomedical Research Centre. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health. French sample collection was performed by the Groupe Française d'Etude Génétique des Spondylarthrites, coordinated by Professor Maxime Breban and funded by the Agence Nationale de Recherche GEMISA grant reference ANR-10-MIDI-0002. We acknowledge and thank the TCRI AS Group for their support in recruiting patients for the study (see below). The authors acknowledge the sharing of data and samples by the BSRBR-AS Register in Aberdeen. Chief Investigator, Professor Gary Macfarlane and Dr Gareth Jones, Deputy Chief Investigator created the BSRBR-AS study which was commissioned by the British Society for Rheumatology, funded in part by Abbvie, Pfizer and UCB. We are grateful to every patient, past and present staff of the BSRBR-AS register team and to all clinical staff who recruited patients, followed them up and entered data—details here: https://www.abdn.ac.uk/iahs/research/epidemiology/spondyloarthritis.php#panel1011. The QIMR control samples were from parents of adolescent twins collected in the context of the Brisbane Longitudinal Twin Study 1992–2016, support by grants from NHMRC (NGM) and ARC (MJW). We thank Anjali Henders, Lisa Bowdler, Tabatha Goncales for biobank collection and Kerrie McAloney and Scott Gordon for curating samples for this study. MAB is funded by a National Health and Medical Research Council (Australia) Senior Principal Research Fellowship (1024879), and support for this study was received from a National Health and Medical Research Council (Australia) program grant (566938) and project grant (569829), and from the Australian Cancer Research Foundation and Rebecca Cooper Medical Research Foundation. We are also very grateful for the invaluable support received from the National Ankylosing Spondylitis Society (UK) and Spondyloarthritis Association of America in case recruitment. Additional financial and technical support for patient recruitment was provided by the National Institute for Health Research Oxford Musculoskeletal Biomedical Research Unit and NIHR Thames Valley Comprehensive Local Research and an unrestricted educational grant from Abbott Laboratories. This research was funded/supported by the National Institute for Health Research (NIHR) Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust and King's College London and/or the NIHR Clinical Research Facility. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval Written informed consent was obtained from all cases, with approval from the relevant research ethics authorities at each participating centre. The overall programme was reviewed and approved by Metro South Hospital Research Ethics Committee (approval reference HREC/05/QPAH/221).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information. Details of the polygenic risk scores will be made available depending on completion of data transfer agreements with King's College London.

ORCID iDs
Zhixiu Li http://orcid.org/0000-0002-2924-9120
Nurullah Akkoc http://orcid.org/0000-0002-3718-171X
Maxime Breban http://orcid.org/0000-0002-6932-9395
Gary J Macfarlane http://orcid.org/0000-0003-2322-3314
Mahdi Mahmoudi http://orcid.org/0000-0002-8164-8831
Helena Marzo-Ortega http://orcid.org/0000-0002-9683-3407
Andrew A Harrison http://orcid.org/0000-0003-4372-3252
Simon Stebbings http://orcid.org/0000-0002-2824-4440
Gareth T Jones http://orcid.org/0000-0003-0016-7591
James Cheng-Chung Wei http://orcid.org/0000-0003-0310-2769
MinJae Lee http://orcid.org/0000-0002-4329-506X
Xiaobing Wang http://orcid.org/0000-0002-4302-2213
Zi-Bing Jin http://orcid.org/0000-0003-0515-698X
Michael M Ward http://orcid.org/0000-0003-1857-9367
Tae-Hwan Kim http://orcid.org/0000-0002-3542-2276
John D Reveille http://orcid.org/0000-0001-5950-0913
Bryan Paul Wordsworth http://orcid.org/0000-0001-7512-3468
Huji Xu http://orcid.org/0000-0002-8588-118X
Matthew A Brown http://orcid.org/0000-0003-0538-8211

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CLINICAL SCIENCE

Quality indicators for systemic lupus erythematosus based on the 2019 EULAR recommendations: development and initial validation in a cohort of 220 patients

Katerina Chavatza ,[1] Myrto Kostopoulou,[2] Dionysis Nikolopoulos ,[1] Ourania Gioti,[3] Konstantina Togia,[1] Laura Andreoli,[4,5] Martin Aringer ,[6] John Boletis,[7] Andrea Doria ,[8] Frederic A Houssiau ,[9] David Jayne,[10] Marta Mosca,[11] Elisabet Svenungsson ,[12] Angela Tincani,[4] George Bertsias,[13] Antonis Fanouriakis ,[1,3] Dimitrios T Boumpas [1,14]

Handling editor Josef S Smolen

► Additional online supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2021-220438).

For numbered affiliations see end of article.

Correspondence to
Dr Dimitrios T Boumpas, Rheumatology and Clinical Immunology, National and Kapodistrian University of Athens, Athens 12462, Greece; boumpasd@uoc.gr

KC, MK, GB, AF and DTB contributed equally.

GB, AF and DTB are joint senior authors.

Received 26 March 2021
Accepted 11 May 2021
Published Online First 23 June 2021

To cite: Chavatza K, Kostopoulou M, Nikolopoulos D, *et al*. *Ann Rheum Dis* 2021;**80**:1175–1182.

ABSTRACT

Background Quality of care is receiving increased attention in systemic lupus erythematosus (SLE). We developed quality indicators (QIs) for SLE based on the 2019 update of European League Against Rheumatism recommendations.

Methods A total of 44 candidate QIs corresponding to diagnosis, monitoring and treatment, were independently rated for validity and feasibility by 12 experts and analysed by a modified Research and Development Corporation/University of California Los Angeles model. Adherence to the final set of QIs and correlation with disease outcomes (flares, hospitalisations and organ damage) was tested in a cohort of 220 SLE patients with a median monitoring of 2 years (IQR 2–4).

Results The panel selected a total of 18 QIs as valid and feasible. On average, SLE patients received 54% (95% CI 52.3% to 56.2%) of recommended care, with adherence ranging from 44.7% (95% CI 40.8% to 48.6%) for diagnosis-related QIs to 84.3% (95% CI 80.6% to 87.5%) for treatment-related QIs. Sustained remission or low disease activity were achieved in 26.8% (95% CI 21.1% to 33.2%). Tapering of prednisone dose to less than 7.5 mg/day was achieved in 93.6% (95% CI 88.2% to 97.0%) while 73.5% (95% CI 66.6% to 79.6%) received the recommended hydroxychloroquine dose. Higher adherence to monitoring-related QIs was associated with reduced risk for a composite adverse outcome (flare, hospitalisation or damage accrual) during the last year of observation (OR 0.97 per 1% adherence rate, 95% CI 0.96 to 0.99).

Conclusion We developed QIs for assessing and improving the care of SLE patients. Initial real-life data suggest face validity, but a variable degree of adherence and a need for further improvement.

Key messages

What is already known about this subject?

► Systemic lupus erythematosus (SLE) is a multisystem disease with considerable morbidity whose care is complicated by its extreme clinical heterogeneity. The European League Against Rheumatism (EULAR) has developed evidence-based and expert opinion-based recommendations for the management of various aspects of SLE. Quality indicators (QIs), a popular tool to measure the degree of quality of care received by patients, have been proposed for SLE, but for the most part they were not based on a comprehensive systematic literature review (SLR).

What does this study add?

► This is the first comprehensive set of QIs in SLE based on an extensive SLR of the various aspects of SLE, performed as part of the EULAR recommendations for SLE. This study further capitalises on this work by developing QIs to detect potential gaps in SLE care and facilitate the implementation of the guidelines. Initial real-life data suggest a variable degree of adherence to the recommendations and identify areas for further improvement.

How might this impact on clinical practice or future developments?

► These QIs can be used towards assessing and improving patient care. QIs may facilitate the implementation of the EULAR recommendations by creating a checklist to be used towards detecting gaps in lupus care and facilitating efforts towards closing them.

INTRODUCTION

Quality of healthcare is defined as 'the degree to which health services for individuals and populations increase the likelihood of desired health outcomes and are consistent with current professional knowledge' (Institute of Medicine 1999).[1] The definition applies both to healthcare practitioners and to all settings of care (hospitals, nursing homes and physicians' offices). Measurement of quality can help to identify problems caused by overuse, underuse or misuse of health resources.

Quality indicators (QIs) is a popular tool to measure the degree of quality of care received by patients. QIs are quantitative measures related to

the structures, processes or outcomes of care,[2 3] derived from guidelines, systematic literature reviews (SLR) or expert panel consensus, through the use of a systematic approach representing the current standard of care. In contrast to most guidelines or recommendations, QIs pertain to measurable aspects of healthcare, describing exactly what to do, when to do it and who is responsible for doing it, with respect to disease management and monitoring.[4 5]

Systemic lupus erythematosus (SLE) is a multisystem disease with considerable morbidity due to both the disease per se and the complications of chronic treatment.[6] Care in SLE is complicated by the profound clinical heterogeneity and differences among individual patients. During the last two decades, the European League Against Rheumatism (EULAR) has developed evidence-based and expert opinion-based recommendations for the management of various aspects of SLE, including general SLE, renal and neuropsychiatric disease, and women's health including fertility and pregnancy.[7–9] These recommendations were recently updated.[10–12] In addition to these recommendations, other initiatives such as the treat-to-target in SLE have also highlighted the importance of a multifaceted care targeting remission or low disease activity.[13]

Towards improving patient care, detect potential gaps in SLE care, and facilitate the implementation of the guidelines, herein we sought to develop QIs based on the 2019 update of the EULAR recommendations for SLE, and perform an initial validation in one academic centre.

METHODS

Overview of the development of preliminary criteria and selection of the final set

QIs were developed using an adaptation of the Research and Development Corporation (RAND)/University of California Los Angeles (UCLA) modified Delphi method, a structured systematic approach that combines the best available evidence from an SLR with the collective expert opinion that has been shown to be valid in similar applications.[14–16] During a two-round process, a panel of experts assessed the validity and feasibility of the proposed indicators. The final set of QIs was used to evaluate the quality of care in an SLE cohort of 220 patients and to explore possible associations with disease outcomes (figure 1).

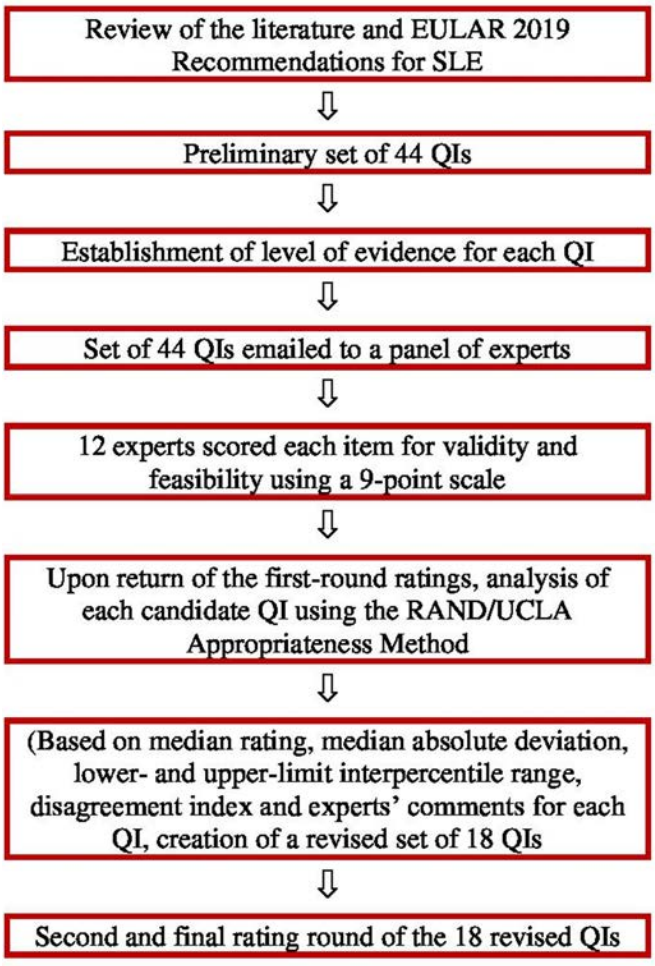

Figure 1 Overview of the development of preliminary criteria and selection of the final set of quality indicators. EULAR, European League Against Rheumatism; QIs, quality indicators; RAND, Research and Development Corporation; SLE, systemic lupus erythematosus; UCLA, University of California Los Angeles.

Identification of potential indicators and rounds of voting

An inventory of candidate QIs was developed based on the 2019 EULAR recommendations for SLE and the corresponding SLR.[7–12 17] A preliminary set of 44 QIs, which addressed seven distinct clinical domains and was graded according to the existing level of evidence, was evaluated by a panel of experts (nine rheumatologists and three nephrologists from five European countries) and one patient representative (see online supplemental appendix and online supplemental table 1). Every panel member was asked to rate each QI item for validity and feasibility, using a 9-point scale, with 9 representing the highest possible rating (definitions for validity and feasibility and voting instructions provided in online supplemental appendix). Panel members were also asked to comment on the draft QIs and suggest amendments as required. Following round 1 ratings, an analysis of the candidate QIs was performed, as described in the RAND/UCLA Method.[14 15] For each candidate QI, the median rating, median absolute deviation, lower and upper limit interpercentile range, were calculated. The Disagreement Index (DI) was calculated using the equations provided in online supplemental appendix. Median validity/feasibility scores of ≤ six were used to exclude QIs. The other measurements (deviation, agreement/disagreement) were used as additional information for the selection of the QIs for round 2 (online supplemental table 2). The moderator (DTB) and two other panel members (GB and AF) convened to discuss the results and revise the initial set, based on the ratings from round 1 (online supplemental table 3). Candidate QIs were modified, merged or eliminated accordingly, and the revised set was sent to the expert panel for round 2 of ratings. Experts were asked to rerate the QIs for validity and feasibility based on the same 9-point scale. Results were analysed and finalised based on the same principles used during round 1, reaching the final set of QIs (table 1).

Validation

To perform an initial validation of the proposed QIs, we used patients from the 'Attikon' lupus cohort, based in the largest tertiary hospital of western Attica and considered as a referral centre for patients with SLE.[18] Patients from the cohort were included if they (1) fulfilled the EULAR/American College of Rheumatology (ACR) 2019 and/or 2012 Systemic Lupus International Collaborating Clinics (SLICC) classification criteria,[19 20] (2) had at least 1 year of follow-up and (3) had at least four visits over the last year. Included patients were derived from an

Table 1 Final set of quality indicators (QIs) and rating during the second round of voting

		Validity		Feasibility	
		Median	Mean absolute deviation	Median	Mean absolute deviation
Screening-diagnostic QIs					
1	**If a patient has SLE** then routine laboratory tests (CBC, serum creatinine and urine analysis) and serological tests (ANA, C3/C4, dsDNA and aPL) should be performed at diagnosis.	9	0	9	0
2	**If a patient has lupus nephritis or is at high risk for nephritis (young, clinical and/or serologic activity)** then follow-up tests with CBC, UPr, urine analysis, serum creatinine and lupus serology (C3/C4 and anti-dsDNA) should be performed every 3–6 months	9	0	9	0
3	**If a patient with SLE has persistent proteinuria ≥500 mg and/or an unexplained decrease in glomerular filtration rate and/or active urine sediment** then kidney biopsy is recommended	9	0	8.5	0.7
4	**If a patient has SLE** then stratification of CVD risk should be performed annually with assessment of both traditional* and disease-related† risk factors and management of modifiable risk factors including smoking	9	0	8	1.5
5	**If a patient has SLE** then osteoporosis risk factors (age, sex, steroid use‡, smoking, low vitamin D, low BMI, family history) should be evaluated and fracture risk§ (high, moderate, low) should be assessed and managed accordingly	9	0	8.5	0.7
Treatment QIs					
6	**If a patient has SLE and is treated with hydroxychloroquine (HCQ)** then the HCQ dose should be ≤5 mg/kg (actual body weight) and be monitored for retinal toxicity with baseline ophthalmologic evaluation (by visual fields and optical coherence tomography (OCT)) and annual follow-up 5 years after the initiation of HCQ-provided that risk factors for HCQ-induced retinopathy¶ are not present	8	1.5	8.5	0.7
7	**If a patient with SLE, receives prednisone ≥7.5 mg for ≥3 months** then prednisone reduction should be attempted to the lowest possible dose	9	0	8	1.5
8	**If a patient with SLE has lupus nephritis III (±V) or IV (±V)** then immunosuppressive agents in combination with glucocorticoids are recommended	9	0	9	0
9	**If a patient with lupus nephritis has proteinuria ≥300–500 mg** then ACE inhibitors or ARB are recommended	9	0	9	0
10	**If a woman with SLE wishes for pregnancy and has traditional risk factors for pre-eclampsia (kidney disease including lupus nephritis, BMI ≥35, age >40, diabetes mellitus, hypertension, nulliparity)** then, low-dose aspirin is recommended during pregnancy	8	0.7	9	0
Monitoring QIs					
11	**If a patient has SLE** then assessment of disease activity, including SLEDAI and PGA, should be recorded in every visit	9	0	8.5	0.7
12	**If a patient has SLE** then the SLICC/ACR damage index should be monitored annually	9	0	9	0
13	**If a patient has SLE** then management should aim at clinical remission or-if remission cannot be achieved-at low disease activity with acceptable dose of steroids and well tolerated immunosuppressive agents at maintenance doses	9	0	8.5	0.7
14	**If a patient has SLE** then baseline tests at drug initiation and monitoring for drug toxicity should be performed	9	0	9	0
15	**If a patient has SLE** then sunscreen protection is recommended	9	0	9	0
16	**If a patient has SLE** then in patients with stable/inactive disease, non-live vaccines such as influenza, pneumococcal and HPV vaccines are recommended while attenuated vaccines (such as HZV vaccine) may be considered	8.5	0.7	8.5	0.7
17	**If a premenopausal woman has SLE** then reproductive health and fertility counselling should be provided including the pitfalls of oestrogen use for contraception in SLE	9	0	9	0
18	**If a woman of reproductive age has SLE and wishes pregnancy** then counselling about pregnancy (eg, stable/inactive disease for at least 6–12 months) should be provided and baseline tests (eg, anti-Ro/La, aPL), should be performed and documented	9	0	9	0

***Traditional risk factors**: family history of premature CVD, primary hypercholesterolaemia, metabolic syndrome, premature menopause, dyslipidaemia and/or elevated hsCRP.
†**SLE-related risk factors**: persistently active or flaring disease, kidney involvement/CKD, moderate-high aPL titres, organ damage, use of GCs >5 mg/day, no use of HCQ.
‡**Steroid use:** with prednisone ≥2.5 mg for ≥3 months, calcium (1000–1200 mg/day) and vitamin D (600–800 IU/day) should be administered.
§**Fracture Risk**:*High Fracture Risk*(Previous Fragility Fracture, T-score ≤−2.5, FRAX score for major osteoporotic or hip fracture, beyond different thresholds according to different countries, very high GC doses) :Antiresorptive treatment with calcium (1000–1200 mg/day) and vitamin D (600–800 IU/day) should be administered. *Moderate fracture risk*(FRAX score for major osteoporotic or hip fracture beyond different thresholds according to different countries, prednisone ≥7.5 mg for ≥6 months AND: Z-score-3 OR rapid bone loss ≥10% at hip or spine over 1 year):Calcium (1000–1200 mg/day) and vitamin D (600–800 IU/day) should be administered and antiresorptive treatment should be considered. *Low fracture risk*(FRAX score for major osteoporotic or hip fracture beyond different thresholds according to different countries):No need for antiresorptive treatment. Calcium (1000–1200 mg/day) and vitamin D (600–800 IU/day) should be considered.**GC doses:** low dose <2.5 mg, medium 2.5–7.5 mg, high >7.5 mg, very high: prednizone ≥30 mg/day or >5 g accumulative dose in the previous year. In high GC doses, FRAX adjustment: multiplication of major osteoporotic fracture risk value (x1.15) and hip fracture value (x1.2).
¶**Major risk factors for retinopathy:** chronic kidney disease with GFR <60 mL/min, pre-existing retinal or macular disease, use of tamoxifen.
ACE, angiotensin-converting enzyme; ANA, antinuclear antibodies; anti-dsDNA, anti-double stranded DNA antibody; aPL, antiphospholipid antibodies; ARB, angiotensin receptor blocker; BMI, body mass index; CBC, complete blood count; CVD, cardiovascular disease; GC, glucocorticoid; HPV, human papilloma virus; hsCRP, high-sensitivity C-reactive protein; HZV, herpes zoster virus; PGA, Physician Global Assessment; QI, quality indicator; SLE, Systemic Lupus Erythematosus; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index; SLICC/ACR, Systemic Lupus International Collaborating Clinics/American College of Rheumatology; UPr, urine protein.

inception cohort (patients followed from January 2016 (year of establishment of the Attikon cohort) to date—60%) and a prevalent cohort (patients with SLE diagnosis before January 2016–40%)].

Chart review and patient interviews were performed retrospectively to assess patient eligibility for each QI (eg, only smokers were eligible for the corresponding QI regarding counselling for smoking cessation), adherence to each QI item, and to document disease outcomes of interest, specifically flares, SLICC/ACR damage index (SDI) and hospitalisations (due to cardiovascular events, infections and flares). Patient interviews were performed by physicians (KC, OG), with the completion of a patient-reported questionnaire, for measures that were not universally available in medical records (eg, counselling for fertility and sun protection).

Table 2 Adherence to 18 selected quality indicators (QIs)

	Function of care	Eligible patients N (%)	Fulfilled n (%)
Screening-diagnostic QIs			
1	**If a patient has SLE** then routine laboratory tests (CBC, serum creatinine and urine analysis) and serological tests (ANA, C3/C4, dsDNA and aPL) should be performed at diagnosis.	132 (60)	64 (48.5)
2	**If a patient has lupus nephritis or is at high risk for nephritis (young, clinical and/or serologic activity)** then follow-up tests with CBC, UPr, urine analysis, serum creatinine and lupus serology (C3/C4 and dsDNA) should be performed every 3–6 months	68 (31)	33 (48.5)
3	**If a patient with SLE has persistent proteinuria≥500 mg and/or an unexplained decrease in glomerular filtration rate and/or active urine sediment** then kidney biopsy is recommended	44 (20)	38 (86.4)
4	**If a patient has SLE** then stratification of CVD risk should be performed annually with assessment of both traditional and disease-related risk factors and management of modifiable risk factors including smoking	220 (100)	89 (40.5)
5	**If a patient has SLE** then osteoporosis risk factors (age, sex, steroid use, smoking, low vitamin D, low BMI, family history) should be evaluated and fracture risk (high, moderate, low) should be assessed and managed accordingly	220 (100)	100 (45.5)
Treatment QIs			
6	**If a patient has SLE and is treated with hydroxychloroquine (HCQ)** then the HCQ dose should be ≤5 mg/kg (actual body weight) and be monitored for retinal toxicity with baseline ophthalmological evaluation (by visual fields and optical coherence tomography (OCT)) and annual follow-up 5 years after the initiation of HCQ provided that risk factors for HCQ-induced retinopathy are not present	189 (86)	139 (73.5)
7	**If a patient with SLE, receives prednisone ≥7.5 mg for ≥3 months** then prednisone reduction should be attempted to the lowest possible dose	141 (64)	132 (93.6)
8	**If a patient with SLE has lupus nephritis III (±V) or IV (±V)** then immunosuppressive agents in combination with glucocorticoids are recommended	41 (18.6)	41 (100)
9	**If a patient with lupus nephritis has proteinuria ≥300–500 mg** then ACE inhibitors or ARBs are recommended	25 (11.3)	22 (88)
10	**If a woman with SLE wishes for pregnancy and has traditional risk factors for pre-eclampsia (Kidney disease including lupus nephritis, BMI ≥35, age >40, diabetes mellitus, hypertension, nulliparity)** then low-dose aspirin is recommended during pregnancy	7 (3)	5 (71.4)
Monitoring QIs			
11	**If a patient has SLE** then assessment of disease activity, including SLEDAI and PGA, should be recorded in every visit	220 (100)	31 (14.1)
12	**If a patient has SLE** then the SLICC/ACR damage index should be monitored annually	220 (100)	63 (28.6)
13	**If a patient has SLE** then management should aim at clinical remission or-if remission cannot be achieved-at low disease activity with acceptable dose of steroids and well tolerated immunosuppressive agents at maintenance doses	220 (100)	59 (26.8)
14	**If a patient has SLE** then baseline tests at drug initiation and monitoring for drug toxicity should be performed	193 (87.7)	186 (96.4)
15	**If a patient has SLE** then sunscreen protection is recommended	220 (100)	201 (91.4)
16	**If a patient has SLE** then in patients with stable/inactive disease, non-live vaccines such as influenza, pneumococcal and HPV vaccines are recommended while attenuated vaccines (such as HZV vaccine) may be considered	220 (100)	105 (47.7)
17	**If a premenopausal woman has SLE** then reproductive health and fertility counselling should be provided including the pitfalls of oestrogen use for contraception in SLE	74 (33.6)	37 (50)
18	**If a woman of reproductive age has SLE and wishes pregnancy** then counselling about pregnancy (eg, stable/inactive disease for at least 6–12 months) should be provided and baseline tests (eg, anti-Ro/La, aPL), should be performed and documented	71 (32.2)	44 (62)

ACE, angiotensin-converting enzyme; ANA, antinuclear antibodies; anti-dsDNA, antidouble stranded DNA antibody; aPL, antiphospholipid antibodies; ARB, angiotensin receptor blocker; BMI, body mass index; CBC, complete blood count; CVD, cardiovascular disease; HPV, human papilloma virus; HZV, herpes zoster virus; PGA, Physician Global Assessment; QI, quality indicator; SLE, Systemic Lupus Erythematosus; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index; SLICC/ACR, Systemic Lupus International Collaborating Clinics/American College of Rheumatology; UPr, urine protein.

Definitions

Performance for each QI was assessed as 'fulfilled', 'not fulfilled' or 'missing', in order to assess the grade of adherence. Because most QIs consisted of more than one individual component, 'fulfilled' denoted that all components were met, 'not fulfilled' that at least one component of the corresponding QI was not met, and 'missing' that data were not recorded in the chart. As an example, QI1 would be labelled as 'fulfilled' if all recommended laboratory and serological tests were obtained at diagnosis (see QI1, table 2). By contrast, QI1 would be labelled as 'not fulfilled', if at least one of the recommended tests was not obtained at diagnosis.

Outcomes

Disease-related outcomes were recorded in all patients. Recorded outcomes included (1) flares, (including major flares), defined as a measurable increase in disease activity leading to therapeutic intervention,[21] (2) SDI increase (an increase in the SDI score during the observation period), (3) adverse outcomes related to glucocorticoids (GC, that is, GC-related complications), (4) cardiovascular events or serious infections necessitating hospitalisation and (5) a composite adverse outcome (CAO), defined as occurrence of at least one of the following: flares, hospitalisations or SDI progression. The phenotype of SLE was categorised as mild, moderate or severe according to the British Isles

Table 3 Adherence to quality indicators (QI) in subgroups of patients

	No of patients	Mean* eligible QIs	Mean adherence, %	P value
Severity pattern				0.006
Mild	56	10.7	49.3	
Moderate	60	11.1	53.9	
Severe	104	12.1	57.2	
Cohort				0.13
Inception	132	9.8	49.5	
Prevalent	88	10.4	52.8	
Disease duration				0.02
<2 years	61	10.2	54.8	
≥2 years	159	9.9	49.3	

*Mean number of QIs for which the patients of each group were eligible.

Lupus Assessment Group 2004 classification of manifestations,[22] combined with expert physician judgement (DTB and AF), as previously described.[18]

Statistical analysis

Descriptive statistics were used for continuous variables and mean/SD or median/IQR values were calculated as appropriate. Adherence to each QI was calculated as the number of patients who received the designated care (numerator) divided by the number of eligible patients for this particular QI (denominator). In addition, a patient-specific mean score was calculated as the number of QIs 'fulfilled' divided by the number of QIs eligible for each patient. Accordingly, the average delivered care for each domain was calculated as a composite score from the cases in which recommended care was successfully delivered divided by the number of eligibility events within each domain.

To detect potential differences in adherence between patient subgroups, we applied three criteria: (1) disease duration (<2 years vs ≥2 years from diagnosis), (2) severity pattern (mild vs moderate vs severe disease) and (3) origin of cohort (inception vs prevalent). A separate analysis was performed to compare the adherence between various QI domains (diagnostic, treatment, monitoring). To compare mean values or the equality of distribution between different categories the one-way analysis of variance and the non-parametric Kruskal-Wallis test were used accordingly. Logistic regression models were performed to estimate the association between adherence to QIs and adverse disease outcomes that occurred in two different time frames (during the total duration of follow-up and during the last year of follow-up). All models were adjusted for age and disease duration. All tests were two tailed and p values less than 0.05 were considered statistically significant. Data management and statistical analyses were performed using STATA/MP V.13.1 (StataCorp).

RESULTS

Final set of QIs

Out of 44 initial candidate QIs (online supplemental table 3), three were removed due to a low median validity/feasibility score. For the remaining, agreement was reached in 31 QIs and disagreement in 10. In the former set, minor edits and discussion based on experts' comments resulted in 4 QIs being included without change, 4 being retained with edits, 17 being merged to 7 separate QIs and 6 being rejected. Of the 10 QIs for which there was disagreement, one was retained without changes, two were retained with edits, four were excluded and three were merged with two previously formed QIs. This resulted in a revised set of 18 candidate QIs, which was available for Round 2 of rating (table 1). The 18 finally selected QIs were further divided into three categories: (1) Screening/diagnosis-related (QIs 1–5), (2) Treatment-related (QIs 6–10) and 3) Monitoring (QIs 11–18). More specifically, selected individual QIs pertain to diagnosis, monitoring, therapy and its targets, fertility and pregnancy and adjunct therapy, including prevention of cardiovascular disease (CVD) and osteoporosis, vaccination, counselling for smoking and sunscreen protection.

Adherence to QIs

The final set of 18 QIs was tested for adherence in all eligible patients of our cohort (N=220)(table 2). Characteristics of the cohort are shown in online supplemental table 4. On average, patients received 54% (95% CI 52.30% to 56.25%) of the indicated care. Complete laboratory work-up at diagnosis was performed in 48.5% (95% CI 39.8% to 57.1%), with antiphospholipid antibodies being the most frequently missed component (68.9%). Disease activity evaluation in at least three out of four visits and annual assessment of organ damage were completed in only 14.1% (95% CI 9.4% to 18.7%) and 28.6% (95% CI 22.6% to 34.6%), respectively. By contrast, lupus nephritis related QIs had excellent overall adherence (88%, 95% CI 66.7% to 96.4% for the use of ACE inhibitor/angiotensin receptor blocker, 100% for the use of immunosuppressive treatment), except for laboratory monitoring (48.5%, 95% CI 36.6% to 60.6%). Overall adherence rate was 50% (95% CI 38.5% to 61.5%) for reproductive health counselling, 62% (95% CI 49.9% to 72.7%) for pregnancy counselling and 91.4% (95% CI 86.8% to 94.4%) for sunscreen protection. Notably, preventive measures for comorbidities had generally low to moderate adherence. More specifically, overall adherence rates for cardiovascular risk modification and vaccination QIs (at least one of the available pneumococcal vaccines in combination with influenza vaccine) were 40.5% (95% CI 34.1% to 47.1%) and 47.7% (95% CI 41.2% to 54.4%), respectively. Regarding osteoporosis prevention and treatment, the corresponding QI was fulfilled in 45.5% (95% CI 38.9% to 52.1%) of patients. A total of 73% of eligible patients had bone mineral density measurement performed at baseline and 58.6% at follow-up (every 2 years), while 60% of patients

Table 4 Adherence to quality indicators (QIs) grouped according to function of care

Function of care	No of QIs	No of times eligible QIs were assessed	No of times recommended care was delivered	Adherence, %	P value
					0.03
Screening-diagnosis*	4	640	286	44.68	
Treatment†	6	447	377	84.34	
Monitoring‡	8	1438	726	50.48	

*QI 1–5.
†QI 6–10.
‡QI 11–18.

belonging in the high fracture risk group received antiresorptive treatment; almost 75% (74.3%) received calcium and vitamin D. Of note, 63.8% of patients on GC received calcium and vitamin D protection.

In a subgroup analysis, patients with severe disease were more likely to receive the indicated care (57.2%) compared with patients with moderate (53.9%) or mild (49.3%) disease (p=0.006). Similarly, higher adherence rates were observed in patients with short (<2 years) vs longer (≥2 years) disease duration (54.8% and 49.3% respectively, p=0.02). No significant differences were observed between the inception and the prevalent cohort (table 3).

In a separate analysis according to the function of care, treatment-related QIs were met in significantly more eligible patients (84.3%) followed by monitoring (50.5%) and diagnostic (44.6%) QIs (p=0.03) (table 4).

Outcomes

Disease-related outcomes are summarised in online supplemental table 5. Patients were followed up for a median of 2 years (IQR 2–4). SDI progression was observed in 22.3% of patients incidence rate (IR)=13/100 patient-years (pys). A total of 310 flares were captured over the follow-up corresponding to 0.58 per py. The IR of hospitalisations was 15.4/100 py, attributed mainly to major flares (7.8/100 py), serious infections (6.1/100 py) and cardiovascular events (1.5/100 py).

Overall, QI adherence did not differ among patients experiencing CAO and patients without CAO throughout the observation period (54.0% vs 54.7%, p=0.71). However, patients with CAO during the last year of follow-up had lower adherence rates in monitoring QIs when compared with patients without a CAO (47.6% vs 53.9%, p=0.02) (online supplemental table 6). We also explored possible associations between adherence to specific QIs and outcomes. Patients who achieved sustained remission or Lupus Low Disease Activity State (LLDAS) (QI13), patients who fulfilled QI16 regarding vaccination and patients who received low-dose GC (QI7) had lower odds of experiencing a flare during the observation period (OR 0.15, 95% CI 0.07 to 0.31 OR 0.46, 95% CI 0.21 to 0.98 and OR 0.23, 95% CI 0.05 to 0.94, respectively). A lower risk of CAO during the last year of follow-up was also found in patients who met QI13 on remission/LLDAS and QI16 on vaccination (OR 0.09, 95% CI 0.04 to 0.18 and OR 0.52, 95% CI 0.28 to 0.99 respectively). As expected, patients who achieved sustained remission or LLDAS (QI13) had lower odds of damage accrual during the observation period (OR 0.35, 95% CI 0.14 to 0.84). Patients assessed for SDI accrual (QI12) and CVD risk stratification (QI4) had higher probability to exhibit any CAO (OR 2.62, 95% CI 1.18 to 5.71 and OR 1.77, 95% CI 1.01 to 3.12, respectively) (table 5).

DISCUSSION

SLE is notorious for its clinical heterogeneity, which may in turn increase the risk of inconsistency and variations in the care received by patients. To ensure improved and more homogeneous care, EULAR has developed evidence-based and expert opinion-based recommendations for the management of various aspects of the disease.[9–12] Nonetheless, since management recommendations are often followed incompletely in real-life settings, efforts have been made to create tools which can transform them into easily applicable, 'user friendly' instructions for daily practice. In this regard, QIs can be useful instruments for the quantification of gaps and shortcomings in medical care. Herein, we created a set of QIs based on the EULAR recommendations for SLE, using a validated, systematic methodology supported by expert opinion. In addition, we examined the adherence to the proposed QIs in 220 patients of the 'Attikon' lupus cohort, a readily available patient cohort, to take an initial 'glimpse' on potential gaps of care in daily practice and assess their impact on disease outcomes.

QIs have been previously proposed for SLE,[5 23] but for the most part they were not based on a comprehensive SLR. This is the first set of QIs based on such a comprehensive SLR of the various aspects of SLE (ie, diagnosis, monitoring and therapy), which was performed in the context of the updated EULAR recommendations. The credibility of the proposed QI set is reinforced by the robust methodology of the procedure (ie, the RAND/UCLA modified Delphi method), which involved assessment of a large number of initial candidate QIs for validity and feasibility, followed by two rounds of voting, all performed by a panel of experts with expertise in SLE.

Our initial findings suggest moderate adherence (54%) with great variability in certain types of QIs. The low rates of CVD protection and reproductive health counselling are consistent with data from previous studies;[24 25] rates for sunscreen protection and individual components for osteoporosis and vaccination (influenza, pneumococcal) QIs are also consistent with published data.[25] Looking for potential explanations, in the case of CVD-related QIs, the complexity of prescribing statins by rheumatologists in some countries and, in case of osteoporosis prophylaxis the plethora of recommendations by various scientific societies, may account at least in part for these low adherence rates. In our view, this reality highlights the need to actively involve nurse specialists in the care of SLE patients, especially in the settings of expert SLE referral centres. Such nurse practitioners could

Table 5 Risk of adverse events associated with the delivered care in an SLE cohort of 220 patients

Quality indicator (QI)	Adverse event	OR	P value
QI12 (If a patient has SLE then the SLICC/ACR damage index should be monitored annually)	CAO	2.6	0.01
QI13 (If a patient has SLE then management should aim at clinical remission or-if remission cannot be achieved—at low-disease activity with acceptable dose of steroids and well tolerated immunosuppressive agents at maintenance doses)	SDI progression	0.4	0.02
	Flares	0.2	<0.001
	CAO*	0.1	<0.001
QI4 (If a patient has SLE then stratification of CVD risk should be performed annually with assessment of both traditional and disease-related risk factors and management of modifiable risk factors including smoking	CAO*	1.8	0.04
QI16 (If a patient has SLE then in patients with stable/inactive disease, non-live vaccines such as influenza, pneumococcal and HPV vaccines are recommended while attenuated vaccines (such as HZV vaccine) may be considered	Flares	0.5	0.04
	CAO*	0.5	0.04

*CAO during the last year of follow-up.
CAO, composite adverse outcome; CVD, cardiovascular disease; HPV, human papilloma virus; HZV, herpes zoster virus; OR, odds ratio; SDI, SLICC/ACR Damage Index; SLE, systemic lupus erythematosus.

monitor the assessment and fulfilment of these QIs, which may not be a priority in a busy physician outpatient clinic.

In reference to potential causes related to better performance in certain indicators, we found that QI adherence rates were higher in patients with disease duration shorter than 2 years and in patients with severe disease. These observations may reflect the fact that physicians are more likely to adhere early after diagnosis to ensure better disease control, and in patients who are more likely to develop irreversible organ damage, respectively. Despite variable rates of adherence, we did not find strong associations between non-adherence to QIs and adverse outcomes, except that patients who were in a low disease activity state had lower rates of flares and damage progression. A possible explanation is that the adherence to a single QI may not suffice to provide a clinically favourable outcome, if not combined with consistent and adequate care. Thus, in a study by Yazdany *et al*, patients who met ≥85% of the eligible QIs had lower odds of damage accrual, however, the difference was not significant for any of the individual QIs alone.[26] The modest associations between quality of care and outcomes in our study may also reflect the relatively short follow-up, especially because many SLE outcomes develop within years, and longer observation time is needed to detect any association. To further address this issue, prospective long-term follow-up studies evaluating a combination set instead of single indicators with varied settings and outcomes are needed.

Our study has several limitations. The duration of follow- up was modest and data represent the experience of a single academic centre. Consequently, our results may not be representative of other clinical settings and in non-academic centres, gaps in patient care may be even greater. Conversely, a tertiary care hospital that serves as a referral centre may follow patients with a higher burden of the disease and higher risk of progression.[27 28] Risk-adjusted and casemix models would help to account for differences in patient-level and hospital-level risk, however, the relatively small study sample, limited access to administrative data and the absence of electronic health record systems prevented us from performing this methodology. Yet, development of the current QIs was based on an extensive systematic review and panels of experts working for over one decade to develop recommendations for SLE. To this end, longitudinal and nationwide population-based studies are warranted to validate these QIs in various time and clinical settings.

In summary, we have developed a set of EULAR recommendations-based QIs for SLE patient care, following a comprehensive SLR and supported by expert opinion. Initial real-life data suggest a variable degree of adherence and areas for further improvement. Nevertheless, these QIs may be used as a 'checklist' to be fulfilled in an outpatient setting, in order to improve SLE patient care by facilitating the implementation of the EULAR recommendations.

Author affiliations
[1]Rheumatology and Clinical Immunology, Medical School, National and Kapodistrian University of Athens, "Attikon" University Hospital of Athens, Athens, Greece
[2]Department of Nephrology, "G. Gennimatas" General Hospital, Athens, Greece
[3]Department of Rheumatology, "Asklepieion" General Hospital, Voula, Athens, Greece
[4]Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy
[5]Unit of Rheumatology and Clinical Immunology, Spedali Civili, Brescia, Italy
[6]Division of Rheumatology, Department of Medicine III, University Medical Center & Faculty of Medicine Carl Gustav Carus at the TU Dresden, Dresden, Germany
[7]Nephrology Department and Renal Transplantation Unit, "Laikon" Hospital, National and Kapodistrian University of Athens, Medical School, Athens, Greece
[8]Rheumatology Unit, Department of Medicine, University of Padova, Padova, Italy
[9]Cliniques Universitaires Saint-Luc, Université catholique de Louvain, Brussels, Belgium
[10]Department of Medicine, University of Cambridge, Cambridge, UK
[11]Rheumatology Unit, Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy
[12]Division of Rheumatology, Department of Medicine Solna, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden
[13]Rheumatology, Clinical Immunology and Allergy, University Hospital of Heraklion, Heraklion, Crete, Greece
[14]Laboratory of Autoimmunity and Inflammation, Biomedical Research Foundation of the Academy of Athens, Athens, Greece

Twitter Dimitrios T Boumpas @none

Acknowledgements We are thankful to the staff physicians and nurses of the Rheumatology and Clinical Immunology Unit of 'Attikon' University Hospital for providing care to the patients with SLE and other rheumatologic diseases.

Contributors KC and OG collected data from patient medical charts and KC drafted the manuscript. MK performed statistical analyses and edited the manuscript. DN edited the manuscript. KT assessed patient medical charts for eligibility in the study. LA, MA, JB, AD, FAH, DJ, MM, ES and AT evaluated the quality indicators, provided voting and critically reviewed the manuscript. GB and AF supervised the study and edited the manuscript. DTB conceived and supervised the study and edited the manuscript.

Funding DTB was supported by the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement no. 742390). DJ was supported by the NIHR Cambridge Biomedical Research Centre (BRC-1215 20014).

Competing interests DTB is an Editorial Board member in the Annals of the Rheumatic Diseases. The remaining authors declare no competing interests relevant to this work.

Patient consent for publication Not required.

Ethics approval The study was approved by the Ethics Committee of 'Attikon' University Hospital of Athens.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as online supplemental information.

ORCID iDs
Katerina Chavatza http://orcid.org/0000-0002-1274-0909
Dionysis Nikolopoulos http://orcid.org/0000-0002-9894-6966
Martin Aringer http://orcid.org/0000-0003-4471-8375
Andrea Doria http://orcid.org/0000-0003-0548-4983
Frederic A Houssiau http://orcid.org/0000-0003-1451-083X
Elisabet Svenungsson http://orcid.org/0000-0003-3396-3244
Antonis Fanouriakis http://orcid.org/0000-0003-2696-031X
Dimitrios T Boumpas http://orcid.org/0000-0002-9812-4671

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CLINICAL SCIENCE

Interaction between the *STAT4* rs11889341(T) risk allele and smoking confers increased risk of myocardial infarction and nephritis in patients with systemic lupus erythematosus

Sarah Reid ,[1] Niklas Hagberg ,[1] Johanna K Sandling ,[1] Andrei Alexsson,[1] Pascal Pucholt ,[1] Christopher Sjöwall ,[2] Karoline Lerang,[3] Andreas Jönsen,[4] Iva Gunnarsson,[5] Ann-Christine Syvänen,[1] Anne Margrethe Troldborg,[6,7] Anne Voss,[8] Anders A Bengtsson,[4] Øyvind Molberg,[3] Søren Jacobsen,[9,10] Elisabet Svenungsson ,[5] Lars Rönnblom ,[1] Dag Leonard[1]

Handling editor Josef S Smolen

► Additional material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2020-219727).

For numbered affiliations see end of article.

Correspondence to
Professor Lars Rönnblom, Medical Sciences and Science for Life Laboratory, Uppsala University, Uppsala 751 85, Sweden;
Lars.Ronnblom@medsci.uu.se

Received 15 December 2020
Revised 10 February 2021
Accepted 6 March 2021
Published Online First 25 March 2021

To cite: Reid S, Hagberg N, Sandling JK, *et al*. *Ann Rheum Dis* 2021;**80**:1183–1189.

ABSTRACT

Objective To investigate how genetics influence the risk of smoking-related systemic lupus erythematosus (SLE) manifestations.

Methods Patients with SLE ($n_{discovery\ cohort}$=776, $n_{replication\ cohort}$=836) were genotyped using the 200K Immunochip single nucleotide polymorphisms (SNP) Array (Illumina) and a custom array. Sixty SNPs with SLE association ($p<5.0\times10^{-8}$) were analysed. Signal transducer and activator of transcription 4 (STAT4) activation was assessed in *in vitro* stimulated peripheral blood mononuclear cells from healthy controls (n=45).

Results In the discovery cohort, smoking was associated with myocardial infarction (MI) (OR 1.96 (95% CI 1.09 to 3.55)), with a greater effect in patients carrying any rs11889341 *STAT4* risk allele (OR 2.72 (95% CI 1.24 to 6.00)) or two risk alleles (OR 8.27 (95% CI 1.48 to 46.27)).

Smokers carrying the risk allele also displayed an increased risk of nephritis (OR 1.47 (95% CI 1.06 to 2.03)). In the replication cohort, the high risk of MI in smokers carrying the risk allele and the association between the *STAT4* risk allele and nephritis in smokers were confirmed (OR 6.19 (95% CI 1.29 to 29.79) and 1.84 (95% CI 1.05 to 3.29), respectively).

The interaction between smoking and the *STAT4* risk allele resulted in further increase in the risk of MI (OR 2.14 (95% CI 1.01 to 4.62)) and nephritis (OR 1.53 (95% CI 1.08 to 2.17)), with 54% (MI) and 34% (nephritis) of the risk attributable to the interaction.

Levels of interleukin-12-induced phosphorylation of STAT4 in CD8+ T cells were higher in smokers than in non-smokers (mean geometric fluorescence intensity 1063 vs 565, p=0.0063).

Lastly, the *IL12A* rs564799 risk allele displayed association with MI in both cohorts (OR 1.53 (95% CI 1.01 to 2.31) and 2.15 (95% CI 1.08 to 4.26), respectively).

Conclusions Smoking in the presence of the *STAT4* risk gene variant appears to increase the risk of MI and nephritis in SLE. Our results also highlight the role of the IL12–STAT4 pathway in SLE-cardiovascular morbidity.

Key messages

What is already known about this subject?

- Neither traditional nor systemic lupus erythematosus (SLE)-related risk factors can fully account for the excess cardiovascular disease risk seen in patients with SLE, but interactions between traditional and SLE-specific risk factors have been scarcely investigated.

What does this study add?

- Our results show that the signal transducer and activator of transcription 4 (*STAT4)* risk allele rs11889341 enhances the effect of smoking on the risk of myocardial infarction and nephritis and that smoking is associated with increased interleukin (IL)-12-induced phosphorylation of STAT4 in CD8+ T cells.
- We further demonstrate that the *IL12A* SLE risk variant rs564799 is associated with an increased risk of myocardial infarction, which further highlights the importance of the IL12-STAT4 pathway in the aetiology of cardiovascular morbidity in SLE.

How might this impact on clinical practice or future developments?

- Our results suggest that genetic profiling of patients with SLE may be useful for predicting comorbidities of the disease, impact of environmental factors and for targeted smoking cessation interventions.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a disease characterised by loss of tolerance to self-antigens, formation of immune complexes and an activated type I interferon (IFN) system.[1] A widely accepted view of the aetiology of SLE is that environmental factors trigger the disease in genetically susceptible individuals. The genetic background is complex, with more than 100 single nucleotide polymorphisms (SNPs) associated with risk

for SLE.[2] Exposure to certain environmental factors, including ultraviolet radiation and viral infections, is associated with SLE development and flare-ups of the disease.[3 4] Several studies have evaluated smoking as a risk factor for SLE, with the largest meta-analysis to date showing a modest risk increase.[5] While the results are not confirmed in prospective studies, both Cozier and Barbhaiya *et al* observed a trend of increased risk in smokers.[6 7] The most extensive prospective study involving 286 cases with SLE demonstrated an association between smoking and development of SLE with increased anti-dsDNA, but no risk of overall SLE.[7]

Although death from active SLE has decreased since the 1950s,[8] the mortality rate still exceeds that of the general population, with cardiovascular morbidity remaining considerably high and a strong risk factor for premature mortality.[9–11] Both traditional and SLE-related risk factors, such as hypertension, nephritis and high disease activity have been identified as risk factors, but cannot fully account for the excess cardiovascular disease (CVD) risk seen in patients with SLE.[12 13] To fully explain the aetiology of SLE or its comorbidities such as CVD, gene–gene or gene–environment interactions may be essential to consider. In rheumatoid arthritis, there is compelling evidence of a strong interaction between the HLA-DRB1*04 shared epitope and smoking on the development of anticitrullinated protein autoantibodies[14] and a high prevalence of cardiovascular events (CVE).[15]

In SLE, a few studies have investigated the interaction between genetic risk factors and smoking on the development of the disease.[16 17] Recently, Cui *et al* demonstrated that an additive interaction between smoking and the cumulative genetic risk of SLE increases the risk of the disease.[18] However, gene–smoking interactions on the development of specific manifestations or co-morbidities of SLE have been scarcely studied. This study, therefore, aims to investigate the effect of smoking on the development of specific manifestations of SLE, including CVE, end-stage renal disease (ESRD) and nephritis, and examine how the effect is modulated by the presence of genetic variants associated with an increased risk of SLE development.

METHODS

Patients of the discovery and replication cohort

The discovery cohort included 774 patients with SLE from Sweden. The replication cohort included 836 patients from Norway and Denmark. All subjects fulfilled ≥4 American College of Rheumatology (ACR)−82 and ACR-97 classification criteria for SLE and were of European descent. Clinical characteristics of the cohorts are described in table 1 and online supplemental

Table 1 Prevalence of clinical manifestations in smokers (n=371) and non-smokers (n=387) in the discovery cohort

	Smokers, n (%)	Non-smokers, n (%)	OR (95% CI)	P value
Age at last follow-up, mean (SD)	55 (15)	50 (17)		**0.000025**
Disease duration, mean (SD)	17 (11)	16 (12)		**0.016**
Male sex	49 (13)	48 (13)	0.94 (0.61 to 1.45)	0.79
Deceased at follow-up	55 (15)	39 (11)	1.19 (0.75 to 1.88)	0.45
ACR 1982 classification criteria[40]				
1 Malar rash	205 (55)	211 (57)	1.00 (0.74 to 1.34)	0.97
2 Discoid rash	83 (22)	80 (21)	0.96 (0.68 to 1.36)	0.81
3 Photosensitivity	262 (71)	246 (65)	1.27 (0.93 to 1.74)	0.13
4 Oral ulcer	103 (28)	96 (25)	1.20 (0.86 to 1.67)	0.27
5 Arthritis	306 (82)	301 (81)	1.11 (0.76 to 1.61)	0.59
6 Serositis	179 (48)	165 (45)	1.10 (0.82 to 1.47)	0.52
7 Renal disorder	129 (35)	132 (36)	1.10 (0.80 to 1.50)	0.56
8 Neurological disorder	33 (9)	40 (10)	0.86 (0.52 to 1.40)	0.54
9 Haematological disorder	213 (57)	258 (70)	**0.62 (0.46 to 0.84)**	**0.0021**
10 Immunological disorder	245 (66)	256 (69)	0.97 (0.71 to 1.33)	0.86
Anti-dsDNA	224 (61)	231 (63)	1.02 (0.76 to 1.38)	0.88
11 ANA	364 (98)	367 (99)	0.69 (0.19 to 2.49)	0.57
Renal variables				
WHO class I–II	12 (5)	20 (8)	0.71 (0.33 to 1.50)	0.37
WHO class III–IV	62 (21)	71 (22)	1.13 (0.75 to 1.69)	0.57
WHO class V	15 (6)	16 (6)	1.05 (0.50 to 2.20)	0.90
Other*	11 (4)	6 (2)	1.24 (0.50 to 3.06)	0.65
ESRD	14 (4)		1.41 (0.62 to 3.24)	0.42
Cardiovascular events				
MI	39 (11)	19 (5)	**1.96 (1.09 to 3.55)**	**0.025**
ICVD	45 (12)	30 (8)	1.37 (0.84 to 2.24)	0.21
VTE	61 (16)	52 (14)	1.14 (0.76 to 1.71)	0.52
Clinical APS	68 (20)	61 (18)	1.08 (0.73 to 1.60)	0.68
Anti-β2GP-I IgG	58 (19)	57 (18)	0.98 (0.69 to 1.39)	0.91
Anti-β2GP-I IgM	8 (11)	11 (12)	0.96 (0.36 to 2.55)	0.93
LA	62 (23)	57 (21)	1.19 (0.79 to 1.80)	0.40
aCL-IgG	86 (26)	90 (28)	1.05 (0.70 to 1.59)	0.080
aCL-IgM	34 (14)	33 (13)	0.96 (0.36 to 2.55)	0.93

Logistic regression models were used to assess differences between smokers and non-smokers. All analyses were adjusted for age at last follow-up and disease duration.
p<0.05 (unadjusted for multiple comparisons) in bold.
*Patients with biopsies displaying signs of nephritis but not meeting the criteria for any of the above classes were classified as *other*.
ACR, American College of Rheumatology;[40] ANA, antinuclear antibodies; Anti-β2GP-I, anti-β_2 Glycoprotein-I; ; APS, antiphospholipid syndrome; dsDNA, double-stranded DNA; ESRD, end-stage renal disease; ICVD, ischaemic cerebrovascular disease; LA, lupus anticoagulant; MI, myocardial infarction; VTE, venous thromboembolism.;

table 1, respectively. Clinical data, including smoking status (ever-smoker, including current or a history of smoking, versus never-smoker), the ACR-82 classification criteria, antiphospholipid syndrome diagnosis, ESRD, renal biopsy data and CVE, defined as myocardial infarction (MI), ischaemic cerebrovascular disease (ICVD) or venous thromboembolism, was collected from medical records. For definitions, see online supplemental file. In the replication cohort, data regarding ESRD were not available, and data on smoking were available from 503 patients.

Genotyping and selection of SNPs

Genotyping of the discovery cohort was performed using the Illumina 200K Immunochip SNP array, for details, see online supplemental file. SNPs previously associated with SLE at genome-wide significance in the European population[2] were selected. For SNPs not included on the Immunochip, the SNP-proxy with the highest linkage disequilibrium (LD) ($r^2 \geq 0.96$) was selected. All SNPs were filtered for independent signals, removing the variant with the lowest SLE-OR for SNPs in LD ($r^2 > 0.2$). In total, 4 HLA and 56 non-HLA SNPs were investigated for associations with MI (online supplemental table 2). Individuals in the replication cohort were genotyped for three single nucleotide variants using a custom assay on the MassARRAY system (see online supplemental file).

Interleukin-12-induced phosphorylation of STAT4

Interleukin 12 (IL-12)-induced phosphorylation of signal transducer and activator of transcription 4 (pSTAT4) was previously determined in 72 healthy blood donors from Uppsala Bioresource using flow cytometry.[19] Smoking data were available from 45 of these donors, of which 20 were past or current smokers and 25 were non-smokers.

Statistical analysis

To investigate associations between smoking and clinical manifestations, logistic regression models were used. As smoking was associated with longer disease duration and higher age at follow-up (table 1), these variables were included as covariates. In analysis of associations between genetic variants and MI, SNPs were first analysed separately. Next, all variants demonstrating a positive association with MI were included in a forwards conditional multiple regression model. All analyses were adjusted for age and disease duration. Results considered statistically significant (unadjusted $p<0.05$) were reanalysed in the replication cohort using the same statistical model and covariates. Meta-analyses were performed on the two datasets and multiplicative and additive interactions between the *STAT4* risk allele and smoking were studied in a combined dataset through addition of a STAT4*smoking interaction term in the logistic models and by calculating the attributable proportion due to interaction, respectively.[20 21] Differences in levels of pSTAT4 were assessed by Student's t-test and by a linear regression model allowing adjustment for age and the *STAT4* risk allele. R[22] was used for all analyses except the meta-analyses which were performed in PLINK.[23]

RESULTS

Smoking is modestly associated with MI

Initially, we assessed the association between smoking and clinical manifestations (table 1). We found no evidence of any associations between smoking and the ACR criteria, except the haematological criterion, which was less prevalent in smokers (table 1). Elevated levels of red and white blood cells in smokers is a well-known phenomenon.[24] Smoking was not associated with a history of DVT or ICVD, however, a significant association between smoking and MI was observed (OR 1.96 (95% CI 1.09 to 3.55), p=0.025) (table 1).

Increased risk of MI in SLE-smokers with the *STAT4* risk allele

Next, we asked whether there are sub-groups of patients in which smoking plays a more prominent role in MI development. We initially examined 60 SNPs with established association with SLE ($p<5.0\times10^{-8}$) for association with MI (online supplemental table 2). We found that the Neutrophil Cytosolic Factor 2 (*NCF2)*, Interleukin-12A (*IL12A)* and *STAT4* risk alleles displayed independent, positive association with MI (table 2). In addition, patients carrying two alleles of both the *STAT4* and the *IL12A* risk variants (n=37, 4.9% of the patients) displayed a substantially higher prevalence of MI compared with those with any other allele combination (27% vs 7%) (OR 5.88 (95% CI 2.44 to 14.17), $p=7.9\times10^{-5}$) (figure 1A).

Next, we stratified patients by smoking status to determine whether each of the three SNPs displayed stronger association with MI in smokers. No significant associations were found for the *NCF2* or *IL12A* risk alleles (OR 1.58 (95% CI 0.89 to 2.78), p=0.12 and OR 1.36 (95% CI 0.89 to 2.08), p=0.15, respectively). However, the *STAT4* risk allele demonstrated a stronger association in smokers (OR 2.45 (95% CI 1.46 to 4.19), p=0.00086) (figure 2A). Next, we assessed the association between smoking and MI in patients carrying the *STAT4* risk allele and observed an almost 3−fold increase in risk for the smokers compared with the non-smokers (OR 2.72 (95% CI 1.24 to 6.00), p=0.013). In patients carrying two risk alleles, the risk was more than eightfold higher for smokers (OR 8.27 (95% CI 1.48 to 46.27), p=0.016). In contrast, we could not demonstrate a significant association between smoking and MI

Table 2 Associations between SLE risk SNPs and myocardial infarction in the discovery and replication cohort

		Discovery cohort (n=763)					Replication cohort (n=836)				
			Risk allele frequency					Risk allele frequency			
SNP	Gene name	HWE p	MI+	MI-	OR	P value	HWE p	MI+	MI-	OR	P value
rs17849502	*NCF2*	0.013	0.12	0.08	**2.00 (1.08 to 3.68)**	**0.027**	8.80×10^{-7}	0.18	0.11	1.94 (0.94 to 4.04)	0.075
rs11889341	*STAT4*	0.81	0.44	0.34	**1.76 (1.18 to 2.63)**	**0.0054**	0.53	0.40	0.31	1.81 (0.94 to 3.47)	0.075
rs564799	*IL12A*	0.29	0.68	0.59	**1.53 (1.01 to 2.31)**	**0.042**	1.00	0.74	0.61	**2.15 (1.08 to 4.26)**	**0.029**

Using a forward conditional multiple logistic regression model, 60 genetic variants with previously established association with SLE ($p<5\times10^{-8}$, online supplemental table 2) were analysed for associations with myocardial infarction in the discovery cohort. The table shows SNPs included in the final model. These SNPs were subsequently analysed in the replication cohort. Age at follow-up and disease duration were included as covariates. P<0.05 (unadjusted for multiple comparisons) in bold. HWE was tested on all patients in the discovery and replication cohorts, respectively.

HWE, Hardy-Weinberg equilibrium; IL12a, Interleukin12A; ; MI, myocardial infarction; NCF2, neutrophil cytosolic factor 2; ; SLE, systemic lupus erythematosus; SNPs, single-nucleotide polymorphisms; STAT4, signal transducer and activator of transcription 4.

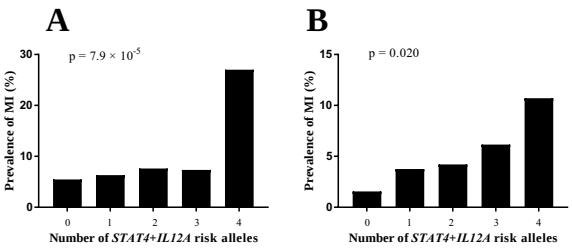

Figure 1 Prevalence of myocardial infarction (MI) in patients with different numbers of *STAT4* and *IL12A* risk alleles. The prevalence of MI for patients in the discovery (A) and the replication (B) cohorts, stratified by number of risk alleles of *STAT4* (rs11889341) and *IL12A* (rs564799). Patients carrying two risk alleles of both *STAT4* and *IL12a* ($n_{discovery\ cohort}$=37 (4.9%), $n_{replication\ cohort}$=28 (3.4%)) were compared with the remaining patients using logistic regression, adjusting for age at follow-up and disease duration. IL12, interleukin-12; STAT4, Signal Transducer and Activator of Transcription 4.

in patients without the risk allele (OR 1.20 (95% CI 0.49 to 2.96) p=0.55).

As patients with nephritis have previously been shown to have a higher prevalence of both MI and the *STAT4* risk allele.[25–27] we hypothesised that the results would be similar if using nephritis, rather than MI, as the outcome variable. Without stratifying for smoking, the association between the *STAT4* risk allele and nephritis reached suggestive significance (OR 1.23 (95% CI 0.98 to 1.54), p=0.072). The effect was more pronounced in the smokers only (OR 1.47 (95% CI 1.06 to 2.03), p=0.020). In addition, we found moderate evidence that patients with nephritis carrying the *STAT4* risk allele were at a greater risk of developing ESRD (OR 1.85 (95% CI 0.96 to 3.59), p=0.068), and this risk was enhanced in smokers (OR 2.52 (95% CI 1.04 to 6.10). p=0.040) (figure 2B). Of note, despite the non-smoking group including more patients with nephritis (n=140 vs n=129), no evidence of an association between the *STAT4* risk allele and nephritis or ESRD could be demonstrated in this group (OR 1.07 (95% CI 0.77 to 1.46), p=0.70 and OR 1.10 (95% CI 0.38 to 3.16), p=0.86, respectively).

To validate our significant findings, we performed the same analyses in an independent cohort of patients with SLE (online supplemental table 1). Analysis of the genetic variants demonstrated that the *IL12A* risk allele was the only gene variant significantly associated with MI when not accounting for smoking

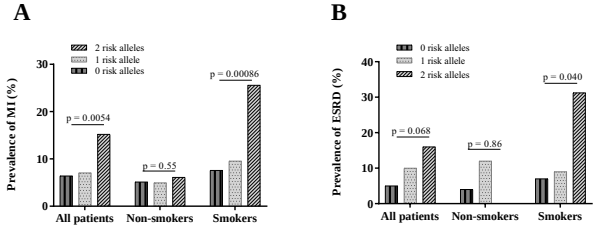

Figure 2 Prevalence of myocardial infarction (MI) and end-stage renal disease (ESRD) in the discovery cohort. Prevalence of MI (A) and ESRD (B) in the discovery cohort in all patients (n=776), smokers only (n=371) and non-smokers only (n=405), stratified by the number of *STAT4* (rs11889341) risk alleles. Patients with 0, 1 or 2 risk alleles in each group were compared using logistic regression, adjusting for age at follow-up and disease duration. STAT4, Signal Transducer and Activator of Transcription 4.

(table 2). Patients with two risk alleles of both *STAT4* and *IL12A* (n=28, 3.2% of the patients) were found to have a significantly higher prevalence of MI than patients without this combination of risk alleles (OR 7.21 (95% CI 1.36 to 38.27, p=0.020) (figure 1B). Similar to in the discovery cohort, we found a significant association between the *STAT4* risk allele and MI in smokers (OR 2.11 (95% CI 1.04 to 4.26), p=0.038). In addition, smoking was associated with MI in patients carrying the *STAT4* risk allele (OR 6.19 (95% CI 1.29 to 29.79), p=0.023). No evidence of these associations could be observed in the non-smoking group (OR 0.58 (95% CI 0.09 to 3.90), p=0.58 and OR 1.32 (95% CI 0.23 to 7.34), p=0.75, respectively). In patients carrying two risk alleles (n=51), the logistic regression could not be performed due to a 'perfect separation' between groups, with 9% of smokers having had a MI compared with 0% of never-smokers. As in the discovery cohort, we found an association between the *STAT4* risk allele and nephritis in smokers (OR 1.84 (95% CI 1.05 to 3.29), p=0.035), whereas the effect size was non-significant in non-smokers (OR 0.78 (95% CI 0.41 to 1.45), p=0.44).

Meta-analysis of the two cohorts showed the risk of MI to more than double with each additional *STAT4* risk allele in smokers (OR 2.28, p=0.00010). In contrast, no association could be detected in the never-smoker group (OR 0.80, p=0.52). Similarly, in meta-analysis of patients with 2 *STAT4* risk alleles (n=282, 22%), smoking was found to be a strong risk factor for MI (OR 8.27, p=0.016). For nephritis, each *STAT4* risk allele increased the risk by ~50% in smokers (OR 1.52, p=0.00051), whereas no increase in risk was found in the non-smoker group (OR 0.82, p=0.33).

Interaction between *STAT4* risk allele and smoking results in a higher risk of MI and nephritis

We subsequently performed interaction analyses on all patients and found a significant multiplicative interaction between the *STAT4* risk allele and smoking on the development of MI (OR 2.14 (95% CI 1.01 to 4.62), p=0.049) as well as nephritis (OR 1.53 (95% CI 1.08 to 2.17), p=0.020) (online supplemental table 3). Next, we examined additive interaction and observed an attributable proportion due to interaction of 0.54 (95% CI 0.24 to 0.83, p=0.00019) and 0.34 (95% CI 0.080 to 0.61, p=0.0051) for MI and nephritis, respectively.

To determine whether the effect of the *STAT4* risk allele on nephritis in smokers could explain the association with MI, we performed stratification of the combined dataset and investigated the association between the *STAT4* risk allele and MI in smokers and non-smokers without nephritis. In the smokers, the association between the *STAT4* risk allele and MI remained significant (n=428, OR 2.43 (95% CI 1.40 to 4.27), p=0.0017) (figure 3). Similarly, the association between the *STAT4* risk allele and nephritis in smokers remained significant after excluding patients with MI from the analysis (n=623, OR 1.54 (95% CI 1.20 to 1.98), p=0.00076).

Lastly, as both SLE risk alleles in *STAT4* and smoking have shown association with the development of aPL in previous studies,[28 29] we assessed the association between the *STAT4* risk allele and aPL in smokers, however, it was not significant (OR 1.56 (95% CI 0.71 to 3.72, p=0.26). Next, we performed a multiple regression model in the smoking group including the *STAT4* risk allele, any aPL, nephritis, age at follow-up, and disease duration as covariates. We found the association between the *STAT4* risk allele and MI to remain significant (OR 3.26 (95% CI 1.15 to 9.20), p=0.026), whereas neither aPL nor

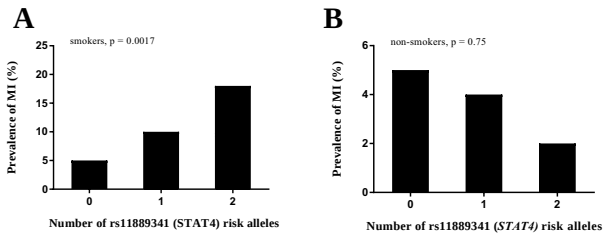

Figure 3 The prevalence of myocardial infarction (MI) in patients without nephritis. To investigate whether the association between the rs11889341 (*STAT4*) allele and MI was dependent on the association between the *STAT4*–nephritis association, all patients with nephritis were excluded and the prevalence of MI was subsequently plotted for smoking (A) and non-smoking (B) patients with 0, 1 or 2 of the *STAT4* risk allele. Patients with 0, 1 or 2 risk alleles in each group were compared using logistic regression, adjusting for age at follow-up and disease duration. STAT4, signal transducer and activator of transcription 4.

nephritis displayed significant association with MI (p=0.87 and p=0.50, respectively), (online supplemental table 4).

Levels of activated STAT4 are increased in smokers

The *STAT4* risk allele is associated with higher levels of pSTAT4 in CD8+ T cells from SLE patients on stimulation with IL-12.[19] Furthermore, higher levels of *STAT4* expression have demonstrated association with increased cardiovascular damage in patients with SLE.[30] To investigate whether smoking also increases the levels of pSTAT4, we analysed IL-12 stimulated CD8+ T cells from healthy blood donors who were smokers (n=20) or never-smokers (n=25). We found the levels of pSTAT4 to be higher in smokers (p=0.0063), with a mean value of 1063 compared with 565 in non-smokers (figure 4). When adjusting for age and the *STAT4* risk allele, which is in LD (r^2=1.00) with a *STAT4* risk variant previously shown to influence levels of pSTAT4 in these individuals,[19 31] the association between smoking and levels of pSTAT4 remained significant (β=396, p=0.023).

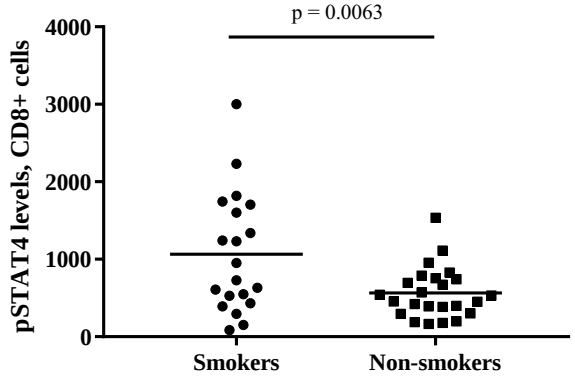

Figure 4 Levels of pSTAT4 in CD8+ T cells after stimulation with IL-12. The levels of IL-12–induced pSTAT4 were compared between healthy blood donors who were smokers (current or past) (n=20) and never-smokers (n=25) by Student's t-test. IL-12, interleukin-12; pSTAT4, phosphorylated Signal Transducer and Activator of Transcription 4.

DISCUSSION

In the present study, we demonstrate that smoking substantially increases the risk of MI in a subset of patients with SLE carrying a variant of the *STAT4* SLE-risk gene. In both the discovery and replication cohorts, the effect size increased with an increasing number of STAT4 risk alleles, with smoking giving rise to a more than 8-fold risk of MI in homozygous individuals. We believe that our results add important knowledge in the understanding of how SLE-risk alleles can modulate the effect of traditional risk factors.

The prevalence of MI is higher in SLE patients with nephritis than patients without renal manifestations[26 32] and SLE-risk alleles in *STAT4* have previously been linked to both nephritis and severe renal insufficiency.[25 27] We, therefore, speculated that the smoking-*STAT4* risk allele interaction did not directly affect MI, but rather, was a consequence of an interaction between the *STAT4* risk allele and smoking on the development of nephritis. Indeed, we found that this gene-environment combination also results in a higher risk of nephritis, as well as ESRD. Interestingly, however, the *STAT4* risk allele/smoking effect on MI did not decrease when adjusting the model for nephritis or when completely removing the patients with nephritis from the analysis. Similarly, the effect of the *STAT4* risk allele/smoking on nephritis remained significant after excluding all patients with MI from the analysis, indicating that the associations were independent.

Based on these results, we hypothesised that the increased risk in individuals who smoke and carry the risk allele is connected with the levels of activated STAT4 in these individuals. Hagberg *et al* have shown that the rs7574865 *STAT4* risk allele—which is in perfect LD (r^2=1.00) with the SNP used in this study[31]—is associated with increased levels of pSTAT4 in activated CD8+T cells of SLE patients.[19] Therefore, we assessed whether smoking elevates pSTAT4 in this cell type and found that the levels were almost twofold higher in smokers. This observation is in line with previous findings by Di Stefano *et al*, who demonstrated higher levels of pSTAT4 in bronchial T cells from healthy smokers compared with non-smokers.[33] When STAT4 is activated and phosphorylated, it homodimerises and translocates to the nucleus where it induces expression of hundreds of genes, resulting in production of IFN-γ, T-helper type 1 and 17 differentiation and activation of monocytes.[34] Increased *STAT4* mRNA expression is associated with increased cardiovascular damage in patients with SLE,[30] and several studies on animal models indicate a link between STAT4 and the development of atherosclerosis.[35] The mechanism of how smoking leads to increased levels of activated STAT4 is unclear, however, we speculate that epigenetics may constitute the bridge between smoking, genetics and SLE. It is well known that smoking affects both overall DNA methylation and specific gene promotors.[36 37] Epigenetic regulation is further believed to play an important role both in cardiovascular biology and in SLE development.[36 38] Whether smoking is associated with epigenetic changes in SLE-specific genes, and if such changes are associated with specific manifestations of SLE, deserves further studies.

The analyses of individual SLE risk alleles identified the SLE-risk SNP *IL12A* to be associated with an increased risk of MI, and that patients in the discovery and replication cohort carrying two alleles of both the *IL12A* and *STAT4* risk SNPs had a more than fivefold and eightfold risk of MI, respectively. The *IL12A* SNP is located within the fourth intron of the *IL12A* gene, which encodes the p35 subunit of the IL-12 protein. On binding to its receptor, IL-12 induces phosphorylation of STAT4.[34] We

believe that the association of the *IL12A* risk allele, in addition to the *STAT4* risk allele, with MI points to the importance of this pathway in the development of the comorbidity. Previous work has demonstrated that JAK-inhibitors efficiently block the increase in pSTAT4 levels, and ameliorate murine lupus as well as its associated vascular dysfunction.[19 39] Due to the potential therapeutic strategy of JAK-inhibitors for patients with SLE displaying an altered activity in this particular pathway, we believe that further studies of the effect of this pathway on development of CVE are warranted.

This study's strength is the large, well-characterised discovery cohort, that results were validated in a second large cohort, and the analysis of healthy control cells, which confirmed that pSTAT4 levels are higher in smokers. In addition, the quality control of genetic data was rigorous, and the patients' long mean follow-up time of 17 years allowed for many outcome variables such as MI to be recorded. There are, however, some limitations. First, our study is based on retrospective data and we lacked data on the year of smoking cessations. As patients who were past smokers at the last follow-up may have been active smokers at the time of their CVE, we could not analyse previous and current smoking separately. Second, we did not have data on number of pack-years, which may have generated more precise results. Third, the study includes only Scandinavian patients with SLE, and whether the associations are generalisable to patients of other ethnicities needs further investigation.

CONCLUSION

We demonstrate that smokers carrying the *STAT4* risk allele are at an increased risk of MI and nephritis and that the IL12-STAT4 pathway may be important for the development of MI. Our results stress the importance of smoking cessation in SLE and particularly among those carrying this risk allele.

Author affiliations
[1]Department of Medical Sciences, Rheumatology and Science for Life Laboratory, Uppsala University, Uppsala, Sweden
[2]Department of Biomedical and Clinical Sciences, Division of Inflammation and Infection, Linköping University, Linkoping, Sweden
[3]Department of Rheumatology, Oslo University Hospital, Oslo, Norway
[4]Department of Clinical Sciences Lund, Rheumatology, Lund University, Skane University Hospital, Lund, Sweden
[5]Division of Rheumatology, Department of Medicine, Karolinska Institutet and Karolinska University Hospital, Stockholm, Sweden
[6]Department of Rheumatology, Aarhus University Hospital, Aarhus, Denmark
[7]Department of Biomedicine, Aarhus University, Aarhus, Denmark
[8]Department of Rheumatology, Odense University Hospital, Odense, Denmark
[9]Center for Rheumatology and Spine Diseases, Copenhagen University Hospital Rigshospitalet, Copenhagen, Denmark
[10]Institute of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark

Acknowledgements We thank the patients and healthy volunteers for participating, clinicians and researches contributing to the replication cohort and Maija-Leena Eloranta, Cane Yaka, Rezvan Kiani and Karolina Tandre.

Contributors SR, DL and LR designed the study. SR, NH, JKS, AA, PP, CS, AJ, IG, A-CS, AMT, AV, AAB, ES, LR and DL collected the data for the discovery cohort. KL, AMT, AV, ØM and SJ collected the data for the replication cohort. SR, DL and LR analysed the data. SR, DL and LR wrote the manuscript. All authors revised the manuscript critically for important intellectual content and approved the final version of the manuscript.

Funding This work was supported by grants from the Swedish Research Council for Medicine and Health (D0283001), the Swedish Rheumatism Association, King Gustav V's 80-year Foundation, the Swedish Society of Medicine and the Ingegerd Johansson donation, Åke Wiberg's foundation, Gustafsson's foundation, Selander's foundation, Norsk Revmatikerforbunds Forskningsfond, Pahles legat og Stortuens legat and the Swedish Society for Medical Research (S20-0127).

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval The study protocol was approved by the Regional Ethical Review Board, Uppsala (DNR 2020–05065) and the local ethics committees.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. Deidentified patient data and statistical code are available on request with project plan.

ORCID iDs
Sarah Reid http://orcid.org/0000-0003-4065-6875
Niklas Hagberg http://orcid.org/0000-0003-2064-2716
Johanna K Sandling http://orcid.org/0000-0003-1382-2321
Pascal Pucholt http://orcid.org/0000-0003-3342-1373
Christopher Sjöwall http://orcid.org/0000-0003-0900-2048
Elisabet Svenungsson http://orcid.org/0000-0003-3396-3244
Lars Rönnblom http://orcid.org/0000-0001-9403-6503

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TRANSLATIONAL SCIENCE

B cell subset composition segments clinically and serologically distinct groups in chronic cutaneous lupus erythematosus

Scott A Jenks ,[1,2] Chungwen Wei,[1,2] Regina Bugrovsky,[1,2] Aisha Hill,[1,2] Xiaoqian Wang,[1,2] Francesca M Rossi,[1,2] Kevin Cashman,[1,2] Matthew C Woodruff,[1,2] Laura D Aspey,[3] S. Sam Lim,[1] Gaobin Bao,[1] Cristina Drenkard,[1] Ignacio Sanz[1,2]

Handling editor Josef S Smolen

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2021-220349).

[1]Department of Medicine, Division of Rheumatology, Emory University School of Medicine, Atlanta, Georgia, USA
[2]Lowance Center for Human Immunology, Emory University, Atlanta, Georgia, USA
[3]Department of Dermatology, Emory University School of Medicine, Atlanta, Georgia, USA

Correspondence to
Dr Ignacio Sanz, Department of Medicine, Division of Rheumatology, Emory University School of Medicine, Atlanta, Georgia, USA; ignacio.sanz@emory.edu

SAJ and CW contributed equally.

CD and IS are joint senior authors.

Received 16 March 2021
Accepted 17 May 2021
Published Online First 3 June 2021

ABSTRACT

Objective While the contribution of B-cells to SLE is well established, its role in chronic cutaneous lupus erythematosus (CCLE) remains unclear. Here, we compare B-cell and serum auto-antibody profiles between patients with systemic lupus erythematosus (SLE), CCLE, and overlap conditions.

Methods B-cells were compared by flow cytometry amongst healthy controls, CCLE without systemic lupus (CCLE+/SLE−) and SLE patients with (SLE+/CCLE+) or without CCLE (SLE+/CCLE−). Serum was analyed for autoreactive 9G4+, anti-double-stranded DNA, anti-chromatin and anti-RNA antibodies by ELISA and for anti-RNA binding proteins (RBP) by luciferase immunoprecipitation.

Results Patients with CCLE+/SLE− share B-cell abnormalities with SLE including decreased unswitched memory and increased effector B-cells albeit at a lower level than SLE patients. Similarly, both SLE and CCLE+/SLE- patients have elevated 9G4+ IgG autoantibodies despite lower levels of anti-nucleic acid and anti-RBP antibodies in CCLE+/SLE−. CCLE+/SLE− patients could be stratified into those with SLE-like B-cell profiles and a separate group with normal B-cell profiles. The former group was more serologically active and more likely to have disseminated skin lesions.

Conclusion CCLE displays perturbations in B-cell homeostasis and partial B-cell tolerance breakdown. Our study demonstrates that this entity is immunologically heterogeneous and includes a disease segment whose B-cell compartment resembles SLE and is clinically associated with enhanced serological activity and more extensive skin disease. This picture suggests that SLE-like B-cell changes in primary CCLE may help identify patients at risk for subsequent development of SLE. B-cell profiling in CCLE might also indentify candidates who would benefit from B-cell targeted therapies.

Key messages

What is already known about this subject?

- While the contribution of B cells to systemic lupus erythematosus (SLE) pathogenesis is apparent, their role in primary chronic cutaneous lupus erythematosus (CCLE) is less clear. Although CCLE is characterised by low serological activity, some patients with this condition can produce autoantibodies and potentially develop systemic phenotypes. However, little is known about B cell phenotype and B cell tolerance in CCLE.

What does this study add?

- B cell phenotypes were found to be heterogeneous in primary CCLE, with some patients resembling healthy donors and others showing an expansion in effector B cells resembling that seen in SLE.
- Patients with a SLE-like B cell phenotype were more likely to have generalised lesions and were more serologically active, with a high prevalence of nucleic acid and RNA-binding protein-specific antibodies.
- Autoreactive VH4.34 9G4+ IgG antibodies were elevated in patients with CCLE; however, these antibodies were not associated with antinucleic acid IgG as typically occurs in SLE.

How might this impact on clinical practice or future developments?

- The heterogeneity in B cell phenotype may reflect fundamentally different disease processes in CCLE. B cell phenotype should be examined as a potential prognostic marker for patients with CCLE that may develop systemic disease.

To cite: Jenks SA, Wei C, Bugrovsky R, *et al*. *Ann Rheum Dis* 2021;**80**:1190–1200.

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disease characterised by heterogeneous clinical manifestations and the production of diverse autoantibodies resulting from defective B cell tolerance and B cell hyper-responsiveness to stimulation.[1] While skin involvement is common in SLE,[2] it may also be present in patients with 'primary' chronic cutaneous lupus erythematosus (CCLE), in the absence of systemic involvement. CCLE includes discoid lupus erythematosus (DLE) and other conditions that often lead to permanent skin scarring,[3] and up to 20% of these patients develop SLE over several years.[3–5] However, the presence of DLE in patients with SLE has been found to reduce the risk of severe systemic manifestations, including lupus nephritis.[6] These findings suggest potential immunopathogenic differences across lupus categories.

In SLE, B cell hyperactivity is illustrated by the diversity and abundance of autoantibodies,[7] the concentration of risk alleles on B cell signalling pathways,[8] and the clinical benefit imparted by anti-B cell therapies.[9 10] In contrast, limited autoantibody production and poor response to B cell depletion in CCLE (relative to the dermatological improvement observed in SLE), have called into question the pathogenic role of B cells in this condition.[11 12]

Multiple B cell abnormalities have been consistently documented in SLE including the expansion of plasmablasts (PB), transitional and pregerminal centre cells[13 14]; increased $IgD^{-}CD27^{-}$ double negative (DN) B cells,[15 16] owing to the preferential expansion of the effector DN2 compartment,[17–19] and the contraction of IgD+CD27+ USM B cells.[19] Moreover, SLE is characterised by profound defects in the censoring of autoreactive B cells both centrally (antinuclear reactivity),[20 21] and peripherally, as illustrated by autoreactive VH4.34 antibodies that are recognised by the rat anti-human idiotypic antibody 9G4 (9G4+),[22] whose expansion is promoted by defective germinal centre censoring.[23] These defects lead to the accumulation of high levels of serum 9G4+ IgG in 45%–70% of patients with SLE with very high disease specificity (>90%).[24] These autoantibodies are associated with higher renal, neurological, haematological and cardiovascular activity, but not skin manifestations.[22] 9G4+ IgG antibodies also correlate with anti-double-stranded DNA (dsDNA) IgG and contribute a substantial proportion of anti-dsDNA antibodies and a majority of autoantibodies recognising apoptotic cells, a major immunogenic source in SLE.[22 25]

In contrast to SLE, little is known about the regulation and potential role of B cells in CCLE. Similarly, little information is available regarding B cell tolerance in this condition. In this study, we compared B cell and autoantibody profiles between patients with primary CCLE and patients with SLE with and without CCLE. Our results demonstrate CCLE heterogeneity with SLE-like abnormalities in a significant fraction of patients. This profile was associated with selective breakdown of B cell tolerance and the expression of autoantibodies. We postulate that B cell profiling may help identify patients with CCLE likely to progress to SLE and more likely to respond to B cell therapies.

PATIENTS AND METHODS

Patient samples

We collected blood samples among participants of the Georgia Organized Against Lupus (GOAL), a population-based cohort of individuals with a validated diagnosis of either SLE or primary CCLE. GOAL recruitment and data collection are described in the online supplemental methods and published elsewhere.[26] Medical records review, physician assessment and picture review were conducted to validate the lupus diagnosis. Cases with a dermatologist-documented diagnosis of either DLE, lupus erythematosus panniculitis (LEP), lupus erythematosus tumidus (LET) or chilblain lupus erythematosus (ChLE) were classified as CCLE. The 1997 Revised American College of Rheumatology Classification Criteria for SLE,[27] and the attending rheumatologist/dermatologist judgement were used to classify cases into three categories: primary CCLE (CCLE+/SLE−), SLE associated with CCLE (SLE+/CCLE+) and SLE without CCLE (SLE+/CCLE−). The Cutaneous Lupus Erythematosus Disease Area and Severity Index (CLASI) scores[28] were assessed within 14 days of the blood draw in a subset of participants. No patients were being treated with B cell depletion therapy. Thirty-nine additional patients with SLE were assessed for serological studies. Additionally, we utilised 46 health control donors for flow cytometry and 69 for serological studies.

Patient and public involvement

We have convened a diverse group of GOAL participants into the Lupus Patient Advisory Research Council (L-PARC). L-PARC members meet at least once a year with researchers to provide feedback on study measures and advice on recruitment, retention and dissemination of findings.

B cell phenotyping by flow cytometry

Blood was collected from patients in BD Vacutainer CPT tubes and peripheral blood mononuclear cells (PBMCs) were isolated and banked in liquid nitrogen until use. For flow cytometry analysis of the B cell subsets, PBMCs were stained at 4°C for 30 min in phopsphate buffered saline (PBS) plus 2% fetal bovine serum with fluorochrome-conjugated antibodies against the markers described in online supplemental table 1 and washed. Cells were then stained with Fixable Viability Dye eFluor506 (eBioscience) and fixed with 0.5% formaldehyde in PBS followed by washing and acquisition on a LSRII Flow Cytometer (BD Bioscience). Flowjo (BD Bioscience) software was used for analysis. Clustering analysis is described in the online supplemental methods.

Apoptotic cells binding assay

Apoptosis was induced in CD45-negative Jurkat cells (J45.1; American Type Culture Collection), which were then incubated with patient serum as described previously,[25] and in the online supplemental methods.

Serological assays

9G4+ IgG, IgM and IgA antibodies were assayed by ELISA as described in the online supplemental methods. Anti-dsDNA IgG and antichromatin IgG levels were tested by QUANTA Lite dsDNA ELISA and Chromatin ELISA kits (INOVA Diagnostics). Anti-RNA was quantified by ELISA,[19] and anti-RNA-binding protein antibodies by luciferase immunoprecipitation assay as described previously,[29 30] and in the online supplemental methods.

Statistical analysis

Statistical analysis was done using Graphpad Prism V.8. Mann-Whitney U test was used to compare differences between two groups and Kruskal-Wallis test with Dunn's multiple comparison test to compare multiple groups. Fischer's exact test was used for contingency testing of distribution in two categories and χ^2 test for more than two categories. Correlation was determined by Pearson correlation coefficient or Spearman's rank correlation coefficient.

RESULTS

Description of patients

We obtained blood samples of 207 patients: 69 CCLE+/SLE−, 53 CCLE+/SLE+ and 85 SLE+/CCLE. Among the CCLE+/SLE− and CCLE+/SLE+ cases, 65 (94%) and 52 (98%) had a diagnosis of DLE, respectively. Of those, 9/65 and 3/52 had DLE associated with LEP or ChLE. The non-DLE cases were diagnosed with LEP (three in the CCLE+/SLE− and one in the CCLE+/SLE+ groups) and one in the CCLE+/SLE− had LET. Table 1 depicts demographic and disease characteristics by group; patients who were CCLE+/SLE− were significantly older and had shorter disease duration than the two other groups.

Table 1 Descriptive characteristics of patient samples

Characteristics	CCLE+/SLE− (n=69)	SLE+/CCLE+ (n=53)	SLE+/CCLE− (n=85)	P value
Age, mean±SD	51.2±13.7	43.0±12.7	47.6±13.6	0.0047
Disease duration, mean±SD	9.9±9.5	10.1±9.1	14.0±9.7	0.013
Gender, n (%)				
Male	11 (15.9)	6 (11.3)	6 (7.1)	0.22
Female	58 (84.1)	47 (88.7)	79 (92.9)	
Race*, n (%)				
Black or African American	63 (92.6)	48 (90.6)	80 (94.1)	0.74
White	5 (7.4)	5 (9.4)	5 (5.9)	
Family history of lupus, n (%)				
No	53 (80.3)	37 (71.2)	65 (76.5)	0.51
Yes	13 (19.7)	15 (28.8)	20 (23.5)	
DLE location†, n (%)				0.034
Above the neck	40 (61.5)	20 (38.5)	NA	
Below the neck	1 (1.5)	3 (5.7)	NA	
Above and below the neck	24 (36.9)	29 (55.8)	NA	
SLE outcomes				
Disease activity, SLAQ score mean±SD	NA	19.5±7.8	16.7±8.7	0.077
Organ damage, n (%)				
No damage (SA-BILD score=0)	NA	4 (9.1)	9 (10.7)	0.4
Mild damage (SA-BILD score=1–2)	NA	20 (45.5)	28 (33.3)	
Severe damage (SA-BILD score≥3)	NA	20 (45.5)	47 (56.0)	
Immunosuppressive drugs‡, n(%)	7 (10.6)	10 (27.0)	34 (40.5)	0.0002

*One participant who self-reported 'other race' is not listed.
†DLE cases per group are n=65 within CCLE+/SLE− and n=52 within CCLE+/SLE+.
‡Comprise any or a combination of azathioprine, cyclophosphamide, cyclosporine,methotrexate and mofetil mycophenolate; there were 3 missing data for CCLE+/SLE−, 16 for SLE+/CCLE+ and 1 for SLE+/CCLE−.
CCLE, chronic cutaneous lupus erythematosus; DLE, discoid lupus erythematosus; SA-BILD, self-administered Brief Index of Lupus Damage ; SLAQ, Systemic Lupus Activity Questionnaire; SLE, systemic lupus erythematosus.

B cell homeostasis in CCLE

Canonical human CD19+ Bcell subsets were defined by the expression of IgD, CD27 and CD38 as combined naive and transitional (N+T; unswitched memory (USM); isotype switched memory (SWM); DN; and PB (figure 1A)).[31] The expression of CD11c and CD21 further discriminated DN1, DN2 and DN3 among DN cells and resting naive (rNAV) and activated naive (aNAV). CD24 expression identified transitional populations (T1+T2) in the N+T compartment (figure 1B).[32–34]

All three lupus groups shared characteristic perturbations of normal B cell homeostasis consisting of loss of USM cells and expansion of PB (figure 1C). DN cells were expanded in all lupus groups, but with higher values in SLE+/CCLE− than in CCLE+/SLE−. Notably, within patients with SLE, the presence of CCLE correlated with DN expansion of lower magnitude (figure 1C), a feature consistent with both, the association between DN2 and LN in SLE and the decreased incidence of LN in SLE+/CCLE+. Consistent with our previous findings in SLE and severe COVID-19 infections,[35] DN expansions were accounted for in all groups by increases of effectors DN2 and DN3 cells with concomitant reversal of the normal predominance of DN1 cells, a population transcriptionally linked to resting SWM cells (figure 1D,E).[19] Similarly, aNAV, representing DN2 progenitors, were also expanded in SLE+/CCLE− and SLE+/CCLE+, relative to CCLE+/SLE− (figure 1F). Immature T1/T2 were expanded in CCLE relative to HCD and SLE+/CCLE− (figure 1G). As further illustrated below, however, all lupus groups were heterogeneous for these populations and included a significant fraction of patients with values above the upper limit of healthy individuals. This was particularly true for SWM and a substantial fraction of patients with SLE+/CCLE− (17%), SLE+/CCLE+ (19%) and CCLE+/SLE− (16%) had a frequency of SWM more than 2SD over the HCD mean.

B cell fingerprinting and disease heterogeneity

Multivariant analysis of B cell profiles revealed significant heterogeneity within patients with lupus (figure 2A). Overall, the combined cohort of HCD and all lupus subsets could be clustered into five separate groups defined by the relative frequencies of three B cell types: (1) early B cell (rNAV, early T1+T2); (2) memory (USM, SWM and DN1); and effector (aNAV, DN2, DN3 and PB). HCD could be separated into two clusters (III/IV) defined by higher frequencies of USM and rN+T3 subsets (figure 2A). In contrast, patients with SLE were concentrated within three clusters with only small fractions (15%–16%), expressing HCD-like B cell profiles. SLE clusters I/II were characterised by a more activated B cell profiles with high frequencies of effector B cells including aN, DN2, DN3 and PB (figure 2A–C), which were most pronounced in cluster II. In turn, SLE cluster V was characterised by the coordinated expansion of T1/T2 and rN/T3 cells in combination with the largest decrease of USM cells (figure 2C). Patients with CCLE+/SLE− displayed the largest degree of B cell heterogeneity of all groups with significant representation within all clusters (figure 2A,B). Overall, while a small majority (58%), expressed SLE-like profiles (I,II,V), 42% had HCD-like B cells. Within the CCLE+/SLE− that clustered with patients with SLE, 64% belonged in clusters I/II with cluster II contributing 12% of all patients with CCLE.

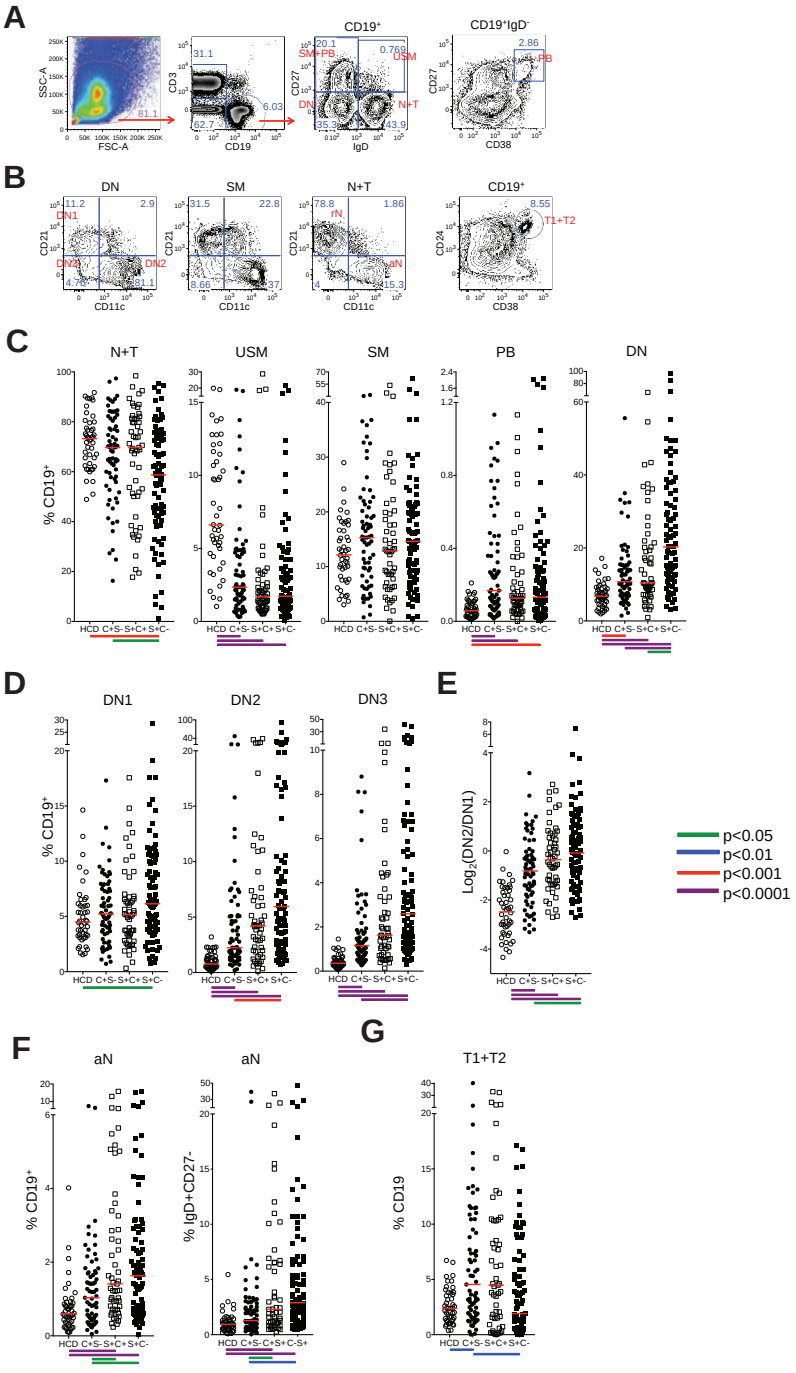

Figure 1 Perturbations of B cell homeostasis in patients with CCLE and SLE. (A) Gating scheme for the flow cytometer analysis of human B cells for a representative SLE sample. CD19+, CD3− B cells are divided into IgD+CD27− naive plus transitional (N+T), IgD+CD27+ USM, IgD−CD27+ memory plus plasmablasts (PB) SM+PB and IgD−CD27− DN, PB were gated as IgD−CD27++CD38++. (B) Separation of DN into DN1 (CD21+CD11c−), DN2 (CD21−CD11c+) and DN3 (CD21−CD11c−). aN were gated from N+T based on CD21−CD11c+. High CD24 and CD38 expression was used to gate transitional (T1+T2). (C) B cell subset frequencies were compared among healthy controls (HCD, n=46), primary CCLE (C+S−, n=69), SLE overlapped with CCLE (S+C+, n=53) and SLE without CCLE (S+C−, n=85). Short horizontal lines indicate the median. The frequency of PB (IgD−CD27++CD38++) was subtracted from that of the IgD−CD27+ compartment to derive the proportion of SM. (D) The percentage of DN1, DN2 and DN3 as a proportion of CD19+. (E) Relative frequencies of DN2 to DN1 is expressed as the $\log^2$ transformed ratio of DN2 to DN1. (F) The percentage of aN as proportion of CD19+ and total naive. (G) The percentage of T1+T2 as a proportion of total CD19+. Bars beneath each plot indicate the statistical significance as determined by a Kruskal-Wallis test followed by Dunn's multiple comparisons test with p<0.05 (green), p<0.01 (blue), p<0.001 (red), p<0.0001 (dark purple). aN, activated naive; CCLE, chronic cutaneous lupus erythematosus; DN, double negative; PB, plasmablasts; SLE, systemic lupus erythematosus; USM, unswitched memory.

Serological autoimmunity in CCLE

Given that subsets of patients with CCLE+/SLE− shared SLE B cell abnormalities, we examined whether tolerance was similarly compromised using serological autoreactivity as a readout. We found that class switched 9G4+ IgG (figure 3A) and 9G4+ IgA (figure 3B) antibodies were elevated in all lupus groups, which

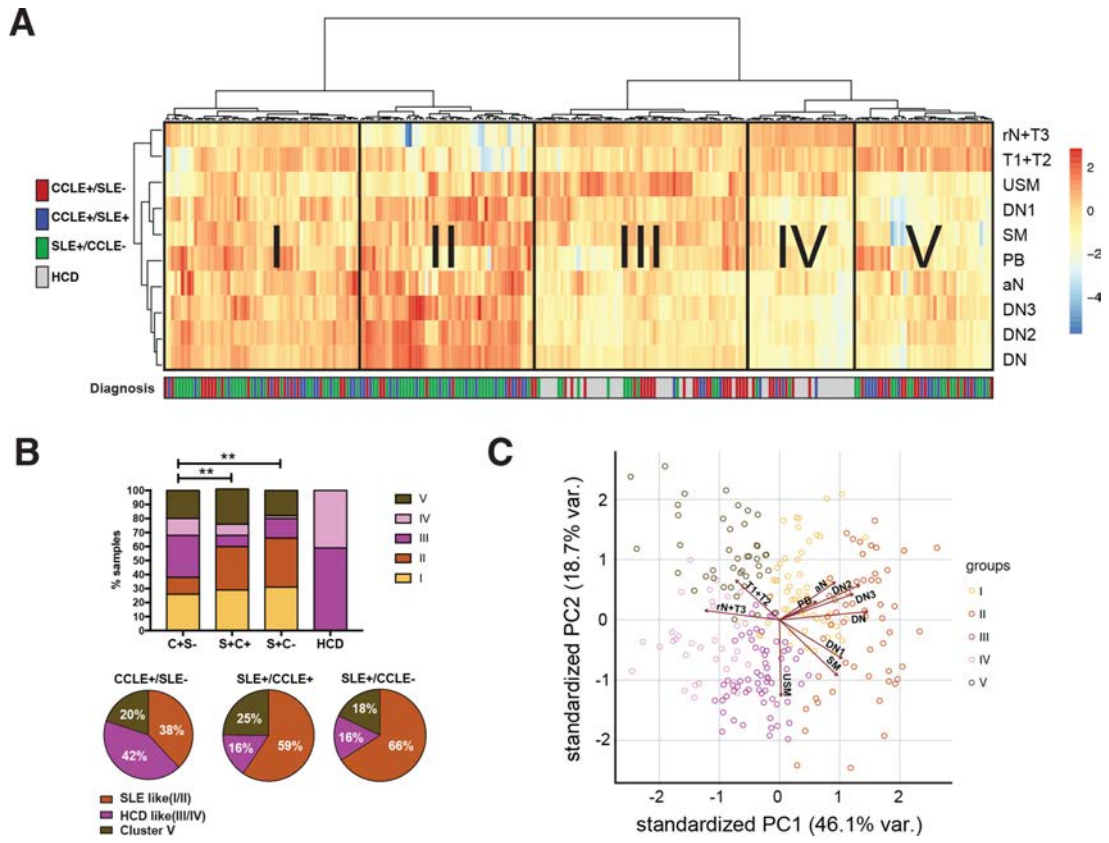

Figure 2 B cell fingerprint of patients with CCLE+/SLE−, SLE+/CCLE+ and SLE+/CCLE−. (A) Hierarchical clustering of samples by B cell subset frequency. Patients are clustered on the top and diagnosis is indicated by colour underneath. B cell subsets are clustered on the right. Patients were divided into five groups as indicated by Roman numeral (I–V). (B) Group distribution for different diagnostic categories, the distribution of patients with CCLE+/SLE− significantly differed from that of SLE+/CCLE+ and SLE+/CCLE−. The majority of SLE+/CCLE+ and SLE+/CCLE− samples were in groups I and II, while HCD samples were only found in groups III and IV. More patients with CCLE+/SLE− were in the HCD-enriched groups III and IV and fewer in SLE-enriched groups I and II. χ^2 test was used to compare frequencies, because no HCD clustered in I,II and V χ^2 tests comparing HCD to patients with lupus were not performed. (C) Principal component plot with cluster group indicated by colour and loading vectors for each B cell subset indicated. X^2 test: **p<0.001. CCLE, chronic cutaneous lupus erythematosus; HCD, healthy controls; SLE, systemic lupus erythematosus

shared similar frequencies (48%–57%) and median values of 9G4+ IgG and IgA antibodies. Only minor differences were observed in 9G4+ IgM (figure 3C).

The canonical autoreactivity of 9G4 antibodies is imparted by its germline sequence and results in binding to the B220 epitope expressed on B cells which results in the majority of naive B cells becoming 9G4+.[36] In addition, 9G4 antibodies also mediate high reactivity against apoptotic cells in patients with SLE through HCDR3-determined binding.[25 37] Of interest, a much larger fraction of 9G4+ antibodies in patients with CCLE+SLE− were autoreactive against apoptotic cell antigens (48% positive) (figure 3D) with a significantly lower level of anti-B cell autoreactivity (17% of samples with serum 9G4+ antibodies) (figure 3E). Anti-dsDNA and anti-chromatin antibodies were present in patients with CCLE+/SLE− but a lower frequency than in SLE (figure 4A,B; anti-dsDNA: 33% of SLE+/CCLE+ and 44% of SLE+/CCLE− compared with only 13% of CCLE+/SLE−) and anti-chromatin antibodies were similar. Anti-dsDNA and anti-chromatin were positively correlated in both groups of patients with SLE (figure 4C). Consistent with previous findings, 9G4+ IgG positively correlated with anti-dsDNA and anti-chromatin IgG in the SLE+ groups (figure 4D,E). In contrast, no correlation was found in patients with CCLE+/SLE−. Instead, anti-dsDNA and anti-chromatin antibodies were both sharply uncoupled from 9G4+ IgG even in patients with elevated values of both types of autoantibodies.

Anti-RNA antibodies are commonly found in SLE and we have shown that this association is particularly strong in patients with expanded effector B cells.[19] Anti-RNA antibodies were elevated in all three lupus groups including in 46% of patients with CCLE+/SLE− compared with 72% of patients with SLE+/CCLE− and 56% of patients with SLE+/CCLE+. As shown in figure 4F, anti-RNA titers were lower in patients with CCLE+/SLE− than in patients with SLE+/CCLE−.

We used a sensitive and highly quantitative luciferase immunoprecipitation assay to quantify serum anti-RNA-binding protein (RBP) antibodies (Sm, RNP, Ro52 and Ro60).[29 30] In all, RBP autoantibodies were more common in SLE groups. Consistent with our previous findings, the frequency of anti-Sm antibodies (71%), was significantly higher than that commonly encountered in cohorts with lower representation of African American patients.[19 38] RBP autoantibodies were also present in patients with CCLE+/SLE− although at a significantly lower frequency. Nonetheless, detectable levels of anti-Sm, were present in 38%, a rate consistent with SLE cohorts with lower representations of African American patients . Anti-RNP (17%), anti-Ro52 (19%) and anti-Ro60 (49%), while substantial, were also lower than observed in SLE+/CCLE− and SLE+/CCLE+ (figure 5A). In patients with CCLE+/SLE−, only anti-Ro52 and anti-Sm titers were higher than HCD and titers for each antigen were higher in patients with SLE+/CCLE− and SLE+/CCLE+ than in patients with CCLE+/SLE− (figure 5B).

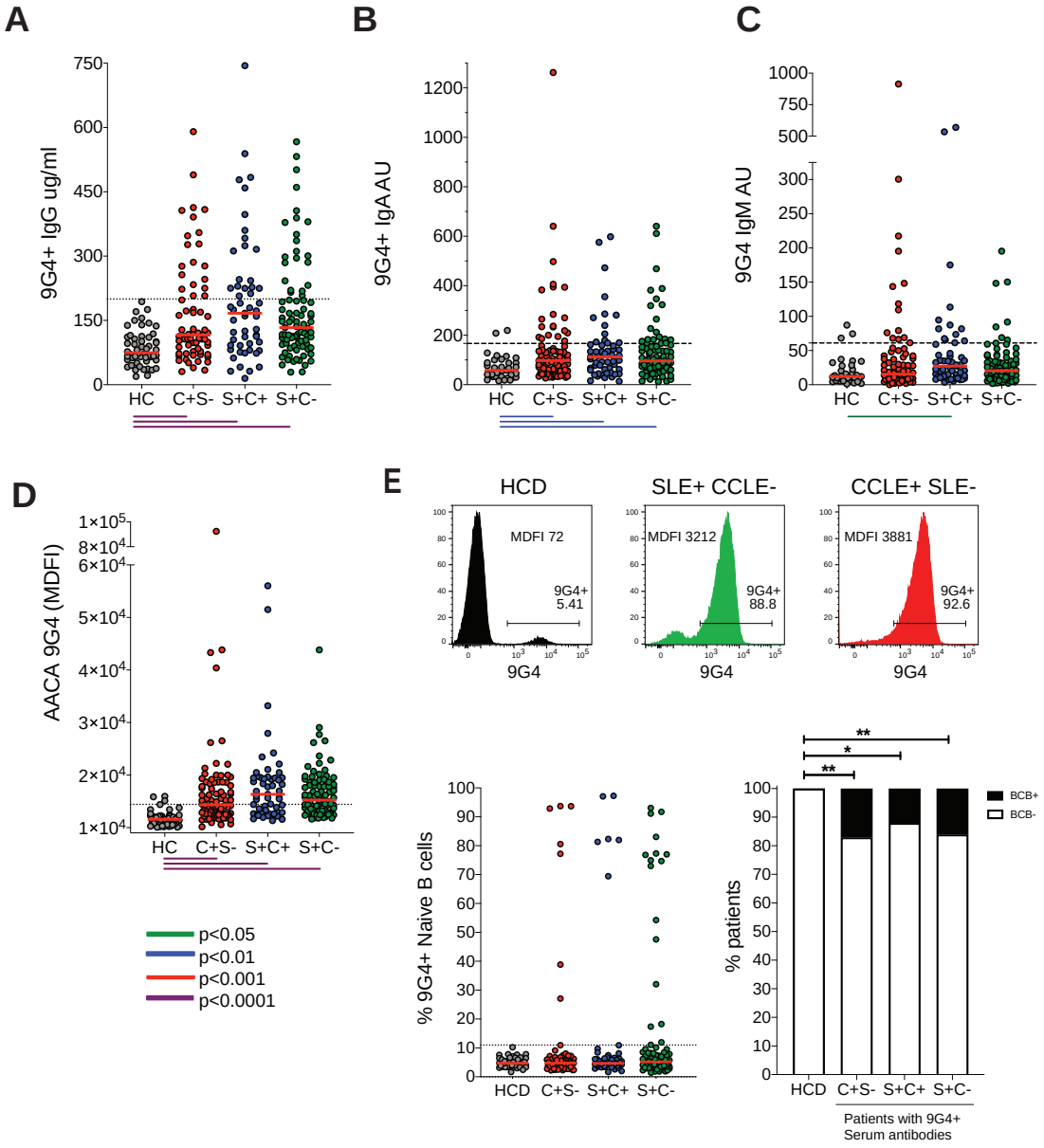

Figure 3 Like in patients with SLE, autoreactive 9G4+ autoantibodies are increased in the serum of patients with CCLE+/SLE−. (A) 9G4+ IgG is increased in in all three groups relative to HCD. (B) 9G4+ IgA is also increased in patients with lupus. (C) 9G4+ IgM was only significantly elevated in the SLE+/CCLE−. (D) 9G4+ AACA are found in the serum of both patients with SLE and CCLE. 9G4 Median fluorescence intensity (MDFI) of apoptotic Jurkat cells are shown after incubation with patient serum. (E) 9G4+ autoreactive antibodies that bind B cells are present in both patients with SLE and CCLE. On top representative 9G4 staining for naive B cells is shown. In HCD only the minor population of Vh4.34 expressing cells are 9G4+, in some patients with SLE+/CCLE− and CCLE+/SLE− almost all naive B cells are 9G4+ due to surface bound anti-B cell antibodies. Below left the frequency of naive B cells that are 9G4+ is shown. Below right the proportion of patients with elevated 9G4+ IgG, IgA or IgM that have greater than 11% naive 9G4+ B cells is shown. Kruskal-Wallis test followed by Dunn's multiple comparisons test: p<0.05 (green), p<0.01 (blue), p<0.001 (red), p<0.0001 (dark purple); Fischer's exact test: *p<0.05, **p<0.01. AACA, anti-apoptotic cell antibodies; CCLE, chronic cutaneous lupus erythematosus; HCD, healthy controls; SLE, systemic lupus erythematosus.

Hierarchical clustering demonstrated a strong association between anti-Ro52 and anti-Ro60 reactivity and between anti-Sm and anti-RNP autoantibodies (figure 5C). The vast majority (95%) of HCD did not have reactivity to any of the tested antigens. In contrast, only 33% of patients with CCLE+/SLE− lacked autoreactivity but only 16% had reactivity against multiple autoantigens compared with 44% and 24% of patients with SLE+/CCLE− and SLE+/CCLE+, respectively (figure 5D). Anti-Sm and anti-RNP were highly correlated in patients with SLE but dissociated in patients with CCLE+/SLE− (figure 5E).

Clinical and immunological associations of B cell heterogeneity in CCLE

As previously indicated (figure 2), patients with CCLE+/SLE− displayed a high degree of B cell heterogeneity which can be more precisely evaluated by restricted analysis of this clinical group. Hence, we determined potential clinical and immunological associations of the two major clusters of CCLE through a comparison of patients with SLE-like and HCD-like B cell phenotypes. Patients with CCLE with a SLE-like B cell phenotype were more likely to have generalised lesions (58%) and less

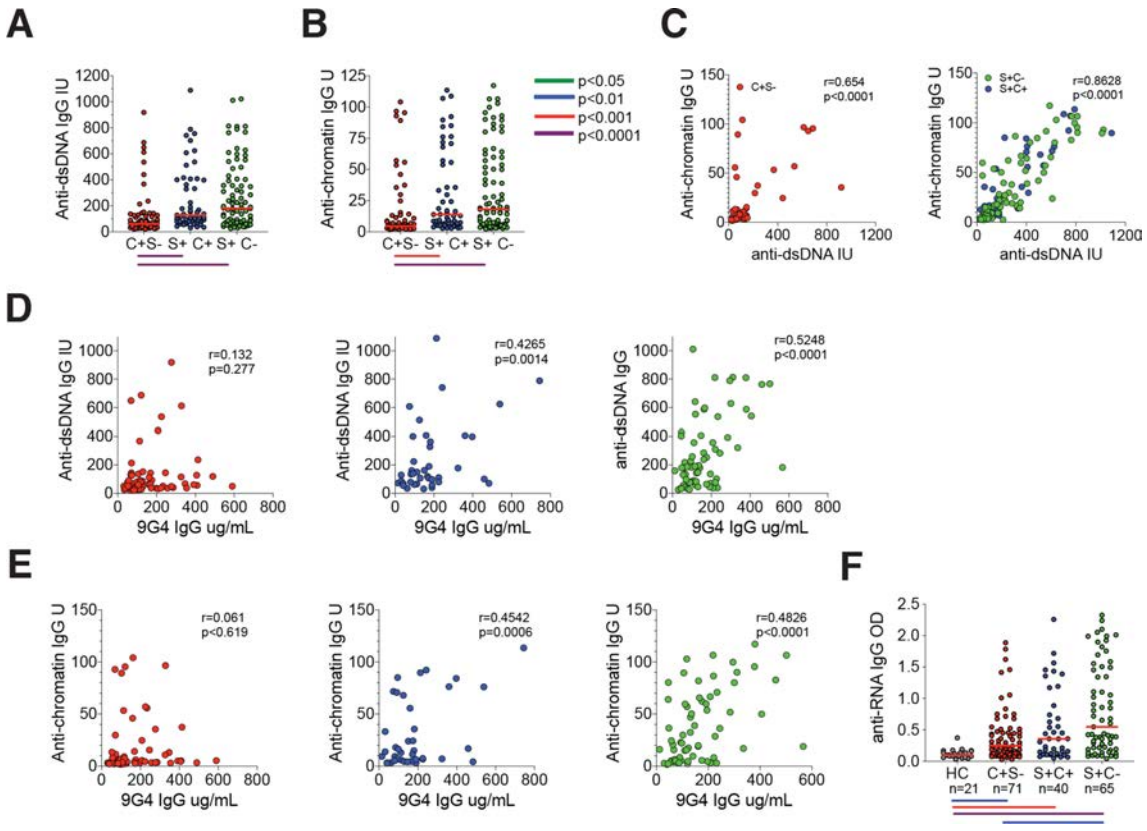

Figure 4 Antinucleic acid antibodies are decreased in patients with CCLE+SLE− relative to patients with SLE and are not correlated with 9G4+ IgG. (A) Anti-dsDNA IgG is decreased in CCLE+/SLE− relative to SLE+/CCLE− and patients with SLE+/CCLE+. (B) Antichromatin IgG is also decreased in CCLE+/SLE− relative to SLE. (C) Anti-dsDNA IgG and antichromatin IgG are correlated in both patients with CCLE+/SLE− (left) and SLE (right). (D) 9G4+ IgG is highly correlated with anti-dsDNA IgG in patients with SLE+CCLE and SLE+CCLE+ but not patients with CCLE+/SLE−. (E) Similarly, antichromatin IgG is correlated with 9G4+ IgG only in patients with SLE. (F) Anti-RNA IgG is increased in patients with CCLE+/SLE−, SLE+/CCLE− and SLE+/CCLE+ but is significantly higher in SLE+/CCLE− and SLE+/CCLE+ than CCLE+/SLE−. Kruskal-Wallis test followed by Dunn's multiple comparisons test: p<0.05 (green), p<0.01 (blue), p<0.001 (red), p<0.0001 (dark purple); Pearson correlation coefficient r and p are shown. CCLE, chronic cutaneous lupus erythematosus; dsDNA, double-stranded DNA; SLE, systemic lupus erythematosus.

likely to be males (8%) than those with a HCD-like B cell phenotype (15% generalised lesions; 31% males) (figure 6A). Disease duration did not differ between the two groups (online supplemental figure 1). CLASI scores were measured in 32 patients with CCLE+/SLE− and skin activity did not differ between the groups. However, there was a trend towards greater skin damage in patients with a SLE-like phenotype (online supplemental figure 2). The group with SLE-like B cells also had more historical serological autoreactivity including ANA, anti-dsDNA, anti-Ro and anti-La (figure 6B,C), as well as more contemporaneous anti-dsDNA and antichromatin reactivity (figure 6D) and higher titers of anti-RNP and anti-Ro52 (figure 6E). Interestingly, patients with CCLE with expanded transitional cells also had more anti-RNP antibodies, particularly anti-Ro60 (data not shown).

DISCUSSION

Our study represents the first systematic B cells description across the spectrum of clinical CCLE phenotypes, spanning from primary CCLE without systemic disease to SLE with or without a CCLE component. Our data establish that a subset of patients with CCLE share B cell abnormalities characteristic of SLE, including contraction of USM B cells and expansion of effector B cell subsets. Why the former population is depleted remains unclear. A similar decrease is observed in Sjögren's syndrome,[39] rheumatoid arthritis,[40] vasculitis[41] and other autoinflammatory diseases including inflammatory bowel disease, in which it may be restored by tumour necrosis factor inhibition.[42] Of note, USM loss is an early feature of Sjögren's syndrome correlated with serological autoimmunity and disease progression.[39] This change may represent the loss of a MZ-equivalent endowed with protective functions such as apoptotic clearance,[43] Interleukin-10-mediated B regulatory activity,[44] and dilution of autoreactivity.[45] Moreover, as in Sjogren's Syndrome,[39] this B cell feature might identify patients with primary CCLE who might be at risk for progression to SLE. In addition to the loss of putatively protective B cells, primary CCLE also shared with other lupus groups an enhancement of effector aN and DN2 B cells and PB, although of lower magnitude. In SLE, effector DN2 and plasma cells localise to the kidney and likely directly contribute to pathology.[18] Similarly, B cells and plasma cells infiltrate the skin in CCLE, particularly in established scarred lesions.[46] Whether the phenotype of skin-infiltrating B cells in CCLE corresponds to that of circulating and kidney-infiltrating B cells remain to be elucidated.

Our findings demonstrate high B cell heterogeneity in lupus, with normal B cell signature in 48% of primary CCLE and 15% of SLE. This data suggest that patients with primary CCLE are more likely to have a B-cell-independent disease, which in turn might identify those with lower risk of

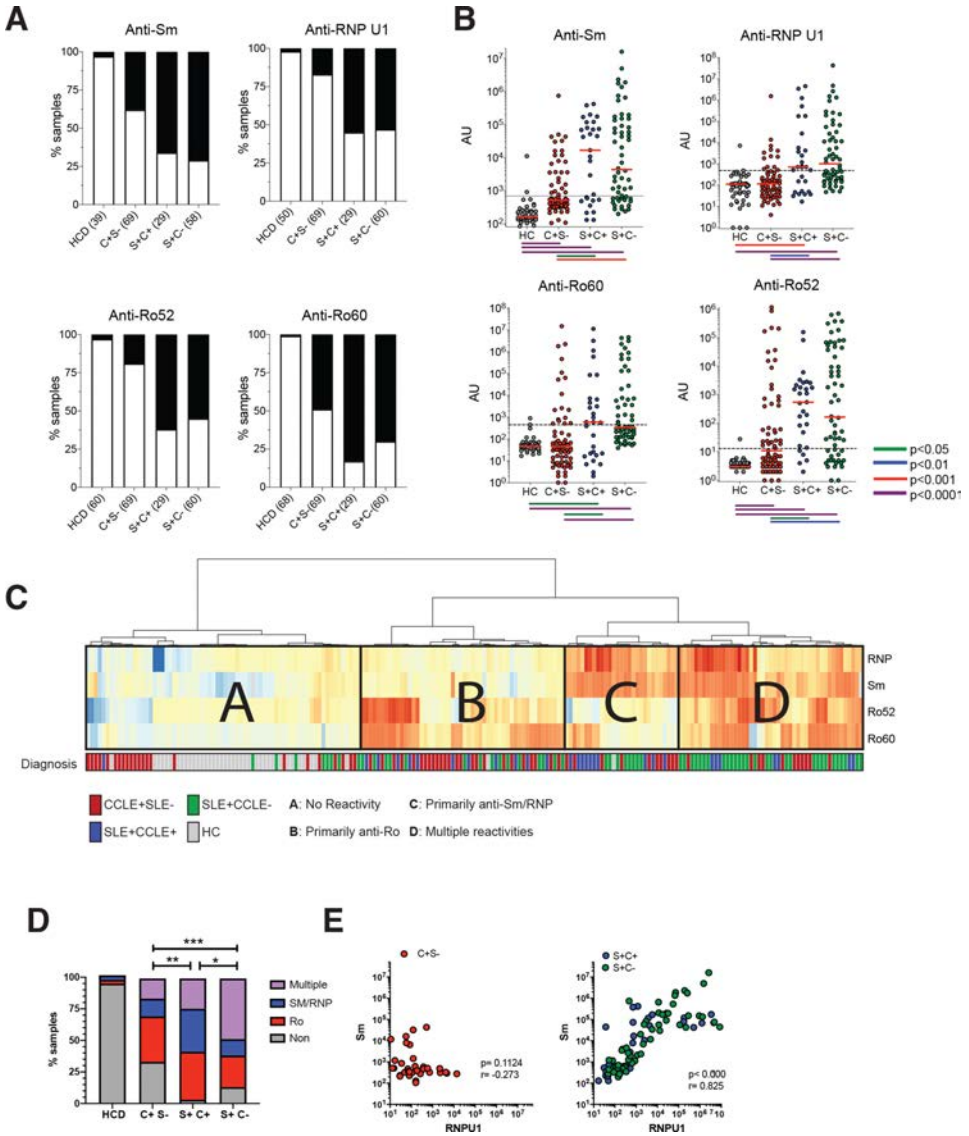

Figure 5 Anti-RNA-binding protein antibodies are elevated in patients with SLE relative to CCLE+/SLE−. (A) The frequency of positive samples for each of the indicated antigen specificities as determined by LIPS for HCD and each patient group. Samples were considered positive if they were higher than the mean value of HCD+3SD. (B) Anti-Sm, anti-RNP, anti-Ro52 and anti-Ro60 levels as assayed by LIPS and expressed as arbitrary units, median values are indicated by the red line, the Kruskal-Wallis test was used to compare each group, p values>0.05 are indicated by colour lines underneath for each comparison. The dashed line indicates the threshold that was considered positive. (C) Hierarchical clustering of luciferase immunoprecipitation system (LIPS) assay values, samples are clustered by patient on top and antigen on the left, diagnosis is indicated by colour underneath. Samples can be grouped into four patterns of reactivity as indicated by letter. (D) Distribution of patients and HCD across the four groups from C above. Differences in distribution between the three patient groups were analysed using the χ^2 test. (E) Anti-Sm and anti-RNP plotted for CCLE+/SLE− (left) or SLE+/CCLE+ and SLE+/CCLE− (right). Kruskal-Wallis test followed by Dunn's multiple comparisons test: p<0.05 (green), p<0.01 (blue), p<0.001 (red), p<0.0001 (dark purple); Spearman correlation coefficient r and p is shown; X^2 test: *p<0.05, **p<0.001, ***p<0.0001. CCLE, chronic cutaneous lupus erythematosus; HCD, healthy controls; SLE, systemic lupus erythematosus.

SLE progression. Conversely, 38% of patients with primary CCLE exhibit a highly activated SLE-like B cell profile. This group of patients is clinically and serologically distinct with more generalised skin lesions and higher autoantibody loads; features that have been reported to increase the risk of SLE among those with primary DLE.[47–51] Our findings suggest that the expansion of effector B cells may identify a distinct CCLE group with higher risk of systemic progression. Long-term longitudinal analysis are warranted to demonstrate the predictive value of B cell phenotype in the development of SLE among patients with primary CCLE.

Also of note, patients with SLE without CCLE lesions had higher proportion of DN2 cells and multiple autoantibodies, features that we previously found to be associated with lupus nephritis.[19] Patients with SLE and DLE are less likely to develop renal disease,[6] and we have shown that SLE B cells are driven by disease-associated epigenetic programmes that promote effector DN2 and PB differentiation.[34] It is therefore possible that CCLE-associated B cells may be regulated by distinct differentiation and effector programmes, resulting in a less pathogenic phenotype, whether in primary CCLE or in the context of SLE. Finally, a subset of patients with CCLE+SLE− clustered together with a group of patients with SLE through their shared frequency of transitional B cells. Patients with SLE may display expanded transitional B cells with increased TLR7 and interferon-regulated gene

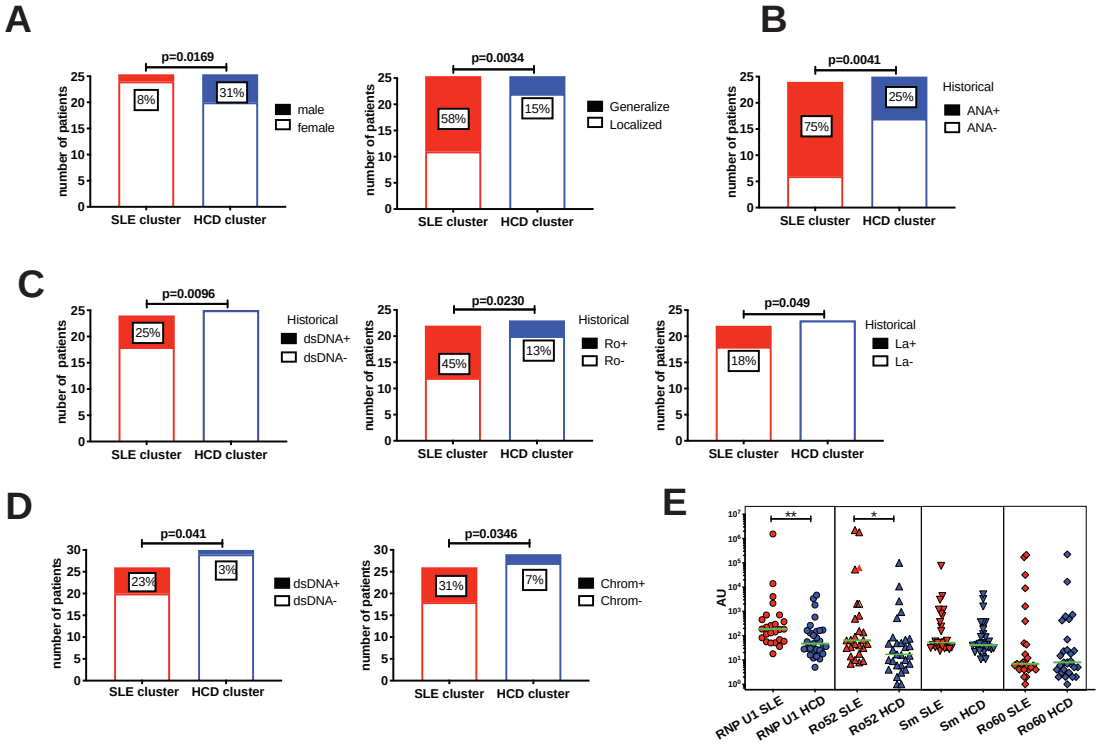

Figure 6 Patients with primary CCLE with a SLE-like B cell subset composition are serologically and clinically distinct from patients with CCLE that resemble HCD. (A) Primary CCLE from clusters III and IV (figure 2A) that resemble patients with SLE (red) are less likely than those from clusters I and II that resemble HCD (blue) to be male (left) and more likely to have generalised skin lesions above and below the neck (right). (B) Frequency of historical anti-nuclear antibody (ANA) reactivity in patients with primary CCLE with a SLE-like (red) or HCD-like B cell subset composition (blue). (C) Frequency of historical anti-dsDNA, anti-Ro and anti-La reactivity in patients with primary CCLE with a lupus-like (red) or HCD-like B cell subset composition (blue). (D) Frequency of anti-dsDNA and antichromatin reactivity at the time of flow analysis for patients with primary CCLE with a lupus-like (red) or HCD-like B cell subset composition (blue). (E) Anti-RNA protein-binding reactivity as assayed by LIPS in patients with primary CCLE with a lupus-like (red) or HCD-like B cell subset composition (blue) at the time of flow analysis. The Mann-Whitney test was used to compare distributions; (*p<0.05; **p<0.005). Fischer's exact test was used to analyse differences in frequencies as indicated by the p value. CCLE, chronic cutaneous lupus erythematosus; dsDNA, double-stranded DNA; HCD, healthy controls; SLE, systemic lupus erythematosus.

expression.[32] It remains to be determined if patients with primary CCLE and transitional B cell expansion represent a distinct disease group and what are the clinical implications of this phenotype. Similarly, an association between any of the B cell profiles demonstrated in our work in CCLE and the interferon signature reported in a fraction of patients with CCLE remains to be investigated.[52]

This study also provides original information regarding the breakdown of B cell tolerance in primary CCLE. Such a defect is demonstrated by the increase in a fraction of patients with one or more SLE-associated autoantibodies including dsDNA, chromatin, RNA and some anti-RNA-binding protein antibodies. Moreover, a significant fraction of primary CCLE also displayed defective tolerance in the autoreactive 9G4 B cell compartment, a feature characteristic of SLE.[23 53] However, meaningful differences were also observed between CCLE and SLE. Thus, unlike patients with SLE, increased class-switched 9G4+ antibodies were uncoupled in CCLE from anti-DNA/chromatin autoantibodies and the autoreactivity of these antibodies was much more pronounced against self-antigens expressed by apoptotic cells than B cells.

Combined, our observations suggest that primary CCLE is characterised by limited breakdown of tolerance that has not yet disseminated to other antigens. This mechanistic scenario is also supported by the dissociation between anti-Sm and U1RNP reactivity in CCLE but not in SLE. This observation has important implications for the development and progression of these diseases. Indeed, these findings are consistent with the notion that concurrent development of antibodies against multiple autoimmune targets is an important component of disease progression, as demonstrated by the progressive development of different autoantibodies during the preclinical phase of SLE prior to full-blown disease and diagnosis.[54 55] Longitudinal studies of epitope spreading in CCLE B cells could represent an informative approach to understand the nature of the triggering antigens responsible for the initial breakdown of tolerance, and of late-target antigens that might be responsible for disease dissemination and SLE development.

Our study has limitations. First, the cross-sectional design does not allow to infer cause–effect. Second, the wide range of disease duration of our sample might impact the results. However, we did not find significant differences between patients with a HCD-like and SLE-like B cell phenotype by disease duration. Third, skin activity and damage were examined in a subset of participants, and we cannot generalise those results to the full sample. Four, findings of this study are best generalised to individuals in the Southeastern USA, where a large majority of patients with lupus are black.

In summary, we demonstrate that CCLE is a heterogeneous condition clinically, serologically and immunologically. B

cell heterogeneity is indicated by both phenotypic and serological diversity with the latter suggesting an early and/or limited breakdown of tolerance. A deeper understanding of these findings should improve our understanding of disease pathogenesis, enhance prognostic power for SLE development, and lead to the development of more precise and effective therapies.

Acknowledgements We would like to express our gratitude to the patients, healthy donors and clinical coordinators and research managers (Charmayne Dunlop-Thomas, Letheshia Husbands, Kelly Williams, Tejal Vashi, Aita Akharume, and LaShawn Baker) who made this work possible. We would also like to thank the CHOA and Emory University Paediatrics and Winship Cancer Centre Flow Core for flow cytometry technical support.

Contributors All authors contributed to this work and approved of the final manuscript. Specific areas of contribution are listed below. Study conceptualisation was done by IS, CD, SAJ, CW and SL. IS, CD, SAJ, CW, SL and GB helped in study design. Data analysis was done by SAJ, CW, MCW, GB, RB and KC. RB, AH, KC, XW and FMR performed experiments. Clinical evaluation and patient recruitment were done by CD, LDA and SL. Drafting and revisions were performed by IS, CD, SAJ, CW, MCW and KC.

Funding This work was supported by the Centers for Disease Control and Prevention, (CDC) grant U01DP005119, National Institute of Health grants R37-AI049660 and Autoimmune Centre for Excellence U19AI110483-06 and the Georgia Research Alliance.

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval Emory Institutional Review Board (3656, 58515, and 58507) approved the study and all participants signed informed consent.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. All data including deidentified patient data,flow cytometry files and R code are available upon reasonable request from Dr Ignacio Sanz, ignacio.sanz@emory.edu.

ORCID iD

Scott A Jenks http://orcid.org/0000-0001-7485-8331

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TRANSLATIONAL SCIENCE

Immune response to dermatomyositis-specific autoantigen, transcriptional intermediary factor 1γ can result in experimental myositis

Naoko Okiyama ,[1] Yuki Ichimura,[1] Miwako Shobo,[1] Ryota Tanaka,[1] Noriko Kubota,[1] Akimasa Saito,[1] Yosuke Ishitsuka,[1,2] Rei Watanabe,[1,2] Yasuhiro Fujisawa,[1] Yoshiyuki Nakamura,[1] Akihiro Murakami,[3] Hisako Kayama,[4] Kiyoshi Takeda,[5] Manabu Fujimoto[1,6]

Handling editor Josef S Smolen

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2020-218661).

For numbered affiliations see end of article.

Correspondence to
Dr Naoko Okiyama, Department of Dermatology, Faculty of Medicine, University of Tsukuba, Tsukuba 305-8575, Japan; naoko.okiyama@md.tsukuba.ac.jp

Received 21 July 2020
Revised 9 March 2021
Accepted 21 March 2021
Published Online First 2 April 2021

ABSTRACT

Objectives To investigate whether autoimmunity to transcriptional intermediary factor 1 (TIF1)γ, a ubiquitous nuclear autoantigen for myositis-specific autoantibodies detected in patients with dermatomyositis (DM) is pathogenetic for inflammatory myopathy.

Methods Wild-type, β_2-microglobulin-null, perforin-null, Igμ-null and interferon α/β receptor (IFNAR)-null mice were immunised with recombinant human TIF1γ whole protein. A thymidine incorporation assay was performed using lymph node T cells from TIF1γ-immunised mice. Plasma was analysed using immunoprecipitation followed by western blot analysis and enzyme-linked immunosorbent assays. Femoral muscles were histologically and immunohistochemically evaluated. $CD8^+$ or $CD4^+$ T cells isolated from lymph node T cells or IgG purified from plasma were adoptively transferred to naïve mice. TIF1γ-immunised mice were treated with anti-CD8 depleting antibody and a Janus kinase inhibitor, tofacitinib.

Results Immunisation with TIF1γ-induced experimental myositis presenting with necrosis/atrophy of muscle fibres accompanied by $CD8^+$ T cell infiltration successfully in wild-type mice, in which TIF1γ-specific T cells and antihuman and murine TIF1γ IgG antibodies were detected. The incidence and severity of myositis were significantly lower in β_2-microglobulin-null, perforin-null, CD8-depleted or IFNAR-null mice, while Igμ-null mice developed myositis normally. Adoptive transfer of $CD8^+$ T cells induced myositis in recipients, while transfer of $CD4^+$ T cells or IgG did not. Treatment with tofacitinib inhibited TIF1γ-induced myositis.

Conclusions Here we show that TIF1γ is immunogenic enough to cause experimental myositis, in which $CD8^+$ T cells and type I interferons, but not $CD4^+$ T cells, B cells or antibodies, are required. This murine model would be a tool for understanding the pathologies of DM.

Key messages

What is already known about this subject?

- A number of autoantibodies have been identified in sera of patients with dermatomyositis (DM), which are not only highly disease specific but are associated with distinct clinical features.
- One of the autoantigens for the myositis-specific autoantibodies, transcriptional intermediary factor 1 (TIF1) γ, is a ubiquitous intracellular molecule that is often mutated or overexpressed in tumours and triggers the development of anti-TIF1γ antibody-positive DM.
- Previously established murine models of experimental autoimmune myositis are dependent on immune responses against muscle tissue-specific antigens.

What does this study add?

- Autoimmunity against TIF1γ results in experimental myositis.
- The initiation of the experimental myositis is completely dependent on autoreactive TIF1γ-specific $CD8^+$ T cells, but not on $CD4^+$ T cells or IgG.
- The type I interferon pathway is partially involved in the pathogenesis of myositis caused by autoimmunity against TIF1γ.

How might this impact on clinical practice or future developments?

- Autoimmunity to TIF1γ is not only a diagnostic marker for a subset of human DM and may play a role in the pathogenesis of the DM seen in patients with these autoantibodies.
- This new murine model of experimental myositis might be a useful tool to investigate pathologic mechanisms of, and to develop specific treatments for, human anti-TIF1γ antibody-positive DM.

To cite: Okiyama N, Ichimura Y, Shobo M, *et al*. *Ann Rheum Dis* 2021;**80**:1201–1208.

INTRODUCTION

Idiopathic inflammatory myopathies (IIMs) include dermatomyositis (DM), polymyositis, immune-mediated necrotising myopathy (IMNM), inclusion body myositis and antisynthetase syndrome (ASS), characterised by inflammation of muscles and other organs.[1 2] Autoimmunity mediates these diseases, as a number of myositis-specific autoantibodies have been identified[3–6] and are associated with distinct clinical features.[7] In DM, five autoantibodies have been identified: anti-Mi-2, antimelanoma differentiation-associated gene 5, antitranscriptional intermediary factor 1 (TIF1),[8 9] antinuclear

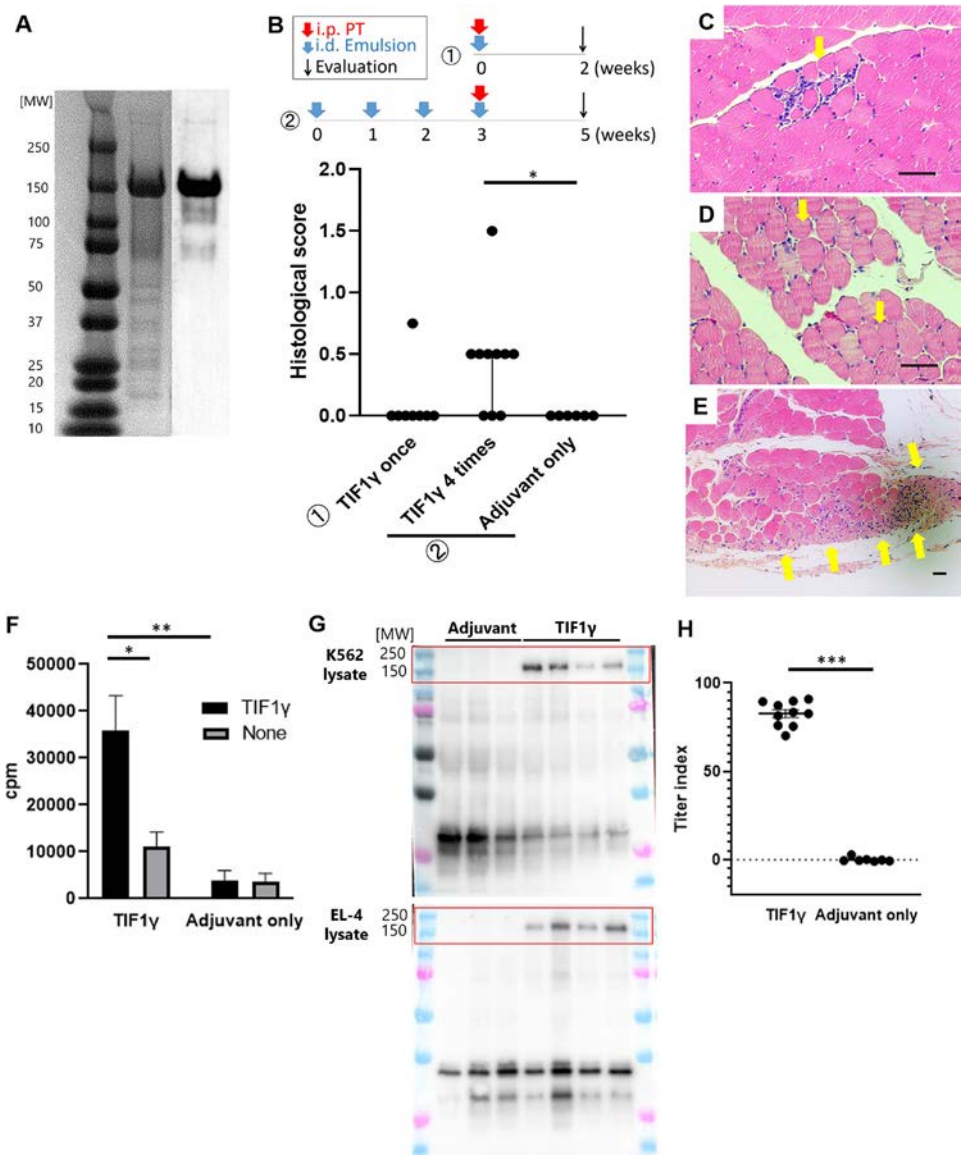

Figure 1 Development of experimental myositis dependent on the immune response to transcriptional intermediary factor 1 (TIF1)γ. (A) Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (second lane) followed by a western blotting assay using anti-TIF1γ antibodies (third lane) revealed that the purified protein has a molecular weight (MW) of about 150 kDa, which is consistent with TIF1γ whole protein. MW markers are shown in first lane. (B) The upper panel shows our immunisation protocols. Mice received intraperitoneal injection (i.p.) of pertussis toxin (PT), and intradermal injection of an emulsion of complete Freund's adjuvant with/without TIF1γ protein at the back and foot pads. The graph shows histological scores for experimental myositis in the hamstrings and quadriceps of mice immunised once (n=8) and four times (n=10) with TIF1γ whole protein, and mice immunised with adjuvant only four times (n=6). Dots and bars represent individuals and medians with interquartile ranges (IRs), respectively. *p<0.05 by Kruskal-Wallis test with Dunn's multiple comparisons test. (C–E) Representative myositis (yellow arrows) with muscle fibre atrophy (C) or necrosis (D), and perifascicular atrophy (E) observed in H&E-stained sections of muscle tissues from TIF1γ-immunised mice. Bars represent 50 µm. (F) ^{3}H thymidine incorporation (cpm) in T cells from regional lymph nodes of TIF1γ-immunised mice (n=3) co-cultured with bone marrow-derived dendritic cells (BMDCs) presenting TIF1γ. T cells from mice immunised BMDCs pulsed with adjuvant only (n=3) or without antigen were used as control T cells and control BMDCs, respectively. Bars represent means with SE of the mean (SEM). *p<0.05, and **p<0.01 by two-way ANOVA with Tukey's multiple comparisons test. (G) IgGs binding TIF1γ antigens from K562 and EL-4 cell lysates, the MWs of which were between 150 and 250 kDa, were detected in the plasma of TIF1γ-immunised mice (n=4), but not in plasma from mice immunised with adjuvant only (n=3). (H) Enzyme-linked immunosorbent assay conducted with plasma from TIF1γ-immunised mice (n=10) and mice immunised with adjuvant only (n=7). The titre index calculation method is detailed in online supplemental materials and methods. Dots and bars represent individuals and means with SEM, respectively. ***p<0.001 by Student's t-test. Data are representative of two independent experiments.

matrix protein 2 and antismall ubiquitin-like modifier activating enzyme.

While autoantibodies against various nuclear/cytoplasmic components serve as diagnostic tools for systemic autoimmune diseases, a direct causative role for most of them has been questioned. TIF1γ, a major antigen of anti-TIF1 antibodies, is a 155 kDa nuclear protein belonging to the tripartite motif superfamily.[8 9] Anti-TIF1γ antibody is present in a quarter of adult/juvenile patients with DM[10 11] and is associated with an increased risk of malignancies in elderly patients.[12–14] TIF1γ was found to be overexpressed not only in tumours[15] but also in muscle tissues, especially in regenerating atrophic perifascicular

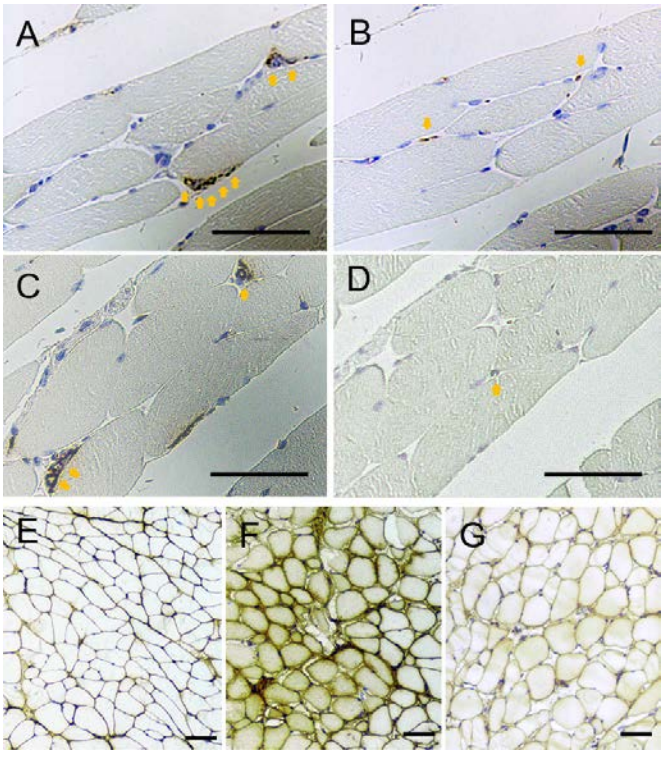

Figure 2 Infiltration of inflammatory cells and upregulation of major histocompatibility complex (MHC) class I molecules in muscle tissues. (A–D) Immunohistochemical (IHC) analyses of CD8 (A), CD4 (B), CD11b (C) and B220 (D) on the mononuclear cells infiltrating into the endomysium areas of the muscle tissues of TIF1γ-immunised mice. (E–G) IHC analyses of MHC class I molecules on the cell membranes of the muscle fibres in control adjuvant-treated mice (E) and in TIF1γ-immunised mice (F), compared with the isotype-control antibody-stained samples from TIF1γ-immunised mice (G) Data are representative of three independent experiments.

myofibers and in skin.[16 17] Our observations revealed that pregnancy might trigger the development of anti-TIF1γ antibody-positive DM,[18] which would be related to overexpression of TIF1γ antigen in the embryo and mammary epithelial cells during pregnancy.[19 20] While this evidence suggests the aetiologic roles of TIF1γ, it remains unknown whether autoimmunity to TIF1γ is directly involved in disease pathogenesis. Here we show that experimental myositis can develop following immunisation with recombinant TIF1γ protein.

METHODS

Mice

Female C57BL/6 (B6) mice 8–10 weeks of age were purchased from Charles River. Beta$_2$-microglobulin (β_2MG)-null, perforin-null and Igμ-null (μMT) B6 mice and interferon α/β receptor (IFNAR)-null B6 mice[21] were purchased from The Jackson Laboratory and B&K Universal. All experiments were carried out under specific pathogen-free conditions in accordance with the University of Tsukuba's ethics and safety guidelines for animal experiments.

Recombinant human TIF1γ protein

A full-length human TIF1γ gene (GenBank accession number: AF119043) was His-tagged at its 3' end and inserted into pFastBac1 vector for baculovirus expression (invitrogen). The detailed protocol for the expression and purification of recombinant TIF1γ protein is described in online supplemental materials and methods. Human TIF1γ whole protein is 93.3% homologous with the murine protein.

Induction of experimental myositis

Mice were immunised intradermally with 200 µg of TIF1γ protein emulsified in complete Freund's adjuvant (CFA) containing 100 µg of heat-killed *Mycobacterium butyricum* (Difco) once in the back and in foot pads along with an intraperitoneal injection of 250 ng of pertussis toxin (PT, Wako Junyaku). Other mice were immunised intradermally with the CFA emulsion containing TIF1γ protein four times weekly at multiple sites of the back and foot pads. The same time as the last (fourth) intradermal injection of the emulsion, 250 ng of PT was once injected intraperitoneally. Mice treated with CFA (weekly, 4 times) and PT were used as controls. These immunisation protocols are presented in figure 1B. Following the evaluation method for C protein-induced myositis (CIM),[22] three H&E-stained sections from the hamstring and quadricep each were blinded to the intervention and examined histologically for necrosis/atrophy of muscle fibres accompanied by mononuclear cell infiltration. The scoring system is detailed in online supplemental materials and methods.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis and western blotting assay and immunoprecipitation followed by western blot assay

Fifty microliters of mouse plasma were combined with 50 µL of Protein G Sepharose 4 Fast Flow (GE Healthcare) for 2 hours at room temperature. Antibody-bound sepharose beads were washed with immunoprecipitation (IPP) buffer (10 mM tris-HCl, pH 8.0; 50 mM NaCl and 0.1% 4-nonylphenyl-polyethylene glycol (BioVision)) and incubated with extracts from 1×10^7 K562 human cells and EL-4 murine cells (American Type Culture Collection (ATCC)), respectively, for 2 hours at 4°C. Purified recombinant TIF1γ protein, K562-precipitated protein and EL-4-precipitated protein were fractionated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) using

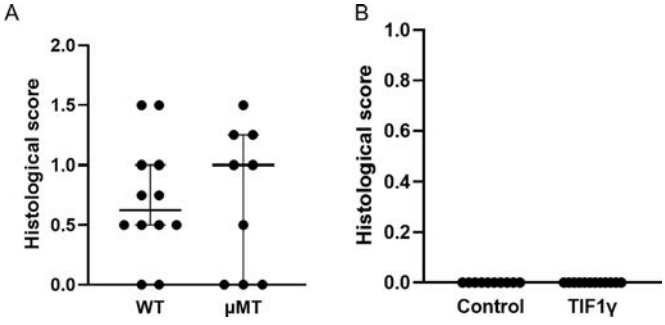

Figure 3 TIF1γ-specific B cell linages and antibodies are not required for the development of TIF1γ-induced myositis. (A) Histologic scores for experimental myositis in hamstrings and quadriceps of µMT mice immunised with TIF1γ whole protein (n=9) were equal to TIF1γ-immunised wild-type (WT) mice (n=12). Dots and bars represent individuals and medians with IRs, respectively. (B) Histologic scores for experimental myositis in the hamstrings and quadriceps of recipient mice (n=12) with adoptive intravenous transfer of IgGs purified from pooled plasma of TIF1γ-immunised mice or control recipients (n=10) of IgGs from pooled plasma of mice immunised with adjuvant only. Dots and bars represent individuals and medians with IRs, respectively. Data are representative of two independent experiments. IRs, interquartile ranges.

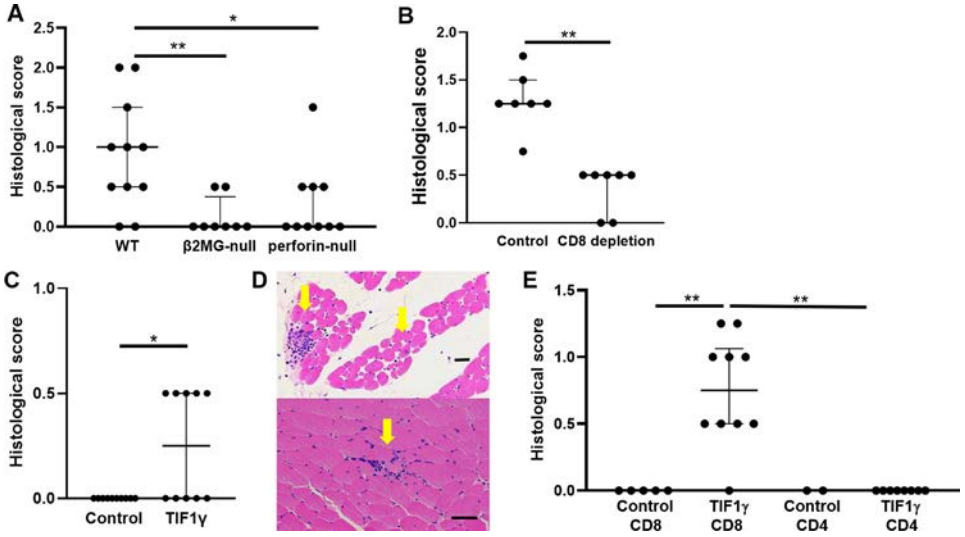

Figure 4 TIF1γ-specific CD8$^+$ T cells are critical for the development of TIF1γ-induced myositis. (A) Histological scores for experimental myositis in the hamstrings and quadriceps of WT mice (n=11), β_2-microgloblin (β_2MG)-null mice (n=8), and perforin-null mice (n=10) 2 weeks after fourth immunisation with TIF1γ whole protein. Dots and bars represent individuals and medians with IRs, respectively. *p<0.05, and **p<0.01 by Kruskal-Wallis test with Dunn's multiple comparisons test. (B) Histological scores for experimental myositis of TIF1γ-immunised mice treated with anti-CD8 depleting antibody (n=7) compared with those treated with control antibody (n=7). Dots and bars represent individuals and medians with IRs, respectively. **p<0.01 by Mann-Whitney *U* test. (C) Histological scores for experimental myositis in the hamstrings and quadriceps of recipient mice (n=10) following adoptive intravenous transfer of TIF1γ-activated T cells originally purified from pooled lymph node cells of TIF1γ-immunised mice compared with TIF1γ-activated T cells originally from pooled lymph node cells of mice immunised with adjuvant only (n=10). Dots and bars represent individuals and medians with IRs, respectively. *p<0.05 by Mann-Whitney *U* test. (D) Representative myositis (yellow arrows) in HE-stained sections of muscle tissues from TIF1γ-specific T cell-recipients. Bars represent 50 µm. (E) Histological scores for experimental myositis in hamstrings and quadriceps of TIF1γ-CD8 recipient mice (n=10) following adoptive intravenous transfer of CD8$^+$ T cells purified from TIF1γ-specific T cells compared with transfer of TIF1γ-CD4 recipient mice (n=8) with adoptive intravenous transfer of CD4$^+$ T cells purified from TIF1γ-specific T cells and control CD8 recipients (n=5) following adoptive intravenous transfer of CD8$^+$ T cells purified from T cells of mice immunised with adjuvant only. Lack of myositis in control CD4 recipients (n=2) following adoptive intravenous transfer of CD4$^+$ T cells purified from T cells of mice immunised with adjuvant only. Dots and bars represent individuals and medians with IRs, respectively. **p<0.01 by Kruskal-Wallis test with Dunn's multiple comparisons test. Data are representative of two independent experiments. IRs, interquartile ranges.

10% polyacrylamide gels, applied to Mini-PROTEANTGX precast gels (4%–15%, Bio-Rad Laboratories). The gel on which recombinant TIF1γ protein was applied was stained with Bio-Safe Coomassie Stain (Bio-Rad Laboratories). For western blotting (WB) assay, proteins were transferred onto nitrocellulose membranes using a wet transfer apparatus (Mini Trans-Blot Cell, Bio-Rad Laboratories) from the gels. The membranes were blocked with 5% skim milk and incubated with rabbit antihuman/murine TIF1γ polyclonal antibody (NB100-57498, Novus Biologicals), antihuman TIF1γ polyclonal antibodies (LS-C408048, LifeSpan Biosciences) and antimurine TIF1γ polyclonal antibodies (ab47062; Abcam), respectively, overnight at 4°C. They were washed with tris-buffered saline with Tween 20, incubated with peroxidase-labelled goat antirabbit IgG polyclonal antibodies (sc-2004, Santa Cruz Biotechnology) and then visualised using SuperSignal West Pico (Thermo Fisher Scientific).

Enzyme-linked immunosorbent assay

The plasma samples collected from immunised mice 2 weeks after the last immunisation were evaluated in our established enzyme-linked immunosorbent assay (ELISA) system (online supplemental materials and methods).

Immunohistochemical analyses

CD8, CD4, CD11b and B220-positive cells and upregulation of H-2Kb molecules were detected on sections from muscle tissue samples (online supplemental materials and methods).

In vivo depletion of CD8$^+$ T cells

To deplete CD8+T cells, mice were intraperitoneally injected with purified rat anti-CD8α depleting monoclonal antibody (53.67.2) in the protocol as shown previously[22] and as described in online supplemental materials and methods.

Adoptive transfer of T cells or IgG

T cells were purified from the inguinal and popliteal lymph node (LN) cells of immunised mice 2 weeks after the fourth immunisation using CD3 T cell enrichment columns (R&D systems). Three million T cells were cultured with 1.5×10^5 TIF1γ-pulsed mature bone marrow-derived dendritic cells (BMDCs, generated in the protocol shown in online supplemental materials) and 100 U/mL recombinant murine IL-2 (PeproTec) in 2 mL RPMI1640 with 10% fetal bovine serum (FBS) for 72 hours using 24-well culture plates. CD8-positive or CD4-positive T cells were sorted with MACS magnetic beads (Miltenyi Biotech). Flow cytometric analyses showed that the purities of the sorted CD8$^+$ and CD4$^+$ T cells were >95%, and that CD11c-positive DCs were absent. IgG was purified from the plasma of immunised mice 2 weeks after the fourth immunisation using protein G columns (Ab-Rapid SPiN Ex, ProteNova). One million whole T cells, 4×10^5 CD8-positive or CD4-positive T cells or 500 µg of IgG were intravenously injected into recipient mice that had been pretreated with CFA.[23] The muscles of the hind legs were evaluated histologically 2 weeks after transfer.

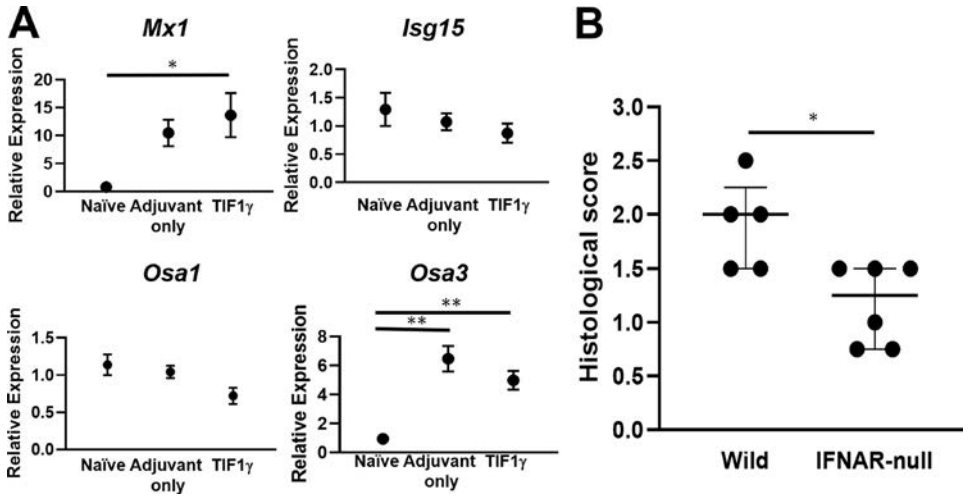

Figure 5 Type I interferon (IFN) signalling in the pathogenesis of TIF1γ-induced myositis. (A) Fold changes in mRNA levels of type I IFN-related genes, *Mx1*, *Isg15*, *Osa1* and *Osa3*, which were normalised against β-actin mRNA levels, in muscle tissues from naïve, adjuvant-treated, and TIF1γ-immunised mice 2 weeks after fourth immunisation. n=2–6 in each group. Bars represent the means with SEMs. *p<0.05, and **p<0.01 by ordinary one-way analysis of variance. (B) Histological scores for experimental myositis in the hamstrings and quadriceps of wild-type (n=5) and IFN α/β receptor-null mice (n=6) 2 weeks after the fourth TIF1γ immunisation. Dots and bars represent individuals and the medians with IRs, respectively. *p<0.05 by Mann-Whitney *U* test.

Real-time quantitative polymerase chain reaction analyses

As shown in online supplemental materials and methods and online supplemental table 1), real-time quantitative polymerase chain reaction (RT-qPCR) analyses were performed on total RNA extracted from the muscle tissue samples.

Treatment with the Janus kinase inhibitor tofacitinib

In accordance with a previous report,[24] tofacitinib (MedChemExpress) in 0.5% methylcellulose/0.025% Tween 20 was orally administrated to mice at 12.5 or 50 mg/kg Twice daily from the day of fourth immunisation of TIF1γ.

Statistical analysis

Data were analysed with Prism 8 (GraphPad Software). *P* values less than 0.05 were considered significant.

RESULTS

TIF1γ-immunised mice develop experimental myositis

SDS-PAGE and WB revealed that the purified recombinant human whole TIF1γ protein contained few contaminants (figure 1A). Wild-type (WT) B6 mice immunised with the TIF1γ protein four times weekly developed myositis 2 weeks after the fourth immunisations in their hamstrings and quadriceps as assessed histologically. The incidence rate and the median ((IQRs) of histologic scores were 70% and 0.5 (0–0.5), while none of the mice treated only with adjuvant was affected (p=0.0143; figure 1B). The mice immunised once with TIF1γ rarely did (12.5% and 0 [0–0]; figure 1B) as well as the mice treated only with adjuvant (p>0.9999). No mice exhibited significant weight loss during the observation period. Histologic analysis of muscles from the mice immunised with TIF1γ four times showed atrophy and necrosis of muscle fibres accompanied by infiltrating mononuclear cells in the perifascicular and endomysial sites of the muscle tissue (figure 1C,D), sometimes (in one per five samples) typical perifascicular atrophy (figure 1E). Myositis was still observed in 60% of the immunised mice 3 weeks after the fourth immunisation (incidence rate, 60%; median (IQRs) of histologic score, 0.500 (0–1.250); n=5), and some mice also presented myositis 1 week later (60%, 0.500 (0–0.625); n=5). No inflammation was observed in other organs, including skin, cardiac muscles and lungs.

Thymidine incorporation assay was performed as shown in online supplemental materials and methods. T cells from mice with TIF1γ-induced myositis (TIM) proliferated significantly more than T cells from control mice treated only with adjuvants when cocultured with TIF1γ-presenting BMDCs (the means±SEMs of ^{3}H thymidine incorporation were 35899±7411 and 3940±2086 (cpm), respectively; p=0.0022). T cells from TIM or control mice did not proliferate when cocultured with BMDCs presenting no specific antigen (11055±3156 and 3600±1782 (cpm), respectively, p=0.6218; figure 1F). IPP-WB analysis demonstrated the existence of not only antihuman TIF1γ antibodies reacting to the human cell (K562) lysate but also antimurine TIF1γ autoantibodies reacting to the murine cell (EL-4) lysate in the plasma from TIM mice, but not in that from CFA-treated control mice (figure 1G). Our ELISA system demonstrated higher titers of anti-TIF1γ antibodies in TIM mice (the mean±SEM of titre index was 82.8±2.2) compared with control mice (0.1±0.5, p=0.0005; figure 1H).

CD8^{+} T cells predominantly adhere to muscle fibres, which upregulate major histocompatibility complex class I molecules, in the muscle tissues of TIM mice

Immunohistochemical analyses of the muscle tissues of TIM mice revealed that CD8^{+} cells predominantly infiltrated into the endomysium areas and adhered to the muscle fibres (figure 2A). On the other hand, only a few CD4^{+} cells (figure 2B), CD11b^{+} macrophages (figure 2C), and B220^{+} B cells (figure 2D) infiltrated into the endomysium areas. Moreover, major histocompatibility complex (MHC) class I molecules were upregulated on the cell membranes of the muscle fibres in TIM mice (figure 2F) compared with those in control adjuvant-treated mice (figure 2E) and the isotype-control antibody-stained TIM samples (figure 2G).

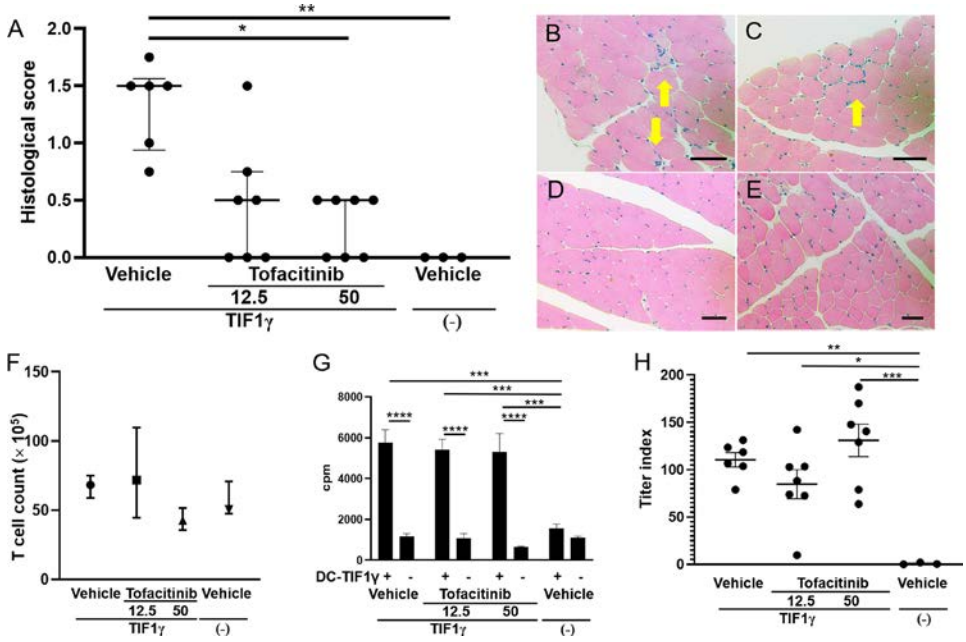

Figure 6 Inhibitory effect of tofacitinib on TIF1γ-induced myositis. (A) Histological scores for experimental myositis in the hamstrings and quadriceps of TIF1γ-immunised mice treated with low dose (12.5 mg/kg, two times per day; n=7) or high dose (50 mg/kg, two times per day; n=7) tofacitinib from the fourth day of TIF1γ immunisation compared with control vehicle-treated TIF1γ-immunised mice (n=6) and control vehicle-treated mice immunised with adjuvant alone. Dots and bars represent individuals and medians with IRs, respectively. *p<0.05, and **p<0.01 by Kruskal-Wallis test with Dunn's multiple comparisons test. (B–E) Representative H&E-stained sections of muscle tissues from TIF1γ-immunised mice treated with control vehicle (B) low-dose tofacitinib, (C) high-dose tofacitinib and (D) control vehicle-treated mice immunised without any antigens (E). Yellow arrows show myositis and bars represent 50 µm. (F) Total T cells in the regional lymph nodes per mouse. TIF1γ-immunised treated vehicle control (n=5), low-dose tofacitinib (n=5) and high-dose tofacitinib (n=5), and vehicle control-treated mice immunised without any antigens (n=3), were counted after T cell purification. Dots/squares/triangles and bars represent medians and interquartile ranges, respectively. (G) Proliferation of purified T cells from TIF1γ-immunised mice treated with vehicle, low-dose tofacitinib, and high-dose tofacitinib compared with those from control vehicle-treated mice immunised without any antigens when co-cultured with bone marrow-derived dendritic cells presenting TIF1γ (DC-TIF1γ) or with dendritic cells lacking antigen. Bars represent means with SEMs. ***p<0.001, and ****p<0.0001 by two-way analysis of variance (ANOVA) with Tukey's multiple comparisons test. (H) ELISA of plasma from TIF1γ-immunised mice treated with control vehicle, low-dose tofacitinib, or high-dose tofacitinib (n=6–7, each) compared with mice immunised with adjuvant alone (n=3). Dots and bars represent individuals and means with SEMs, respectively. **p<0.05, **p<0.01, and ***p<0.0001 by ordinary one-way ANOVA.

TIF1γ-specific B cell linages and antibodies are not required for the initiation of TIM

µMT mice, which completely lack B cell lineages, developed myositis (the incidence and the median±IQR of histologic scores were 67% and 1.000 (0–1.250)) at a similar incidence and severity as observed in WT mice (83% and 0.625 (0.500–1.000), p=0.9014) when immunised with TIF1γ emulsion (figure 3A). Moreover, intravenous adoptive transfer of the IgG purified from pooled plasma of TIM mice did not induce myositis in recipient mice (figure 3B).

TIF1γ-specific CD8$^+$ T cells are involved in the initiation of TIM

β_2MG-null mice lacking MHC class I expression and perforin-null mice rarely developed TIM (figure 4A). While the incidence rate and the median histologic score with IQRs for TIM were 82% and 1.000 (0.500–1.500) in WT mice, they were 29% and 0 (0–0.375) in β_2MG-null and 40% and 0 (0–0.500) in perforin-null mice (p=0.0054 and p=0.0123 vs WT mice, respectively). Mice treated with anti-CD8 depleting antibody rarely presented TIM (the incidence rate and the median histologic score with IQRs, 71.4% and 0.500 (0–0.500)) compared with control mice (100% and 1.250 (1.250–1.500), p=0.0006; figure 4B).

Moreover, adoptive transfer of enriched TIF1γ-specific T cells derived from TIM mice-induced TIM-like myositis with an incidence of 50% in naïve recipient mice (the median (IQRs) of histologic scores was 0.250 (0–0.500)), while transfer of CFA-treated mouse-derived T cells stimulated by TIF1γ-presenting BMDCs did not (p=0.0325, figure 4C,D). Adoptive transfer of CD8$^+$ T cells from TIM mice-induced myositis with a high incidence (90%) as well as muscle damage (the median (IRs) of histologic scores was 0.750 (0.500–1.063)); however, transfer of CD4$^+$ T cells from TIM mice did not (p=0.0010) nor did CD8$^+$ T cells from CFA-treated control mice (p=0.0067, figure 4E).

Type I interferon partially mediates the pathogenesis of TIM

Our RT-qPCR analyses revealed that the mRNA expression of type I interferon (IFN)-related genes, *Mx1* and *Osa3*, was significantly upregulated in the muscle tissues of the TIM mice (the mean±SEM, 11.3±4.49 and 3.66±0.11) compared with those of naïve mice (0.86±0.14 and 0.95±0.11; p=0.0205 and 0.0063, respectively); however, *Osa3* mRNA expression was also upregulated in adjuvant-treated control mice (5.96±1.00, p=0.0019 vs naïve mice, figure 5A). Upregulation of mRNA expression for other type I IFN-related genes, *Isg15* and *Oas1*, was not observed in the muscle tissues of TIM mice or control mice (figure 5A). IFNAR-null mice developed milder myositis (the medians (IQRs) of the histological scores, 1.250 (0.750–1.500)) than WT mice after TIF1γ immunisations (2.000 (2.250–1.500), p=0.0433, figure 5B).

Treatment with a JAK inhibitor, tofacitinib, inhibits the development of TIM

TIF1γ-immunised mice treated with high dose (50 mg/kg, two times per day) or low dose (12.5 mg/kg, two times per day) of tofacitinib starting on the fourth day of TIF1γ immunisation developed milder myositis (the medians (IQRs) of histologic scores were 0.500 (0–0.500) and 0.500 (0–0.7500), respectively) at a lower incidence rate (57% and 57%, respectively) than vehicle control-treated mice (1.500 (0.938–1.563); p=0.0075 and 0.0873, respectively; 100% incidence; figure). T cell counts in regional LNs of mice treated with low/high-dose tofacitinib did not differ from those of vehicle-treated mice (the medians (IRs) of T cell counts were 72 [45 – 110] and 43 [36 – 52] vs 68 [59 – 75] [$\times 10^5$], p>0.9999 and p=0.0909, figure 6F). Moreover, there was no inhibition of ex vivo proliferation of T cells purified from regional LNs of tofacitinib-treated TIF1γ-immunised mice when cocultured with TIF1γ-presenting BMDCs (figure 6G). This effect was significant when compared with that of mice immunised with adjuvant alone (p=0.0002, 0.0007 and 0.0009 for TIF1γ-immunised mice treated with vehicle, low-dose and high-dose tofacitinib, respectively). Index values of TIF1γ-specific antibodies in low-dose and high-dose tofacitinib-treated TIF1γ-immunised mice (the means±SEMs were 84.7±15.3 and 131.0±17.1, respectively) were also equal to those in vehicle-treated TIF1γ-immunised mice (110.5±7.6; p=0.3713 and 0.3713, respectively; figure 6H).

DISCUSSION

Our findings indicate that immunity to TIF1γ can contribute to the development of myositis in mice. This is the first study to demonstrate the immune response to a DM-specific autoantigen that can induce myositis. Therefore, this new experimental murine model, which we termed TIM, closely mimics human pathogenesis, especially in the initiation phase.

A number of animal models for IIMs have been established, including infectious, genetic and antigen-induced models.[25 26] While experimental autoimmune myositis[27 28] and CIM[22 29] completely depend on immune responses specific to muscular antigens, myosin and C protein, TIM is induced via autoimmunity generated in response to a ubiquitous intracellular molecule, which has been identified as an autoantigen in humans suffering DM. In addition to a previous report showing that muscle and lung inflammation could be induced by immunisation with purified epitopic peptides derived from conspecific histidyl-tRNA synthetase (Jo-1) as a murine model for ASS,[30] our results indicate that experimental myositis can be induced by immunisation with the DM-specific autoantigen. In our TIM model, mice immunised by xenogeneic (human) TIF1γ protein developed autoimmunity to conspecific TIF1γ resulting in experimental myositis, which might be due to epitope spreading to a counterpart conspecific molecule as shown in experimental autoimmune encephalomyelitis.[31]

TIM is initiated by cytotoxic $CD8^+$ T cells, which evokes infiltration of $CD8^+$ T cells and MHC class I upregulation in muscle fibres of patients with IIM.[32–36] While it has also been proven that genetically modified mice with overexpression of MHC class I in muscle tissues naturally develop myositis via endoplasmic reticulum (ER) stress,[34 37] our experiments showed that adoptive transfer of TIF1γ-specific $CD8^+$ T cells, but not of TIF1γ-specific $CD4^+$ T cells, caused myositis in recipient mice. This suggests that autoaggressive $CD8^+$ T cells are indicative of the development of myositis.

In contrast, B cells and autoantibodies themselves are not required for the development of TIM. Previous clinical reports indicated that the titres of anti-TIF1γ antibody were related to the conditions of DM[38] and/or the presence of internal malignancies.[39 40] Our findings indicate that while the immune response against TIF1γ is likely to mediate the induction of myositis, the development of anti-TIF1γ autoantibodies may be an epiphenomenon lacking direct pathogenic roles. In contrast, transfer of human IgGs from patients with IMNM, which contained antisignal recognition particle or anti-3-hydroxy-3-methylglutaryl-CoA reductase antibodies, corroborated the idea that complement can provoke muscle deficiency in recipient mice.[41] The difference between our experiments and this study may clarify the differences in pathogenesis of DM and IMNM.

Immunohistochemistry and gene-expression analyses of muscle and skin biopsy samples revealed that type I IFN expression correlates with DM pathogenesis.[42 43] Janus kinase (JAK)1 mediates downstream effects of type I IFN, and it has been reported that ruxolitinib, a JAK1/2 inhibitor, is effective for the treatment of DM case, some of which were positive for anti-TIF1γ antibody.[44–46] In addition, a report presented that treatment with tofacitinib, a JAK1/3 inhibitor, also improved myositis in a case of anti-TIF1γ antibody-positive DM.[47] Our experiments revealed that deficiency of IFNAR partially inhibits the development of TIM. Treatment of myositis with tofacitinib after the initiation of immunity to TIF1γ was effective; however, it did not result in significant inhibitory effects on the TIF1γ-specific T cells and antibodies. The mechanism underlying these results could be that the activation of type I IFN pathway induces myotube atrophy and impairs endothelial cells angiogenesis.[45]

Collectively, the limitations of this murine model include the lack of several DM-like phenomenons (specific rash, define muscle weakness with persistent myositis and upregulation of some type I INF-related genes in the muscle) and predominant infiltration of CD8 T cells to muscle fibres, which is not usually observed in patients with DM. Nevertheless, our new model based on autoimmunity against the ubiqutous interacellular antigen, TIF1γ, provides a useful tool to investigate the pathologic mechanisms of anti-TIF1γ antibody-positive DM.

Author affiliations
[1]Department of Dermatology, Faculty of Medicine, University of Tsukuba, Tsukuba, Ibaraki, Japan
[2]Department of Integrative Medicine for Allergic and Immunological Disease, Graduate School of Medicine, Osaka University, Suita, Osaka, Japan
[3]Medical and Biological Laboratories Co Ltd, Nagoya, Aichi, Japan
[4]Institute for Advanced Co-Creation Studies, Osaka University, Suita, Osaka, Japan
[5]Department of Microbiology and Immunology, Graduate School of Medicine, Osaka University, Suita, Osaka, Japan
[6]Department of Dermatology, Graduate School of Medicine, Osaka University, Suita, Osaka, Japan

Acknowledgements Parts of this work were presented at the 3 rd Global Conference on Myositis, 2019.

Contributors Study concept and design: NO and MF. Acquisition and analysis and interpretation of data: all authors. Drafting of the manuscript: NO and MF. Critical revision of the manuscript for important intellectual content: NO and YI. Obtained funding: NO and MF.

Funding Supported by KAKENHI from the Japan Society for the Promotion of Science (JSPS, 18K08263 for Naoko Okiyama).

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval All animal studies were approved by the Institutional Animal Care and Use Committee of University of Tsukuba. This study did not involve human participants.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information.

ORCID iD
Naoko Okiyama http://orcid.org/0000-0002-5398-0773

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CLINICAL SCIENCE

circPDE4B prevents articular cartilage degeneration and promotes repair by acting as a scaffold for RIC8A and MID1

Shuying Shen,[1,2] Yute Yang,[1,2] Panyang Shen,[1,2] Jun Ma,[1,2] Bin Fang,[3] Qingxin Wang,[4] Kefan Wang,[1,2] Peihua Shi,[1,2] Shunwu Fan ,[1,2] Xiangqian Fang [1,2]

Handling editor Josef S Smolen

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2021-219969).

[1]Department of Orthopaedic Surgery, Sir Run Run Shaw Hospital, Zhejiang University school of medicine, Hangzhou, China
[2]Key Laboratory of Musculoskeletal System Degeneration and Regeneration Translational Research of Zhejiang Province, Hangzhou, China
[3]Department of Spine Surgery, The Central Hospital Affiliated to Shaoxing University, Shaoxing, China
[4]Department of Spine Surgery, The Hospital of the Marine Police Corps of the Chinese people's Armed Police Force, Jiaxing, China

Correspondence to
Dr Xiangqian Fang and Dr Shunwu Fan, Department of Orthopaedic Surgery, Sir Run Run Shaw Hospital, Zhejiang University school of medicine, Hangzhou, People's Republic of China; orthofxq@zju.edu.cn, 0099203@zju.edu.cn

SS, YY and PS contributed equally.

Received 25 January 2021
Accepted 13 May 2021
Published Online First 26 May 2021

To cite: Shen S, Yang Y, Shen P, *et al*. *Ann Rheum Dis* 2021;**80**:1209–1219.

ABSTRACT

Objectives Circular RNAs (circRNAs) have emerged as significant biological regulators. Herein, we aimed to elucidate the role of an unidentified circRNA (circPDE4B) that is reportedly downregulated in osteoarthritis (OA) tissues.

Methods The effects of circPDE4B were explored in human and mouse chondrocytes in vitro. Specifically, RNA pull-down (RPD)-mass spectrometry analysis (MS), immunoprecipitation, glutathione-S-transferase (GST) pull-down, RNA immunoprecipitation and RPD assays were performed to verify the interactions between circPDE4B and the RIC8 guanine nucleotide exchange factor A (RIC8A)/midline 1 (MID1) complex. A mouse model of OA was also employed to confirm the role of circPDE4B in OA pathogenesis in vivo.

Results circPDE4B regulates chondrocyte cell viability and extracellular matrix metabolism. Mechanistically, FUS RNA binding protein (FUS) was found to promote the splicing of circPDE4B, while downregulation of circPDE4B in OA is partially caused by upstream inhibition of FUS. Moreover, circPDE4B facilitates the association between RIC8A and MID1 by acting as a scaffold to promote RIC8A degradation through proteasomal degradation. Furthermore, ubiquitination of RIC8A at K415 abrogates RIC8A degradation. The circPDE4B–RIC8A axis was observed to play an important role in regulating downstream p38 mitogen-activated protein kinase (MAPK) signalling. Furthermore, delivery of a circPDE4B adeno-associated virus (AAV) abrogates the breakdown of cartilage matrix by medial meniscus destabilisation in mice, whereas a RIC8A AAV induces the opposite effect.

Conclusion This work highlights the function of the circPDE4B–RIC8A axis in OA joints, as well as its regulation of MAPK-p38, suggesting this axis as a potential therapeutic target for OA.

Key messages

What is already known about this subject?

- Circular RNAs broadly participate in normal physiology and disease, including functioning as miRNA sponges in osteoarthritis (OA).
- Protein post-translational modifications are necessary for proteins to perform physiological or pathological functions, including knee cartilage homeostasis.

What does this study add?

- circPDE4B serves as a scaffold to facilitate RIC8 guanine-nucleotide exchange factor A (RIC8A)–midline 1 binding, thereby decreasing RIC8A-dependent activation of the p38 mitogen-activated protein kinase signalling pathway and regulating OA progression.
- The role of RIC8A is first reported in chondrocytes, and K415 is found as the most important ubiquitination site of RIC8A regulated by circPDE4B.

How might this impact on clinical practice or future developments?

- The circPDE4B–RIC8A axis may serve as a potential therapeutic target for OA.

INTRODUCTION

The aetiology of osteoarthritis (OA), the most common type of arthritis, is multifactorial and is associated with obesity, ageing, strain, trauma, congenital joint abnormalities and joint deformities.[1 2] Although OA involves pathological changes in joint sites, including subchondral osteosclerosis, synovitis and osteophyte formation, destruction of cartilage represents its landmark.[3] Considering that the extracellular matrix (ECM) accounts for 90% of the dry weight of cartilage,[4] changes in its physiology directly impact the function of cartilage. Moreover, as the only cell type in cartilage, chondrocytes play an important role in maintaining ECM homeostasis.[5] Thus, characterising the molecular mechanisms of chondrocytes involved in OA development and pathogenesis is crucial for improving prognosis and developing effective therapies.[6–8]

Recently, a growing number of studies have identified various functional non-coding RNAs, including circular RNAs (circRNAs), many of which are present in the human transcriptome.[9] The expression of these circRNAs exhibits tissue specificity, while their heterocyclic structure makes them highly stable.[10] Although circRNAs are believed to participate in cell differentiation and pluripotency,[11–13] their specific functions remain largely uncharacterised. Moreover, although most identified circRNAs are non-coding, some have been recently described as protein coding.[14 15] CircRNAs also have various biological functions related to different diseases.[16] In fact, our previous report,[17] as well as those of others,[18–20] have reported a significant role for circRNAs in chondrocyte

regulation of OA development and progression. However, these studies focused primarily on the function of circRNAs as miRNA sponges; hence, it remains unclear whether other molecular mechanism are also associated with the role of circRNAs in OA.

In the current study, we investigated the functions and molecular mechanisms of circPDE4B in OA. We believe that our study paves the way for future research investigating circRNA as a promising therapeutic target for OA.

METHODS

Detailed experimental procedures are described in the online supplemental materials and methods and tables.

RESULTS

circPDE4B exhibits lower expression in OA tissue

We previously performed RNA-seq analyses on the chondrocytes total RNA of ribosomal RNA deletion in three clinical OA and three control samples (SRA accession: PRJNA516555). Among the 50 most abundant significantly dysregulated circRNAs, the expression level of circPDE4B ranked first, the expression of which was significantly downregulated in chondrocytes of patients with OA ($p<0.05$, online supplemental table S1). In the current study, collected cartilage was assigned to one of three groups (total n=20): normal medial, OA lateral and OA medial. The OA severity for each case was assessed using the preoperative Kellgren-Lawrence, Outerbridge and Osteoarthritis Research Society International (OARSI) grading systems for the region of interest (ROI) (figure 1A). Meanwhile, histomorphological and western blot analyses accompanied by fluorescence in situ hybridisation (FISH) staining of ROI cartilage indicated that increased degradation of cartilage corresponded to decreased expression of circPDE4B in chondrocytes (figure 1B and online supplemental figure S1A). These results were confirmed by quantitative reverse transcription PCR (RT-qPCR) analysis which detected downregulated circPDE4B RNA levels in the chondrocytes of severe OA tissues, whereas mPDE4B mRNA level remained relatively consistent (figure 1C). Taken together, these results revealed that circPDE4B expression was negatively associated with OA severity.

Considering that circPDE4B is conserved between humans and mice, we also detected circPDE4B expression in human/mouse chondrocytes (circPDE4B in human chondrocytes (HCs); circPde4b in mouse chondrocytes (MCs)) and found that interleukin-1β (IL-1β; 10 ng/mL) and tumor necrosis factor-α (TNF-α; 50 ng/mL) treatment significantly decreased circPDE4B/circPde4b expression in HCs/MCs in a time-dependent manner (figure 1D and online supplemental figure S1B). Moreover, Sanger sequencing displayed the splicing sequence of circPDE4B/circPde4b (online supplemental figure S1C,H). Meanwhile, circPDE4B/circPde4b was amplified by divergent primers from cDNA, but not in gDNA (online supplemental figure S1D,I). circPDE4B/circPde4b also exhibited a remarkable resistance to RNase R digestion (online supplemental figure S1E,J) and actinomycin D treatment (online supplemental figure S1F,K). Besides, mPDE4B/mPde4b was amplified by random primer and oligo(dT) primer, whereas circPDE4B/circPde4b was only amplified using random primers (online supplemental figure S1G,L). Nuclear separation experiments coupled with RT-qPCR analysis and FISH revealed that circPDE4B/circPde4b is primarily located in the cytoplasm of HCs/MCs (figure 1E,F and online supplemental figure S1M,N). Cumulatively, these results indicate that circPDE4B is downregulated in OA and, thus, may contribute to OA progression.

FUS RNA binding protein (FUS) regulates circPDE4B expression through direct binding to pre-mRNA

We next sought to identify circPDE4B upstream regulators. We first performed RNA pull-down (RPD)-MS assay of circPDE4B flanking sequence and found two RNA splicing related RBPs, including DExH-box helicase 9 and FUS (figure 1G). RT-qPCR results indicated that following FUS knockdown, circPDE4B was downregulated in HCs, while pPDE4B and mPDE4B did not exhibit significant changes (figure 1H and online supplemental figure S2A). In addition, infection with two FUS shRNA lentivirus served to only decrease the expression of circPDE4B (figure 1I and online supplemental figure S2B), whereas overexpressed FUS upregulated the expression of circPDE4B (figure 1I). Next, RNA immunoprecipitation (RIP) assays revealed that FUS binds to exon-adjacent sites, while remote regions elsewhere were negligible (figure 1J,K). We also searched for potential FUS response elements and found two potential motifs, A located upstream and B located downstream. We further engineered two short circPDE4B minigenes, including circPDE4B-s and circPDE4B-s-del (figure 1L). RIP revealed an overt interaction between FUS and circPDE4B-s, but not with circPDE4B-s-del (figure 1M), indicating that FUS requires the putative sites in surrounding introns for binding. We next knocked down FUS in circPDE4B-s/del expressed HCs and found that circPDE4B-s had significantly reduced circPDE4B transcripts on FUS knockdown, compared with circPDE4B-del (figure 1N). Notably, FUS was downregulated by TNF-α in HCs (figure 1O). Cumulatively, the downregulation of circPDE4B in OA was, at least in part, caused by the inhibition of FUS.

circPDE4B regulates chondrocyte cell viability and ECM metabolism

To assess the involvement of circPDE4B/circPde4b in ECM metabolism, we transfected HCs/MCs with three circPDE4B/circPde4b siRNAs, respectively (figure 2A and online supplemental figure S3A). Knockdown of circPDE4B/circPde4b expression did not affect PDE4B/Pde4b mRNA levels (online supplemental figure S3B,C).

We then assessed the influence of circPDE4B/circPde4b on chondrocytes viability using a cell counting kit-8 (CCK-8) assay. Results showed that knockdown of circPDE4B/circPde4b expression reduced chondrocytes viability (figure 2B and online supplemental figure S3D). In addition, the inhibition of circPDE4B/circPde4b by shRNA adenovirus (online supplemental figure S3E,F) significantly enhanced the expression of MMP3, MMP13 and ADAMTS4, whereas the expression of SOX9, COL2A1 (or COL2 protein) and aggrecan was downregulated in HCs/MCs, as revealed by RT-qPCR (figure 2C and online supplemental figure S3G) and western blot (figure 2D and online supplemental figure S3H). Immunofluorescence further confirmed that circPDE4B/circPde4b knockdown affected MMP3, MMP13, COL2 and aggrecan levels in HCs/MCs (figure 2E,F and online supplemental figure S3I,J). Meanwhile, Alcian blue staining of HCs/MCs revealed that circPDE4B/circPde4b inhibition led to a chondrocytes dysfunction with less blue-stained proteoglycan. (figure 2G and online supplemental figure S3K).

We then performed gain-of-function experiments (online supplemental figure S3L,M) and found that overexpression of circPDE4B/circPde4b increased the viability of chondrocyte cells, as revealed by a CCK-8 assay (figure 2H and online supplemental figure S3N). Besides, mRNA and protein levels of MMP3, MMP13 and ADAMTS4 were downregulated, whereas those of SOX9, COL2A1 (or COL2 protein) and aggrecan

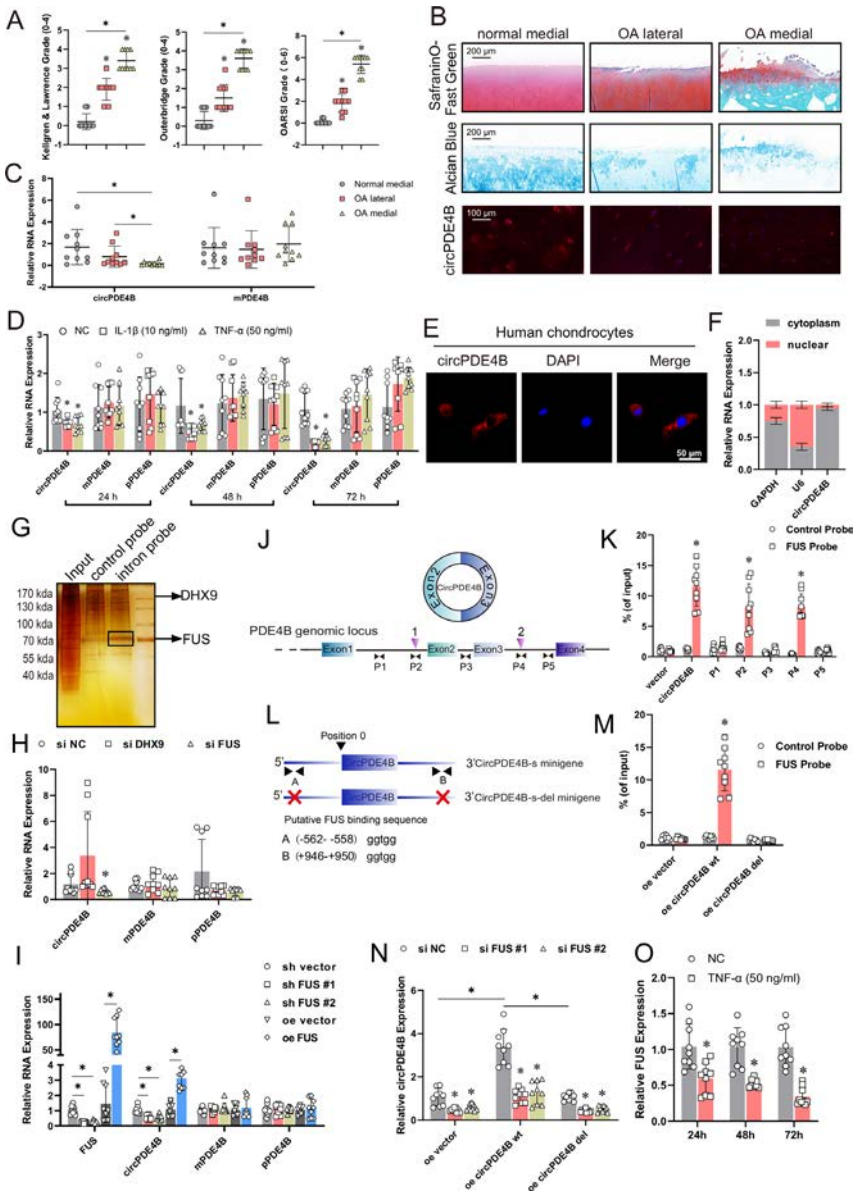

Figure 1 Characterisation of circPDE4B in human chondrocytes (HCs) and osteoarthritis tissues. (A) Preoperative Kellgren-Lawrence, Outerbridge and OARSI grading based for region of interest (ROI) cartilage (n=10 per group). *p≤0.05. (B) Histomorphological analysis and circPDE4B-labelled FISH staining for ROI cartilage. Scale bars, 200 μm. (C) circPDE4B and mPDE4B expression in ROI chondrocytes via RT-qPCR (n=10); *p≤0.05. (D) Changes in circPDE4B, mPDE4B and pPDE4B RNA levels, treated with IL-1β and TNF-α, assessed via RT-qPCR (n=9, 3 donors for three replicates); *p≤0.05. (E) Representative images of FISH staining for circPDE4B localisation in HCs. Scale bars, 50 μm. (F) Expression of circPDE4B assessed by RT-qPCR in the nuclear and cytoplasmic fractions (n=9, 3 donors for three replicates); *p≤0.05. (G) Silver staining of purified interaction proteins in the circPDE4B flanking sequence RPD experiment. (H) circPDE4B expression in HCs transfected with DExH-box helicase 9 and FUS siRNA or a negative control (n=9, 3 donors for three replicates); *p≤0.05. (I) circPDE4B, mPDE4B and pPDE4B expression after FUS inhibition or overexpression (n=9, 3 donors for three replicates); *p≤0.05. (J) Schematic of PDE4B pre-mRNA showing the locations of the two putative sites (inverted blue triangles) and amplicons (P1–P5) used for the RIP assay. (K) RIP assay performed with the PCR primers indicated in the schematic on the left. (n=9, 3 donors for three replicates); *p≤0.05. (L) Schematic illustrating the putative FUS-binding sites on the flanking introns in the circPDE4B-s minigene. The 5′ terminus of the circular exons of circPDE4B was defined as position 0. Putative FUS-binding sites A and B are located in the intron at the 5′ terminus of the circPDE4B exon (position: −562 to −558) and on the intron at the 3′ terminus of the circPDE4B exon (position: 946–950). (M) RIP analysis of FUS binding to circPDE4B-s and circPDE4B-s-del minigenes in HCs (n=9, 3 donors for three replicates); *p≤0.05. (N) Expression of circPDE4B relative to β-actin in HCs infected with circPDE4B-s or circPDE4B-s-del lentivirus followed by transfection with FUS siRNA or control siRNA (n=9, 3 donors for three replicates); *p≤0.05. (O) FUS mRNA expression level in HCs after TNF-α treatment (n=9, 3 donors for three replicates); *p≤0.05. DAPI, 4′,6-diamidino-2-phenylindole; FISH, fluorescence in situ hybridisation; FUS, FUS RNA binding protein; IL-1β, interleukin-1β; NC, negative control; RIP, RNA immunoprecipitation; RPD, RNA pull-down; RT-qPCR, quantitative reverse transcription PCR; TNF-α, tumour necrosis factor-α.

were upregulated in circPDE4B/circPde4b-overexpressing HCs/MCs (figure 2I–L and online supplemental figure S3O–R). Furthermore, Alcian blue staining of HCs/MCs indicated that circPDE4B/circPde4b overexpression and IL-1β cotreatment reduced cartilage destruction compared with IL-1β treatment alone (figure 2M and online supplemental figure S3S). These data demonstrate that circPDE4B/circPde4b in HCs/MCs can promote cell viability and inhibit the catabolic effect.

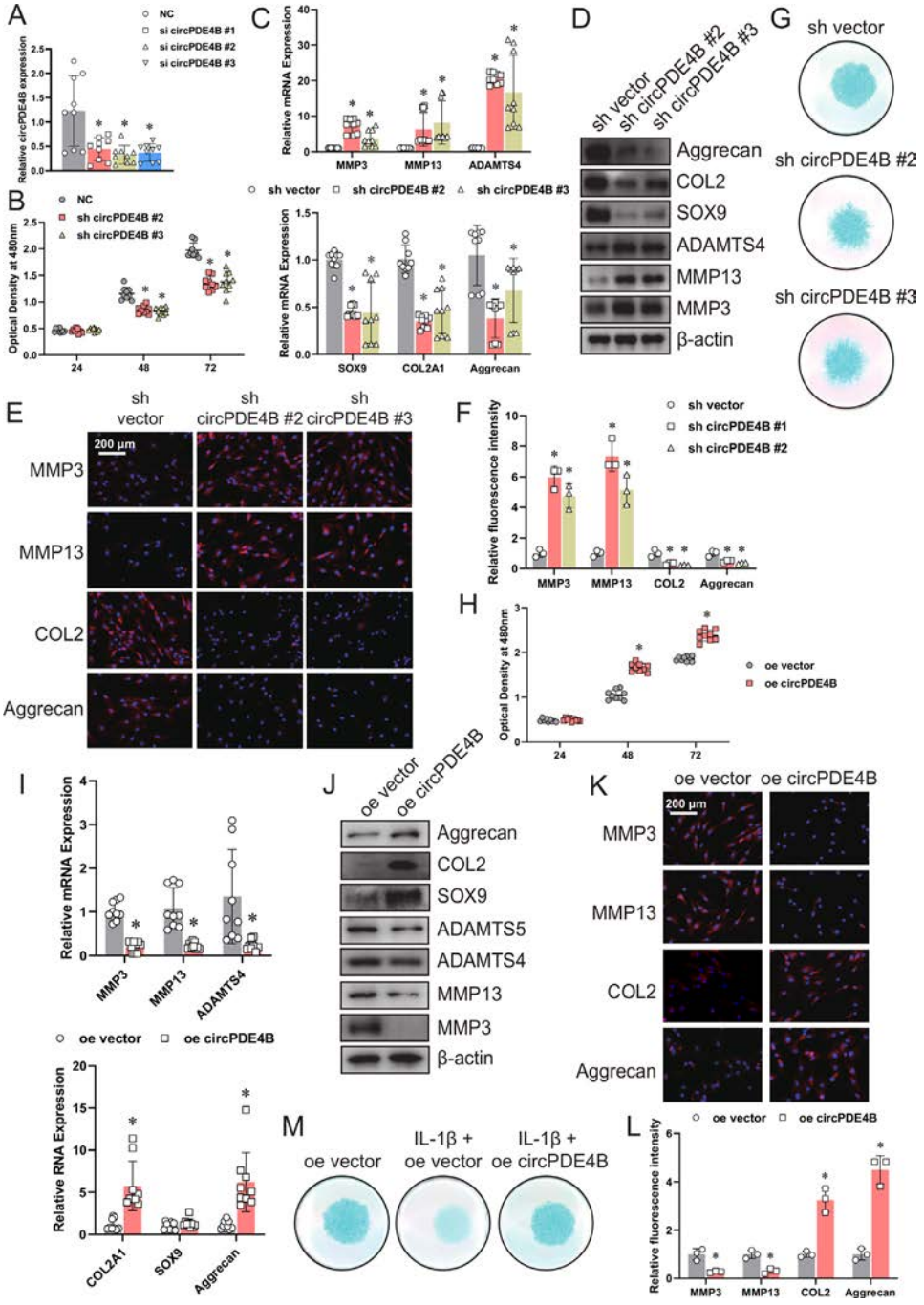

Figure 2 Targeting circPDE4B expression affects matrix-degrading and anabolic factors in human chondrocytes (HCs). (A) circPDE4B expression in HCs transfected with circPDE4B siRNAs or negative control siRNA (n=9, 3 donors for three replicates); *p≤0.05. (B) Viability of HCs infected with circPDE4B shRNA #2/#3 adenovirus or control shRNA adenovirus (n=9, 3 donors for three replicates); *p≤0.05. (C,D) mRNA and protein levels of MMP3, MMP13, ADAMTS4, COL2A1 (or COL2 protein), SOX9 and aggrecan in HCs infected with circPDE4B shRNA #2/#3 adenovirus or control shRNA adenovirus (n=9, 3 donors for three replicates); *p≤0.05. (E) Immunofluorescence analysis of MMP3, MMP13, COL2 and aggrecan. (F) Quantification of immunofluorescence analysis (n=9, 3 donors for three replicates); *p≤0.05. (G) Alcian blue staining of shRNA-treated HCs. (H) Viability of HCs infected with a circPDE4B overexpression adenovirus or control adenovirus (n=9, 3 donors for three replicates); *p≤0.05. (I,J) mRNA and protein levels of MMP3, MMP13, ADAMTS4, COL2A1 (or COL2 protein), SOX9 and aggrecan (n=9, 3 donors for three replicates); *p≤0.05. (K) Immunofluorescence of MMP3, MMP13, COL2 and aggrecan. (L) Quantification of immunofluorescence analysis (n=9, 3 donors for three replicates); *p≤0.05. (M) Alcian blue staining of HCs treated with IL-1β with or without circPDE4B overexpression. IL-1β, interleukin-1β; NC, negative control.

RIC8 guanine-nucleotide exchange factor A (RIC8A) interacts with circPDE4B and participates in OA

Cytoplasm-localised circRNAs participate in translational regulation by acting as ceRNAs, coding RNAs or as a scaffold for RBPs. AGO2 RIP assay revealed that circPDE4B does not bind to AGO2 (online supplemental figure S4A). Bioinformatics analysis of circPDE4B further revealed that it has an open reading frame (ORF) fragment (online supplemental figure S4B). Therefore, two full-length (FL) predicted ORFs were cloned into a eukaryotic expression vector, however, circPDE4B was not found to encode a protein (online supplemental figure S4C).

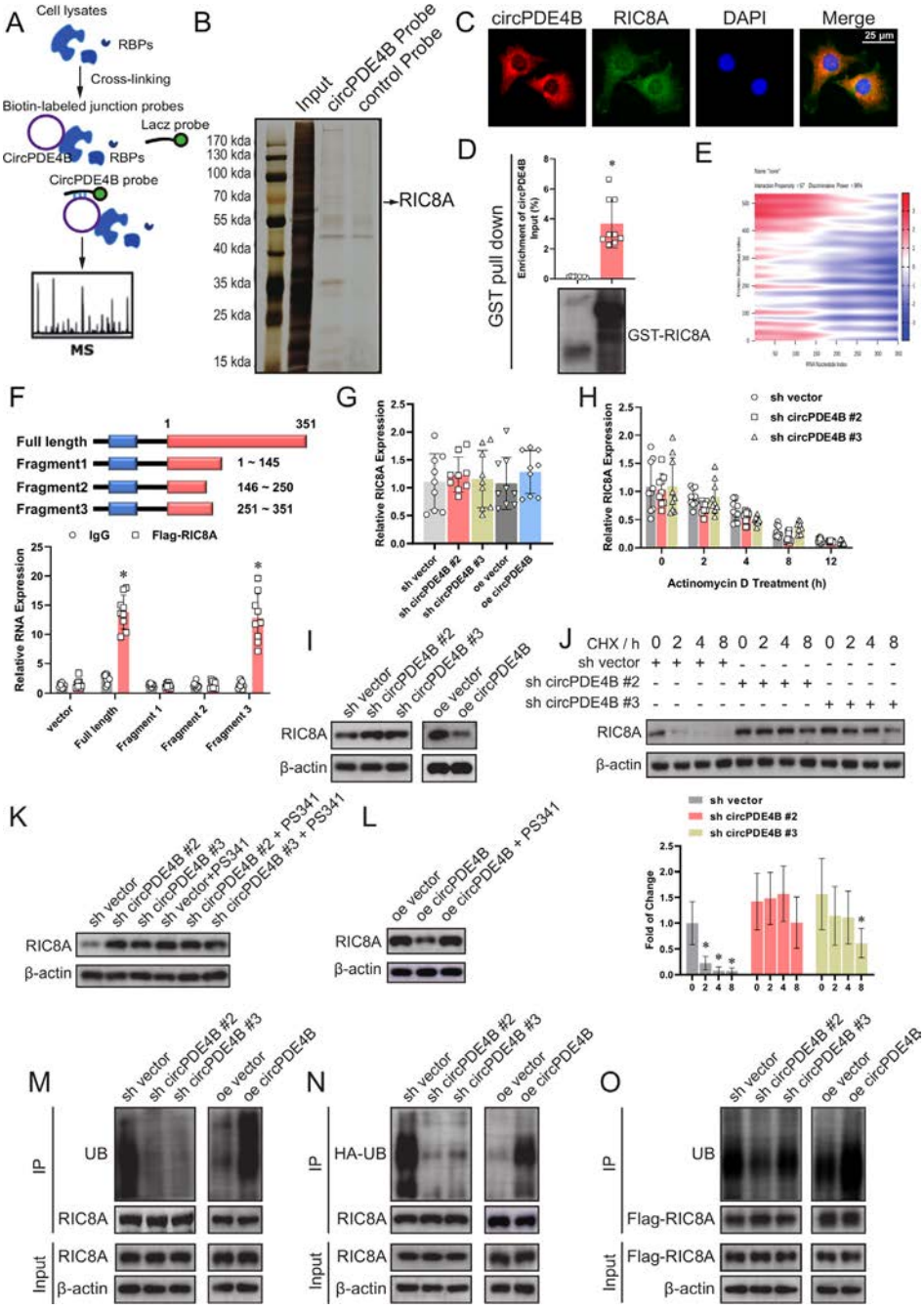

Figure 3 circPDE4B interacts with RIC8A and affects RIC8A ubiquitylation. (A) Schematic of RPD-MS experiments. (B) Silver staining of proteins binding to circPDE4B. (C) circPDE4B and RIC8A interaction in human chondrocytes (HCs) confirmed via an RNA-protein colocalisation assay. Scale bars, 50 µm. (D) RIC8A–circPDE4B interaction detected by GST pull-down assays. GST was used as a pull-down control. (E) Predicted binding sites of circPDE4B and RIC8A (catRAPID graph). (F) Binding sequence of circPDE4B for RIC8A identified by an RIP assay (n=9, 3 donors for three replicates); *p≤0.05. mRNA levels (G), mRNA stability (H) and protein levels (I) of RIC8A after circPDE4B knockdown and overexpression (n=9, 3 donors for three replicates); *p≤0.05. (J) Western blot of RIC8A in HCs treated with the transcription inhibitor CHX (200 µg/mL). (K) Effect of PS341 treatment on RIC8A protein level alteration mediated by circPDE4B knockdown. (L) Effects of PS341 treatment on RIC8A protein expression mediated by circPDE4B overexpression. (M) Immunoprecipitation (IP) analysis of ubiquitinated RIC8A in HCs treated with PS341. The lysates of circPDE4B overexpression or knockdown cells were treated with an anti-RIC8A antibody. (N) HCs were infected with HA-UB lentivirus and then treated with PS341. The lysates of circPDE4B overexpression or knockdown cells were treated with an anti-HA antibody. (O) HCs were infected with Flag–RIC8A lentivirus and then treated with PS341. The lysates of circPDE4B overexpression or knockdown cells were treated with an anti-Flag antibody. CHX, cycloheximide; DAPI, 4',6-Diamidino-2-Phenylindole; GST, glutathione-S-transferase; RBPs, RNA binding proteins; RIC8A, RIC8 guanine-nucleotide exchange factor A; RIP, RNA immunoprecipitation; UB, ubiquitination.

To identify proteins that interact with circPDE4B, we employed RPD-MS (figure 3A and online supplemental figure S5A). A total of 112 proteins interacting with circPDE4B were identified (online supplemental table S2 and figure 3B). We selected five of the highest pep _score proteins and verified their role in the regulation of ECM metabolism in HCs by siRNA knockdown. RT-qPCR results revealed that only RIC8A and ENO1 had an obvious effect on regulating MMP13 and

COL2A1 (online supplemental figure S5B). However, the RIP assay indicated that only RIC8A binds to circPDE4B (online supplemental figure S5C). We further confirmed the binding of RIC8A and circPde4b by RIP assay in MCs (figure S5D). RNA-protein colocalisation in HCs also verified the interaction between RIC8A and circPDE4B (figure 3C). RPD assay showed that in vitro linearly transcriptional circPDE4B was able to pull down recombinant RIC8A (figure 3D). We then used the catRAPID tool to predict the interacting regions of circPDE4B and RIC8A (figure 3E and online supplemental figure S5E). To identify the predicted binding sites, we truncated the FL circPDE4B into three segments (S1: 1–145 nt, S2: 146–250 nt, S3: 251–351 nt). In line with the prediction, RIP results indicated only FL and S3 were pulled down by RIC8A (figure 3F). Interestingly, the S3 truncation is reflected as a hairpin region 2 loop in the predicted RNA stem-loop structure (online supplemental figure S5F). Taken together, these results indicate that circPDE4B interacts with RIC8A in HCs.

To further investigate the function of RIC8A in the ECM metabolism of HCs, we infected HCs with two RIC8A shRNA adenoviruses (online supplemental figure S6A). CCK-8 assay indicated that RIC8A knockdown increased HCs viability (online supplemental figure S6B). Moreover, RIC8A knockdown cells displayed a significant decrease in the expression of MMP3, MMP13 and ADAMTS4 and increased expression of SOX9, COL2A1 (or COL2 protein) and aggrecan (online supplemental figure S6C–F).

We also performed gain-of-function experiments (online supplemental figure S6G). RIC8A overexpression decreased the viability of chondrocytes as revealed by CCK-8 assay (online supplemental figure S6H). Besides, the mRNA and protein expression of MMP3, MMP13 and ADAMTS4 were downregulated, while SOX9, COL2A1 (or COL2 protein) and aggrecan were upregulated in RIC8A-overexpressing HCs (online supplemental figure S6I–L). We further performed western blot and RT-qPCR to assess the influence of mmu_RIC8A on ECM metabolism in MCs. RIC8A also impaired ECM anabolic processes in MCs (online supplemental figure S6M,N). These data collectively support inhibition of cell viability and procatabolic effects of RIC8A in chondrocytes.

circPDE4B regulates RIC8A function through proteasome-mediated degradation

Our further investigation indicated that circPDE4B regulates RIC8A protein levels, however, not mRNA levels or stability (figure 3G–I). We also blocked RIC8A protein synthesis and observed obvious differences in RIC8A protein half-life between sh-negative control (NC) and sh-circPDE4B HCs (figure 3J), suggesting that circPDE4B decreased RIC8A protein stability. Moreover, in MCs, circPde4b also regulated mmu_RIC8A protein levels (online supplemental figure S6O). To confirm whether circPDE4B affects RIC8A function via changes in post-translational modification, we introduced a proteasome inhibitor named PS341. Accordingly, RIC8A was observed non-changed in both circPDE4B overexpression and knockdown cells after treatment with PS341 (figure 3K,L), indicating that circPDE4B regulates RIC8A through proteasomal activity. Consistently, the polyubiquitination of RIC8A decreased following circPDE4B depletion and increased following circPDE4B overexpression, regardless of endogenous or exogenous RIC8A (figure 3M–O). Cumulatively, these results showed that circPDE4B post-translationally impacts the degradation and turnover of RIC8A mediated by the proteasome.

circPDE4B facilitates the formation of a ternary complex between RIC8A and midline 1 (MID1) that promotes RIC8A degradation

We next sought to identify E3 ligases involved in the proteasomal degradation of RIC8A. Interestingly, MS results revealed that circPDE4B also binds two E3 ligases, including RNF2 and MID1. We, therefore, inferred whether circPDE4B could act as a scaffold for RIC8A and E3 ligases complex. However, since RNF2 is localised within the nucleus, we choose MID1 for further investigation. Indeed, MID1 was found to bind RIC8A, as indicated by an immunoprecipitation (IP) assay (figure 4A). Immunofluorescence staining of RIC8A and MID1 also proved their colocalisation in HCs (figure 4B).

Western blot results showed that MID1 decreased RIC8A protein levels (online supplemental figure S7A,B), while IP results indicated that MID1 knockdown effectively impaired the ubiquitylation of RIC8A and MID1 overexpression and increased RIC8A ubiquitylation (figure 4C). Co-immunoprecipitation (Co-IP) assay also revealed that binding of RIC8A and MID1 decreased in circPDE4B knockdown cells compared with control cells, while circPDE4B overexpression had the opposite effect (figure 4D). Moreover, circPDE4B did not affect MID1 levels (figure 4E). Both RPD and sequential IP assays revealed that circPDE4B promotes the binding of RIC8A and MID1 (figure 4F,G). In line with this finding, circPDE4B increased the association between recombinant RIC8A and MID1 proteins in an in vitro binding assay (figure 4H).

To further investigate these interactions, we performed domain truncation of RIC8A and MID1 for binding assays. The simple modular architecture research tool (SMART) prediction website indicated that RIC8A contains only a Pfam domain (online supplemental figure S7C). Thus, we divided the protein into two fragments, an N-terminal (1–153 amino acids) and C-terminal (154–537 amino acids) domain. Via co-IP, as expected, MID1 was shown to bind to RIC8A at the N-terminal regulatory domain (figure 4I). In addition, we detected RIC8A functional sites. RIC8A was immunoprecipitated in HCs and subjected to MS analysis, which confirmed ubiquitylation of amino acid residues in RIC8A (figure 4J). Ten ubiquitylation sites were identified in RIC8A, K143 and K187 and were not conserved between humans and mice (figure 4J). We thus mutated conserved RIC8A sites from lysine (K) to arginine (R), to exclude ubiquitylation. IP results indicated that substitution of K415 greatly reduced RIC8A ubiquitylation compared with WT (figure 4K), identifying K415 as the major ubiquitylation site of RIC8A (online supplemental figure S7D). Interestingly, K415 is highly conserved among mammals (figure 4L,M). Further, circPDE4B overexpression or inhibition no longer regulated the ubiquitylation levels of RIC8A following K415 mutation (figure 4N). These results suggest that circPDE4B serves as a scaffold to facilitate the association between RIC8A and MID1.

circPDE4B and RIC8A regulate the p38 signaling pathway in chondrocytes

To elucidate the signalling pathways downstream of RIC8A, we investigated the phosphorylation levels of mitogen-activated protein kinases (MAPKs), NF-κB and mTOR in RIC8A knockdown HCs. The phosphorylation level of p38 was significantly decreased by two RIC8A shRNAs (figure 5A). Next, HCs were pretreated with signalling molecule inhibitors, including PD98059 (extracellular regulated protein kinases 1/2 (ERK1/2) inhibitor), SB203580 (p38 inhibitor) and SP600125 (c-Jun N-terminal kinase (JNK) inhibitor), followed by RIC8A

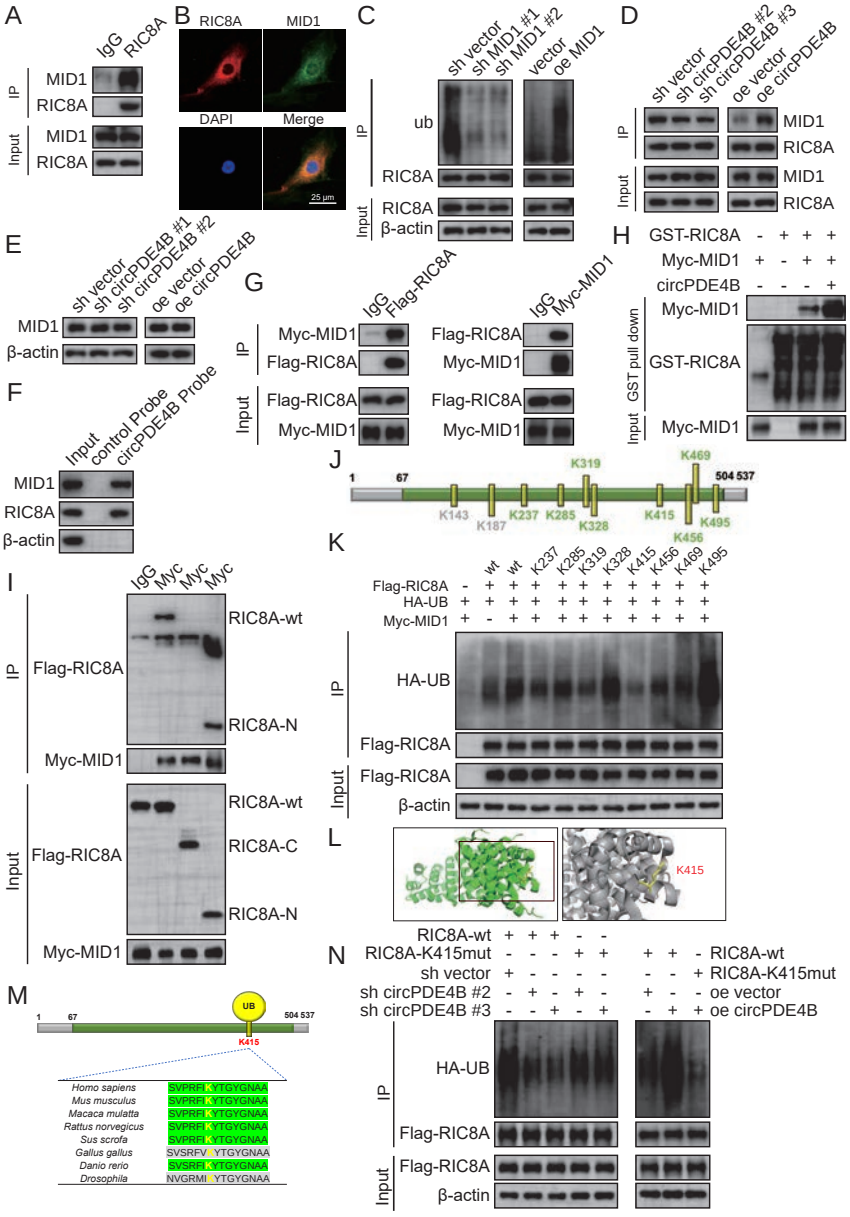

Figure 4 MID1 is an E3 ligase of RIC8A, and K415 is the primary ubiquitylation site of RIC8A. (A) Immunoprecipitation (IP) assay to verify whether E3 ligase MID1 binds to RIC8A. (B) Colocalisation in human chondrocytes (HCs) labelled with anti-RIC8A or anti-MID1 by immunofluorescence. Scale bars, 50 µm. (C) Effect of MID1 overexpression or knockdown on RIC8A ubiquitylation. (D) Effect of circPDE4B overexpression or knockdown on the interaction between RIC8A and MID1. (E) Protein expression of MID1 after circPDE4B knockdown and overexpression. (F) RPD assays using biotin-labelled linear circPDE4B probes in HC lysate before western blotting. (G) HEK-293T cells were infected with Myc–MID1 or Flag–RIC8A before consecutive IP. Rinsing with Flag peptides at first-stage Flag IPs and then eluates were subjected to secondary IP with Myc antibodies or homotypic matching IgG. Western blot was then performed to detect samples. (H) GST–RIC8A and Myc–MID1 overexpressed and purified from cells. RIC8A–MID1 interactions with or without circPDE4B were detected by GST pull-down assays. GST was used as a pull-down control. (I) Myc–MID1 and Flag–RIC8A WT, N-terminal domain and C-terminal domain plasmids were transfected into HEK-293T cells, a co-IP assay was performed and Flag expression was examined by western blotting. (J) HCs were subjected to RIC8A IP and LC–MS/MS analysis of RIC8A ubiquitylation peptide spectra. Ubiquitylated sites were identified by LC–MS analysis. (K) HCs expressing Flag-tagged wild-type or mutant RIC8A KR were first exposed to PS341 and subsequently treated with Flag IP. RIC8A ubiquitylation was analysed via western blot analysis. (L) Crystal structure of RIC8A proteins with K415. (M) Conservation ability of the K415 site of RIC8A. (N) Effect of circPDE4B inhibition and overexpression on the K415R RIC8A ubiquitylation level, as detected by an IP assay. co-IP, co-immunoprecipitation; GST, glutathione-S-transferase; HA-UB, HA-tagged ubiquitination; KR, mutation of lysine (K) to arginine (R); LC, liquid chromatography; MID1, midline 1; MS, mass spectrometry; RIC8A, RIC8 guanine-nucleotide exchange factor A; RPD, RNA pull-down.

overexpression. The overexpression of RIC8A pretreated with p38 MAPK inhibitors inhibited OA, however, it was not affected by ERK or JNK inhibitors (figure 5B). Moreover, after being infected with RIC8A shRNA or overexpression adenovirus, p38 MAPK phosphorylation and its localisation were dysregulated (figure 5C–E). These results suggest that RIC8A functions through the p38 signalling pathway in chondrocytes.

We next investigated the role of circPDE4B in regulation of the p38 signalling pathway in OA. circPDE4B overexpression decreased while circPDE4B knockdown activated p38 MAPK

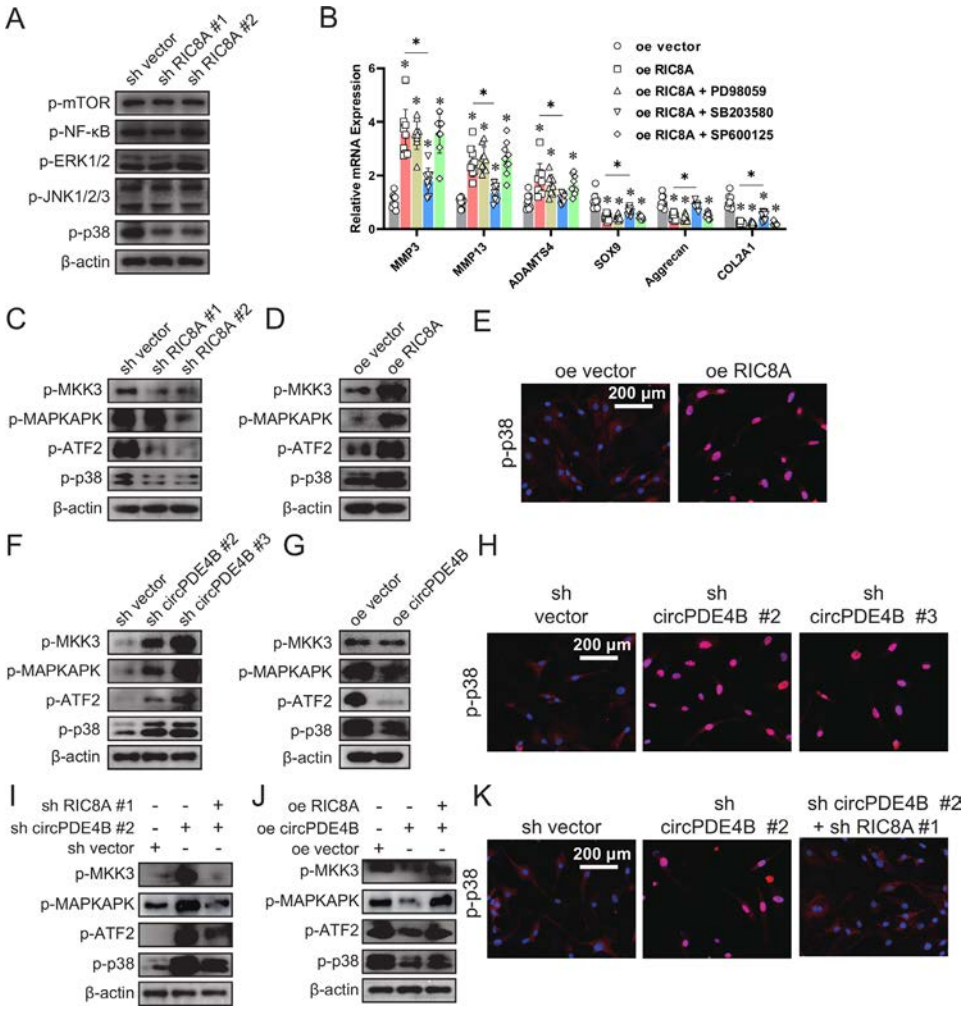

Figure 5 The p38 MAPK pathway is the downstream target of the circPDE4B–RIC8A axis. (A) Phosphorylation of MAPK, NF-κB and mTOR in human chondrocytes (HCs) infected with the vector or RIC8A shRNAs. (B) Relative mRNA expression levels of MMP3/13, ADAMTS4, SOX9, aggrecan and COL2A1 in HCs infected with RIC8A adenovirus and pretreated for 1 hour with PD98059 (ERK inhibitor), SB203580 (p38 MAPK inhibitor) or SP600125 (JNK inhibitor) (n=9, 3 donors for three replicates); *p≤0.05. (C) Phosphorylation levels of p38 MAPK signalling pathway members in HCs infected with RIC8A shRNA or vector adenovirus. Phosphorylation levels of p38 MAPK signal pathway members (D) in HCs with overexpressed RIC8A and (E) associated translocation of p38. (F) Phosphorylation levels of p38 MAPK signalling pathway members in HCs infected with circPDE4B shRNA or vector adenovirus. (G) Phosphorylation levels of p38 MAPK signalling pathway members in HCs infected with circPDE4B adenovirus. (H) Associated translocation of p38 in HCs infected with circPDE4B shRNA or vector adenovirus. (I,J) Phosphorylation levels of p38 MAPK signal pathway members in HCs coinfected with sh circPDE4B and sh RIC8A adenovirus. (I) or circPDE4B and RIC8A overexpression adenovirus. (K) Associated translocation of p38 in HCs coinfected with sh circPDE4B and sh RIC8A adenovirus. ERK, extracellular regulated protein kinases; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; mTOR, mechanistic target of rapamycin kinase; NF-κB, nuclear factor kappa B; RIC8A, RIC8 guanine-nucleotide exchange factor A.

signalling together with p38 phosphorylation and nuclear translocation (figure 5F–H). We then performed rescue assays. As shown in figure 5I–K, RIC8A overexpression rescued the downregulation of the p38 signalling pathway induced by circPDE4B overexpression, while RIC8A inhibition rescued the activation of p38 signalling pathway induced by circPDE4B knockdown, together with p38 phosphorylation and nuclear translocation. Based on these findings, the circPDE4B–RIC8A axis plays an important role in regulating the downstream p38 MAPK signalling pathway in chondrocytes.

circPde4b and RIC8A affect OA pathogenesis in mice

To corroborate the abovementioned findings, we further assessed the effects of circPde4b on OA in mice (online supplemental figure S8A). The specific adeno-associated virus (AAV) (approximately 1.0×10^{10}vg) efficiently infected the cartilage and synovium in the four groups (online supplemental figure S8B,C), but in vitro study showed that overexpressed circPde4b and RIC8A did not obviously promoted the inflammation of synovium (online supplemental figure S8D,E). Figure 6A shows the RNA expression of circPde4b and RIC8A after infection with the different AAV in the four groups. RT-qPCR and western blot analyses of ECM-associated proteins extracted from cartilage also suggested more severe OA in the medial meniscus destabilisation (DMM)+vector group and DMM+circPde4b+RIC8A group (figure 6B,C). Using Safranin O fast green staining (figure 6D), marked proteoglycan loss was observed in the DMM+vector group and DMM+circPde4b+RIC8A group compared with the SHAM+vector and DMM+circPDE4B groups, indicating that circPde4b AAV could rescue the OA progression caused by DMM, while RIC8A AAV could reverse this rescue. OARSI grade (figure 6E) further suggested that mice

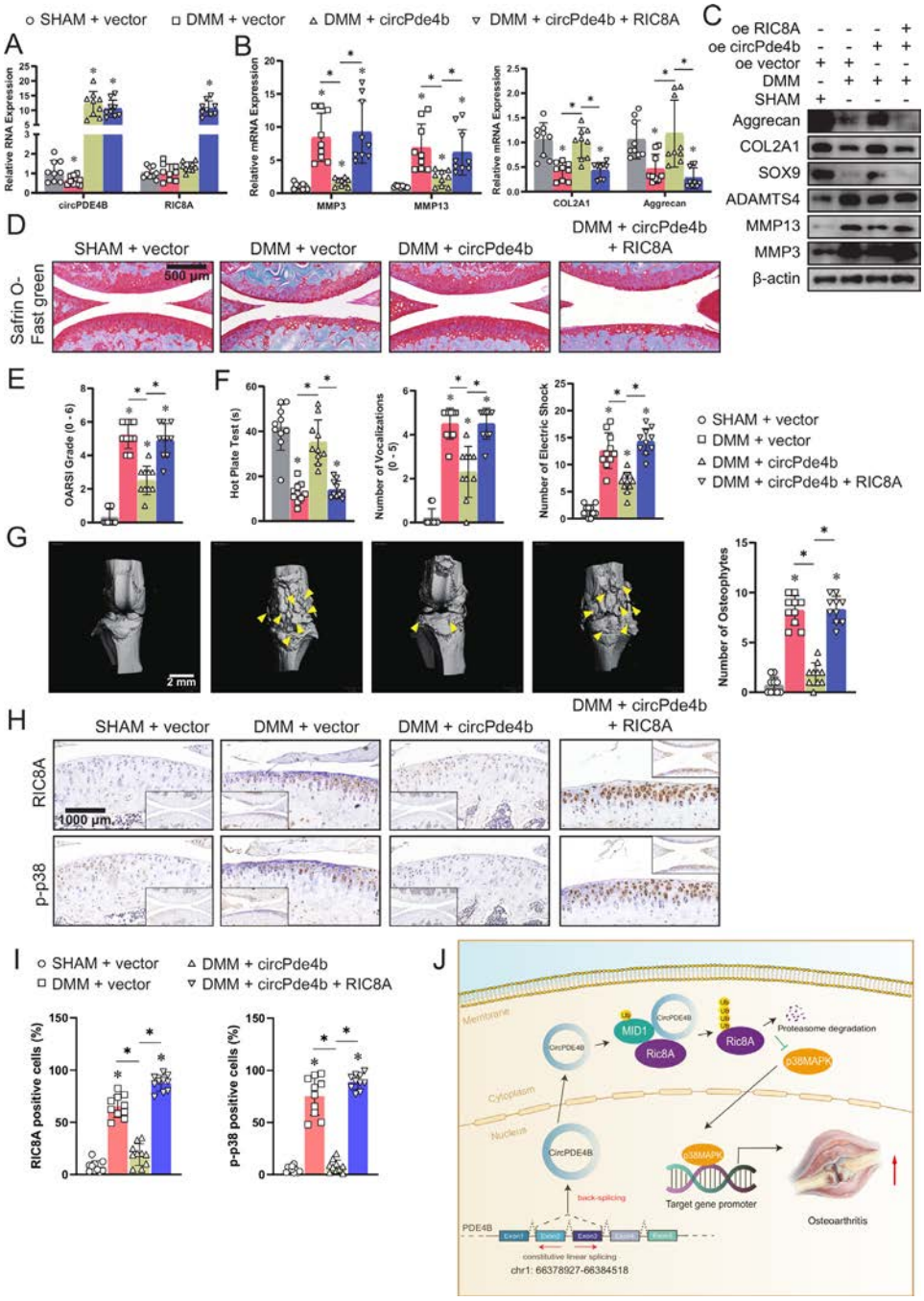

Figure 6 circPDE4B and RIC8A modulates osteoarthritis pathogenesis in a murine model. (A) RT-qPCR quantification of circPDE4B and RIC8A expression in mouse chondrocytes extracted from knee cartilage in the four groups (n=3); *p<0.05. (B) RT-qPCR quantification of MMP3, MMP13, COL2A1 and aggrecan expression in the four groups (n=3); *p<0.05. (C) Western blot analysis of extracellular matrix-associated proteins in the four groups. (D) Representative images of Safranin O fast green staining of cartilage in the four study groups. Scale bars, 500 µm. (E) OARSI grade used for evaluation of the cartilage degradation in the four groups (n=10); *p<0.05. (F) Hot plate test, knee extension test and electric shock-stimulated treadmill test used for the evaluation of knee pain (n=10); *p<0.05. (G) Left, 3D reconstruction images of micro-CT scanning of the knees and osteophytes (yellow arrow). Scale bars, 2 mm. Right, the number of osteophytes (n=10); *p<0.05. (H) Representative images of RIC8A and p-p38-labelled IHC staining. Scale bars, 1000 µm. (I) Quantitative analysis of RIC8A and p-p38 expression in the cartilage with IHC. (n=10); *p<0.05. (J) Graphic abstract of our study. DMM, medialmeniscus destabilisation; IHC, immunohistochemistry; MCs, mouse chondrocytes; OARSI, Osteoarthritis Research Society International; RIC8A, RIC8 guanine-nucleotide exchange factor A. RT-qPCR, quantitative reverse transcription PC.

in the SHAM+vector and DMM+circPde4b group displayed less cartilage degradation, whereas those in the DMM+vector and DMM+circPde4b+RIC8A exhibited the opposite. The hot plate test, knee extension test and electric shock stimulated treadmill test demonstrated more discomfort and knee pain in the DMM+vector group and DMM+circPde4b+RIC8A group than in the SHAM+vector and DMM+circPde4b groups (figure 6F). 3D reconstruction of the micro-CT of mouse knees revealed much more osteophytes in the DMM+NC group and DMM+circPde4b+RIC8A group than in the SHAM+vector and DMM+circPde4b groups (figure 6G). MMP3, MMP13, COL2 and aggrecan expression in cartilage from the four groups was

consistent with the above staining results (online supplemental figure S9A). Moreover, RIC8A and p-p38 labelled immunohistochemistry (IHC) staining in the four groups showed that overexpressed circPde4b downregulated the RIC8A and p-p38 expression, therefore inhibited the OA progression caused by DMM operation (figure 6H,I). Together, these results indicate that, in mice, circPde4b and RIC8A are involved in OA pathogenesis and their underlying mechanism is presented in figure 6J.

DISCUSSION

The OA pathogenesis is primarily underpinned by an imbalance in joint metabolism, for example, when catabolism exceeds anabolism, leading to the degradation of the cartilage matrix.[21] Emerging evidence has suggested several key catabolic regulators that contribute to cartilage destruction.[22] However, the mechanism underlying the cessation of matrix anabolism remains largely unknown.

Recent studies have begun to shed light on the various roles of circRNAs including a crucial role in the occurrence, development, diagnosis, prognosis and treatment of diseases.[23] Specifically, we previously reported that circSERPINE2 could inhibit the occurrence and development of OA by regulating ERG gene as ceRNA.[17] Zhou *et al*[19] reported a basic role for circRNA33186 in OA development, thus providing a latent drug target for OA therapy. However, when it comes OA, relatively few reports have focused on the importance of circRNAs. Here, we reported that circPDE4B was the most highly expressed among differentially regulated circRNAs obtained through sequencing data. We also observed that circPDE4B is downregulated in chondrocytes treated with IL-1β, as well as the cartilage of OA mice induced by DMM. Further, circPDE4B was inversely related to cartilage degeneration, suggesting that circPDE4B is likely associated with OA development. Further functional experiments revealed that circPDE4B has a key role in OA progression and could represent a therapeutic target.

circRNAs reportedly function through three well-established mechanisms: (1) regulation of parental gene expression and splicing events; (2) complex formation within proteins to perform biological functions and (3) regulating gene expression via miRNA sponging.[24–26] Herein, we describe the potential mechanism by which circPDE4B can act as a scaffold for RIC8A–MID1 complex, thus promoting RIC8A ubiquitylation. Therefore, we have discovered a distinctive function through which circRNAs can modulate protein stability in OA.

As a guanine nucleotide exchange factor for G-protein alpha subunits, RIC8A was initially identified in *Caenorhabditis elegans*.[27] RIC8A has been described as an essential protein for G-protein signalling and in centrosome movements during early embryogenesis in *C. elegans*.[28–31] In mammals, RIC8A disruption in neural progenitors leads to germinal matrix haemorrhage,[32 33] suggesting that RIC8A activation may represent a key event in human OA pathogenesis. The role of RIC8A in OA, however, remains unclear. Herein, we found that RIC8A plays an important role in OA pathogenesis by regulating p38 MAPK signalling. Previous reports have indicated that the activation of p38, ERK and JNK signalling pathways is strongly correlated with OA cartilage damage.[34–36] Moreover, MAPKs serve as pivotal signalling molecules that participate in the production of matrix metalloproteinases and regulate viability and differentiation of chondrocytes.[37] Hence, considering that circPDE4B was found to function through the RIC8A–p38 axis, disruption of this pathway may cause dysregulation of cartilage homeostasis.

Post-translational modifications are associated with disease development and may influence protein function, immunogenicity and subcellular localisation.[38–41] Ubiquitylation, a major post-translational modification, plays an important role in signal transduction, apoptosis and cell proliferation.[42–44] Herein, we demonstrated that circPDE4B could disrupt the protein stability of RIC8A and possibly functions by regulating RIC8A post-translational modification. However, such modifications of RIC8A have not been previously reported. Interestingly, we found that circPDE4B also binds to an E3 ligand protein MID1 and RIC8A is ubiquitylated by MID1, thus we infer that circPDE4B promotes RIC8A ubiquitylation by acting as a scaffold to facilitate MID1 binding to RIC8A. Besides, employing acetyl-deficient (K→R) mutants, K415 was identified as a major ubiquitylation site of RIC8A and circPDE4B overexpression or inhibition had no effect on RIC8A K415R ubiquitination.

In summary, our research describes a new circRNA mechanism in OA. We demonstrated that circPDE4B could function as a scaffold for protein degradation and play a crucial role in the progression of OA. circPDE4B was found to regulate ECM metabolism and prevent cartilage matrix construction, validating its latent therapeutic influence on OA development in preclinical animal models. Mechanistically, circPDE4B served as a scaffold to facilitate RIC8A–MID1 binding which decreased RIC8A-dependent activation of p38 signal pathway, thus regulating OA progression. Cumulatively, the results of this study provide prospects for developing novel OA therapies by focusing on reducing the imbalance between matrix synthesis and degradation.

Correction notice This article has been corrected since it published Online First. Figure 4 has been replaced.

Contributors SS and XF conceived and designed the project. SS, YY, PS and JM performed all cell and animal experiments. SS, YY and BF collected the specimens and data from humans and mice. QW, KW and PS graded the histological changes. SF, XF and SS supervised the project and wrote the manuscript.

Funding The study was supported by the National Natural Science Foundation of China (grant numbers 81871797, 81472064,81873985, 81874015 and 81601925); Key Project of Zhejiang Medical Science and Technology Plan (grant numbers 2015145597 and 2016145597); Key Project of Zhejiang Provincial Natural Science Foundation (grant number LZ15H06002) and Medical Science and Technology Project of Zhejiang Province (grant number 2017179447). No benefits in any form have been, or will be, received from a commercial party related directly, or indirectly, to the subject of this study.

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval All experimental procedures involving mice were conducted with the approval of the Zhejiang University Ethics Committee. Cartilage samples were collected following informed written patient consent with approval from Sir Run Run Shaw Hospital Ethics Committee.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available in a public, open access repository. All data relevant to the study are included in the article or uploaded as supplementary information.

ORCID iDs
Shunwu Fan http://orcid.org/0000-0002-8785-0280
Xiangqian Fang http://orcid.org/0000-0003-4500-8914

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EPIDEMIOLOGICAL SCIENCE

Variants in urate transporters, ADH1B, GCKR and MEPE genes associate with transition from asymptomatic hyperuricaemia to gout: results of the first gout versus asymptomatic hyperuricaemia GWAS in Caucasians using data from the UK Biobank

Gabriela Sandoval-Plata [1,2,3] Kevin Morgan,[3] Abhishek Abhishek [1,2]

Handling editor Josef S Smolen

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2020-219796).

[1]Academic Rheumatology, University of Nottingham, Nottingham, UK
[2]Nottingham Biomedical Research Centre, NIHR, Nottingham, UK
[3]Human Genetics, School of Life Sciences, University of Nottingham, Nottingham, UK

Correspondence to
Gabriela Sandoval-Plata, University of Nottingham, Nottingham, UK; mbxgs2@nottingham.ac.uk

Received 24 December 2020
Revised 22 March 2021
Accepted 23 March 2021
Published Online First 8 April 2021

ABSTRACT

Objectives To perform a genome-wide association study (GWAS) of gout cases versus asymptomatic hyperuricaemia (AH) controls, and gout cases versus normouricaemia controls, and to generate a polygenic risk score (PRS) to determine gout-case versus AH-control status.

Methods Gout cases and AH controls (serum urate (SU) ≥6.0 mg/dL) from the UK Biobank were divided into discovery (4934 cases, 56 948 controls) and replication (2115 cases, 24 406 controls) cohorts. GWAS was conducted and PRS generated using summary statistics in discovery cohort as the base dataset and the replication cohort as the target dataset. The predictive ability of the model was evaluated. GWAS were performed to identify variants associated with gout compared with normouricaemic controls using SU <6.0 mg/dL and <7.0 mg/dL thresholds, respectively.

Results Thirteen independent single nucleotide polymorphisms (SNPs) in ABCG2, SLC2A9, SLC22A11, GCKR, MEPE, PPM1K-DT, LOC105377323 and ADH1B reached genome-wide significance and replicated as predictors of AH to gout transition. Twelve of 13 associations were novel for this transition, and rs1229984 (ADH1B) was identified as GWAS locus for gout for the first time. The best PRS model was generated from association data of 17 SNPs; and had predictive ability of 58.5% that increased to 69.2% on including demographic factors. Two novel SNPs rs760077(MTX1) and rs3800307(PRSS16) achieved GWAS significance for association with gout compared with normouricaemic controls using both SU thresholds.

Conclusion The association of urate transporters with gout supports the central role of hyperuricaemia in its pathogenesis. Larger GWAS are required to identify if variants in inflammatory pathways contribute to progression from AH to gout.

Key messages

What is already known about this subject?

- Previous genome-wide association study (GWAS) identified loci in inflammatory genes (CNTN-5, ZNF724 and MIR302F) as risk factors for transition from asymptomatic hyperuricaemia (AH) to gout, and was conducted in Japanese population.

What does this study add?

- This is the largest GWAS of gout cases and AH controls, and the first in Caucasian population.
- Thirteen variants in urate transporters and metabolic genes, but none in inflammatory genes associated with transition from AH to gout.
- A novel GWAS-significant gout risk locus was identified in ADH1B gene.
- Genetic and demographic factors performed moderately well in predicting gout status in AH.

How might this impact on clinical practice or future developments?

- Adults with AH should be advised lifestyle and dietary interventions that lower their serum urate levels in order to reduce their risk of gout.

INTRODUCTION

Gout is a common form of inflammatory arthritis caused by the deposition of monosodium urate (MSU) crystals. Elevated serum urate (SU) concentration is the precursor to MSU crystal deposition, and the onset of gout.[1] However, the majority of people with hyperuricaemia do not develop gout. For instance, in the USA, the prevalence of hyperuricaemia (defined as SU >7.0 mg/dL) is 20%, while that of gout is 3.9%.[2] The reason(s) why only some people with hyperuricaemia develop gout is poorly understood. Genome-wide association studies (GWAS) have improved the understanding of the pathophysiology of hyperuricaemia and gout over the last 10–15 years. For instance, genetic variants located in urate transporters such as the ABCG2, SLC2A9 and SLC22A11 genes have been identified as risk loci for both hyperuricaemia and gout.[3–6] Additional genetic variants such as GCKR and ALDH2 that play important roles in carbohydrate and alcohol metabolism respectively have been associated with both phenotypes.[5 7–9] However, the genetic variants associated with progression from hyperuricaemia to gout remain poorly understood. To date, only a single GWAS (n=6009 Japanese adults, 2860 with gout) has examined this and revealed two novel loci: CNTN5 and MIR302F, which participate in immune and inflammatory

To cite: Sandoval-Plata G, Morgan K, Abhishek A. *Ann Rheum Dis* 2021;**80**:1220–1226.

responses.[10] However, the identified polymorphism in CNTN5 is intronic while the SNP near MIR302F is intergenic. Further analyses in independent populations and larger sample sizes are needed to improve the understanding of the molecular mechanisms involved in transitioning from asymptomatic hyperuricaemia (AH) to gout.

Thus, the purpose of this study was to examine the genetic variants associated with transition from hyperuricaemia to gout. In order to meet this objective, we performed a GWAS using gout cases and (1) AH controls (SU ≥6.0 mg/dL), (2) normouricaemia controls with SU <6.0 mg/dL and (3) normouricaemia controls with SU <7.0 mg/dL derived from the UK Biobank resource. Genotype data were used to develop a polygenic risk score (PRS) to predict gout-case and AH-control status. We chose a threshold of ≥6.0 mg/dL to define AH as the risk of incident gout increases above this SU level.[11]

METHODS

Data source

This study was conducted using data from the UK Biobank resource (project ID 45987). Briefly, the UK Biobank is a prospective study of ~500 000 participants, aged 40–60 years and recruited across England, Wales and Scotland between the years 2006 and 2010. Data were collected on lifestyle and sociodemographic information, cognitive function, health status and family medical history. Participants had standard physical and functional measurements, and provided blood samples for genetic analyses. Details about recruitment and samples processing for genotyping are described elsewhere.[12 13]

Subjects

For this research, three phenotypes were derived from the UK Biobank cohort.

Gout cases

Gout was defined as present if any of the following criteria were met[14]: self-reported physician-diagnosed gout; urate-lowering therapy (ULT) prescription without a hospital diagnosis of lymphoma or leukaemia (International Classification of Diseases (ICD)-10 codes C81-C96) or a primary or secondary diagnosis of gout in hospital discharge letters using the ICD-10 codes M10, M100-M14 and M109. Participants with self-reported physician-diagnosed gout were excluded if their SU was <6.0 mg/dL and they did not report prescription of ULT at the UK Biobank visit.

AH controls

Participants with SU ≥6.0 mg/dL and not classified as gout. A threshold of ≥6.0 mg/dL was chosen as it associates with incident gout in prospective studies.[11]

Normouricaemia controls

Participants with SU <6.0 mg/dL and not classified as gout[14] were considered as normouricaemia controls. Given the uncertainty around definition of normal SU, for example, SU <6.0 mg/dL being the treatment threshold for treat-to-target ULT while epidemiological studies use a cut-off of <7.0 mg/dL, another group of normouricaemia controls was ascertained with SU <7.0 mg/dL and not classified as gout.[14]

Genotyping and quality control

UK Biobank samples were genotyped by Affymetrix using two arrays: The UK BiLEVE Axiom array (n=49 950; 807 411 markers), and the UK Biobank Axiom array (n=438 427; 825 927 markers). These arrays shared 95% of content, resulting in >805 000 genotyped variants for 488 288 participants. For this study, participants with non-European ancestry were excluded to avoid population stratification. Thus, genotyping data for 409 629 European descendants were available following UK Biobank centrally performed quality control (QC) procedures. Detailed information about genotyping and QC have been described previously.[13 15] Further stringent QC filters were applied using PLINK V.1.9.[16] Individuals with a kinship coefficient equivalent to second-degree (or greater) relatives were excluded. Individuals were also excluded if they had a heterozygosity ±3 SD from the mean, a call rate <90% or were identified as gender mismatches. Markers with a call rate <95%, or those deviating from Hardy-Weinberg equilibrium (Bonferroni-corrected p value threshold=6.82×10^{-8}) were removed from the dataset.

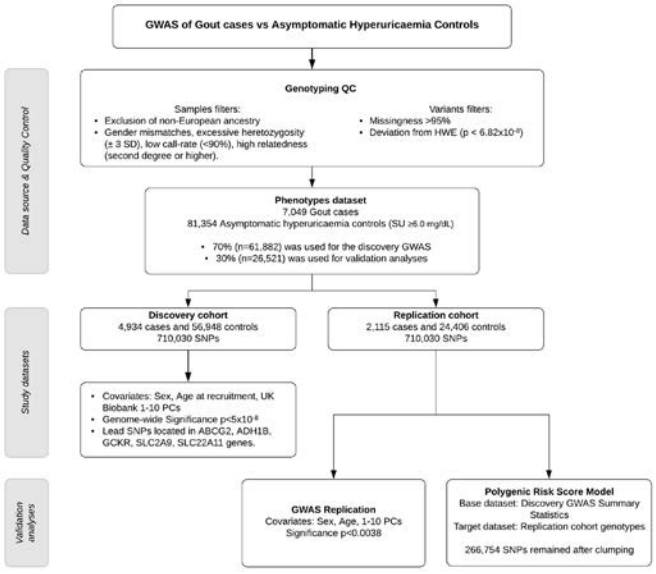

Figure 1 Study design. Workflow for the discovery and replication analyses. GWAS, genome-wide association study; HWE, Hardy-Weinberg equilibrium; PC, principal component; QC, quality control; SNPs, single nucleotide polymorphisms.

Gout versus AH

A sample of 354 825 individuals with 717 091 genotyped SNPs were included in this analysis, from which the phenotypes of interest were derived. The cohort comprised 7049 cases and 81 354 controls that were divided into two datasets: 70% (n=61 882) was used as the discovery dataset, and the remaining 30% (n=26 521) was used as the replication dataset (figure 1).

Gout versus normouricaemia

Two separate GWAS were conducted, using gout cases and 64 424 controls with SU <6.0 mg/dL, and 79 531 controls with SU <7.0 mg/dL, respectively.

Statistical analyses

Baseline data were summarised using mean (SD) for continuous variables, and number (%) for categorical variables. Independent sample t-test and χ^2 test were used to compare continuous and categorical data, respectively.

Gout versus AH GWAS

Discovery and replication association tests were performed using PLINK V.1.9. ORs and 95% CIs were computed using additive logistic regression. We adjusted for sex, age at recruitment and 10 principal components (PCs). To determine the number of independent loci from the GWAS analysis, linkage disequilibrium (LD) clumping was performed using PLINK V.1.9. SNPs with a p value $<1\times10^{-5}$, $r^2>0.1$ and a 500 kb window from the index SNPs were assigned to the clump. Annotation of lead SNPs was conducted using the SNP2GENE tool of the Functional Mapping and Annotation of GWAS.[17] Pairwise LD patterns from SNPs identified as independent, located in the same gene or <500 kb apart, were further analysed using the R package LDlinkR,[18 19] which uses the 1000 Genomes Project data as the reference panel. HaploView was used to generate the LD plot.[20] For the discovery analysis, genome-wide significance was set at $p=5\times10^{-8}$.

For replication analysis, the 13 variants that reached genome-wide significance in the discovery analysis were tested for association with gout in the replication cohort. Logistic regression was adjusted for sex, age and 10 PCs. A Bonferroni-corrected p value of <0.004 (0.05/13) was used to determine significant associations in the replication analysis. The results from the discovery GWAS and the replication analysis were combined by meta-analysis using PLINK. The fixed-effects model was used to estimate pooled ORs and 95% CI, and Cochran's Q test p values and I^2 values were used to assess heterogeneity.

Linear regression was used to examine the effect of GWAS hits on SU levels. This was performed using the full cohort, and adjusted for sex and age at recruitment. Beta-coefficients and SEs, and adjusted beta-coefficients and SEs were calculated. As previous GWAS[10 21] have used a cut-off of 7.0 mg/dL to define hyperuricaemia, a sensitivity analysis was conducted to evaluate if the association of GWAS hits and gout remained significant if controls had a SU ≥7.0 mg/dL.

Polygenic risk score

PRS was calculated using PRSice-2.[22] The discovery GWAS summary statistics were used as the base dataset, while the replication cohort genotype-phenotype data were used as the target dataset. Clumping parameters in PRSice were set to an $r^2>0.1$ and a 500 kb window from the index SNPs, which generated a final number of 266 754 SNPs available for PRS calculation. ORs and p values from the GWAS summary statistics were used to calculate the best PRS model, which was generated from testing different p value thresholds. The best-fit model was defined by the largest Nagelkerke's R^2 value. Logistic regression was used to estimate the effect of the demographic variables for inclusion into the predictive models using SPSS Statistics 24. The area under the receiving operatic characteristic curve (AUROC) was used to evaluate the predictive ability of the PRS, demographic characteristics (age, sex and body mass index (BMI)) and combined models.

Gout versus normouricaemia GWAS

Two GWAS were conducted. Prior to conducting these analyses, both datasets underwent the same genotyping QC filters as described earlier. The association tests were performed with PLINK V.1.9, using age, sex and the first 10 PCs as covariates.

RESULTS

Demographic characteristics

Following genotype QC filters, data for 7049 gout cases and 81 354 AH controls were included. The entire cohort comprised 80.77% men, and their mean (SD) age, BMI and SU were 57.87 (7.77) years, 29.63 (4.81) kg/m^2 and 6.92 (0.88) mg/dL, respectively. This cohort was divided into the discovery and replication datasets (table 1, figure 1). The two datasets had comparable disease and demographic characteristics.

Gout versus AH

GWAS

An additive logistic regression was performed to test the association between gout and 710 030 variants. Thirty-four SNPs reached genome-wide significance and after filtering for tight LD ($r^2<0.2$), 13 SNPs were identified as independent associations (figure 2). These lead SNPs were selected for the replication study, where they were tested for association with gout in the remaining 30% of the dataset. Successful replication was defined if the p value was <0.004. Summary results for both the discovery and the replication analyses are shown in table 2. The SNP with the greatest effect was rs2231142 in ABCG2 gene with OR=1.66 (2.05×10^{-78}) in the discovery stage, and OR=1.64 (1.17×10^{-32}) in the replication stage. This was followed by a novel locus: rs1229984 in ADH1B gene (OR=1.51, $p=5.00\times10^{-12}$; OR=1.44, $p=4.77\times10^{-5}$). The remaining SNPs were located in or near GCKR, PPM1K-DT, SLC2A9, MEPE, LOC105377323 and SLC22A11. Pairwise LD parameters were evaluated for SNPs located within the same gene or in genes <500 kb apart (online supplemental figure S1).

Genetic variants and SU

All lead SNPs associated with SU, with rs2231142 (ABCG2) and rs16890979 (SLC2A9) showing the greatest effects: adjusted β=0.107 and $p=1.21\times10^{-80}$, and adjusted β=−0.055 and $p=1.67\times10^{-43}$, respectively (online supplemental table S1). On sensitivity analysis examining the association between 13 lead SNPs and gout, excluding AH controls with SU <7.0 mg/dL, the ORs diminished in magnitude but remained significant (online supplemental table S2).

PRS model

A PRS for all cases and controls was constructed with PRSice using the replication cohort as the test dataset. The best-fit p value threshold that gave the highest Nagelkerke's R^2 (0.016) was 4.0×10^{-6}, and included 17 SNPs (online supplemental table S3). The mean (±SD) PRS for cases was 0.018 (±0.017), and 0.013 (±0.016) for controls (p<0.0001). The predictive ability of this PRS model was evaluated using the AUROC curve, and compared with the demographics model (age, sex and BMI) and combined model (age, sex, BMI and PRS). The AUC for each model was 58.5%, 66.7% and 69.2%, respectively (figure 3).

Gout versus normouricaemia

We conducted two GWAS of gout versus normouricaemia using SU cut-off values <6.0 mg/dL and <7.0 mg/dL, respectively. The first GWAS identified 52 lead SNPs, while the second identified 46 lead SNPs (online supplemental table S4). Three novel SNPs (rs760077, rs3800307 and rs11227299 in MTX1, PRSS16 and AP5B1 genes, respectively) associated with gout compared with SU <6 mg/dL with GWAS significance. Two (rs760077 and rs3800307) remained GWAS significant when a higher SU threshold of <7 mg/dL was used to define normouricaemia.

Table 1 Demographic, life-style and comorbidities for gout cases and asymptomatic hyperuricaemia controls of the UK Biobank

		Discovery GWAS		Replication stage	
	All (88 403)	Gout cases (4934)	Controls (56 948)	Gout cases 2115	Controls 24 406
Age at recruitment, years, mean (SD)	57.87 (7.77)	60.11 (6.88)	57.67 (7.82)	60.06 (6.81)	57.69 (7.80)
Male sex, n (%)	71 401 (80.77)	4529 (91.79)	45 506 (79.91)	1950 (92.20)	19 416 (79.55)
BMI, kg/m^2, mean (SD)	29.63 (4.81)	30.79 (4.96)	29.53 (4.78)	30.62 (4.93)	29.53 (4.78)
Waist circumference, cm, mean (SD)	99.56 (11.74)	103.76 (12.14)	99.23 (11.64)	103.49 (12.12)	99.16 (11.64)
SU, mg/dL, mean (SD)	6.92 (0.88)	6.74 (1.78)	6.94 (0.76)	6.77 (1.74)	6.93 (0.75)
Alcohol intake, n (%)*					
Never	4397 (4.97)	221 (4.48)	2822 (4.96)	85 (4.02)	1269 (5.20)
Special occasions	6698 (7.58)	272 (5.51)	4405 (7.74)	115 (5.44)	1906 (7.81)
<1/week	7588 (8.58)	278 (5.63)	5006 (8.79)	128 (6.05)	2176 (8.92)
1–2/week	21 908 (24.78)	1093 (22.15)	14 190 (24.92)	496 (23.45)	6129 (25.11)
3–4/week	23 370 (26.44)	1351 (27.38)	15 035 (26.40)	589 (27.85)	6395 (26.20)
Daily or almost daily	24 365 (27.56)	1710 (34.66)	15 448 (27.13)	701 (33.14)	6506 (26.66)
Smoking status, n (%)*					
Non-smoker	41 778 (47.26)	1998 (40.49)	27 243 (47.84)	862 (40.76)	11 675 (47.84)
Ex-smoker	37 705 (42.65)	2481 (50.28)	23 871 (41.92)	1065 (50.35)	10 288 (42.15)
Current smoker	8590 (9.72)	434 (8.80)	5613 (9.86)	183 (8.65)	2360 (9.67)
Comorbidities, n (%)†					
Metabolic syndrome, n (%)	40 049 (45.30)	2999 (60.78)	24 900 (43.72)	1325 (62.65)	10 825 (44.35)
Diabetes mellitus	5389 (6.10)	589 (11.94)	3205 (5.63)	245 (11.58)	1350 (5.53)
Hypertension	35 776 (40.47)	2824 (57.24)	22 206 (38.99)	1241 (58.68)	9505 (38.95)
Hypercholesterolaemia	15 831 (17.91)	1368 (27.73)	9737 (17.10)	585 (27.66)	4141 (16.97)
Ischaemic heart disease	6817 (7.71)	666 (13.50)	4051 (7.11)	290 (13.71)	1810 (7.42)
Cardiac failure	133 (0.15)	28 (0.57)	62 (0.11)	18 (0.85)	25 (0.10)
CKD stages*					
G1 (>90 mL/min)	39 800 (45.02)	1962 (39.76)	25 849 (45.39)	855 (40.43)	11 134 (45.62)
G2 (60–90 mL/min)	43 774 (49.52)	2434 (49.33)	28 260 (49.62)	1026 (48.51)	12 054 (49.39)
G3a (45–59 mL/min)	3722 (4.21)	343 (6.95)	2258 (3.97)	159 (7.52)	962 (3.94)
G3b (30–44 mL/min)	792 (0.89)	138 (2.80)	419 (0.74)	40 (1.89)	195 (0.80)
G4 (15–29 mL/min)	213 (0.24)	41 (0.83)	106 (0.19)	22 (1.04)	44 (0.18)
G5 (<15 mL/min)	51 (0.06)	13 (0.26)	19 (0.03)	12 (0.57)	7 (0.03)

*The following data were missing: alcohol intake for 0.09%, smoking satus for 0.73% and CKD information for 0.06%.
†Diabetes, hypertension, hypercholesterolemia,ischaemic heart disease and cardiac failure were defined as present if they were self-reported as diagnosed by a doctor. Chronic Kidney Disease stages were defined as per the National Institute of Heath and Care Excellence (NICE) guidelines CG182.[43] Metabolic syndrome was calculated as recommended by the International Diabetes Federationin 2006.[44]
BMI, body mass index; CKD, chronic kidney disease; GWAS, genome-wide association study; SU, serum urate.

We then plotted the OR of each lead SNP in the gout versus AH-control GWAS and the gout versus SU <6 mg/dL GWAS (figure 4A). To be consistent with our sensitivity analysis, we plotted the OR of the 13 lead SNPs on excluding AH controls with SU 6–7 mg/dL, with the gout versus SU <7 mg/dL GWAS (figure 4B). The same loci were responsible for transition from AH and normouricaemia to gout.

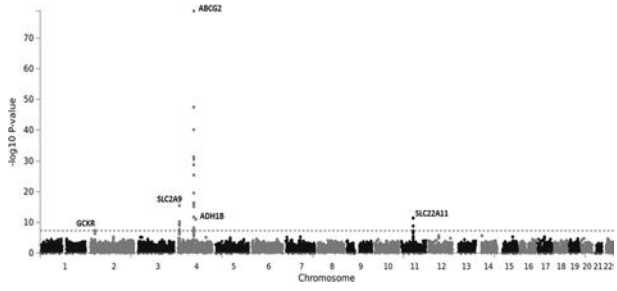

Figure 2 Manhattan plot of the discovery genome-wide association study of gout versus asymptomatic hyperuricaemia (serum urate ≥6.0 mg/dL) controls. The y-axis shows –log10 p values ordered by chromosomal position on the x-axis. The horizontal dashed line represents genome-wide significance threshold (5.0×10^{-8}).

DISCUSSION

This is the largest GWAS to date and the first in Caucasians to examine the SNPs associated with transition from AH to gout. Using UK Biobank data, it identified 13 independent SNPs from 8 loci that reached genome wide significance for association with gout versus AH, and replicated. These loci include urate transporters, metabolic pathway genes (eg, GCKR, ADH1B) and *MEPE* gene that regulates renal phosphate handling and skeletal mineralisation.[23] The latter may promote progression to gout via pro-mineralising osteopontin-like function or via low phosphate levels that associates with incident hyperuricaemia.[24] The identified loci in PPM1K-DT and LOC105377323 were in non-coding regions and their molecular mechanism is unclear.

Of the eight loci, ABCG2, SLC2A9, SLAC22A11, PPM1K-DT, GCKR and MEPE have previously been associated with gout or SU levels in different populations but never in the transition from AH to gout in a GWAS.[5 21 25 26] This is the first such report. In a previous study, Tin *et al* generated a genetic risk score using variants associated with SU and examined their ability to predict gout cases in 334 800 UK Biobank participants not specifically selected for high SU levels. Ours is the first study to attempt to generate a PRS for predicting gout status in an AH population that is, those with SU ≥6.0 mg/dL, and reports an AUC of

Table 2 Summary of GWAS and replication analysis of 13 lead SNPs in gout cases and AH controls (SU ≥6 mg/dL)

SNP	Chr	bp	Gene	A1	Freq	Discovery GWAS		Replication stage		Meta-analysis*			
						aOR (95% CI)†	P value	aOR (95% CI)†	P value	aOR (95% CI)†	P value	Cochrane's Q p value	I^2
rs1260326	2	27 730 940	GCKR	T	0.41	1.13 (1.08 to 1.17)	3.54×10^{-08}	1.15 (1.08 to 1.23)	2.54×10^{-05}	1.14 (1.10 to 1.18)	5.10×10^{-12}	0.61	0
rs2231142	4	89 052 323	ABCG2	T	0.14	1.66 (1.58 to 1.76)	2.05×10^{-78}	1.64 (1.51 to 1.78)	1.17×10^{-32}	1.65 (1.58 to 1.73)	3.33×10^{-109}	0.75	0
rs13120400	4	89 033 527	ABCG2	C	0.28	0.82 (0.78 to 0.86)	1.56×10^{-16}	0.84 (0.78 to 0.91)	3.43×10^{-06}	0.83 (0.79 to 0.86)	3.66×10^{-21}	0.50	0
rs7672194	4	89 126 647	ABCG2	T	0.48	1.16 (1.11 to 1.21)	3.21×10^{-12}	1.15 (1.08 to 1.23)	1.13×10^{-05}	1.16 (1.12 to 1.20)	1.58×10^{-16}	0.88	0
rs4693211	4	89 249 061	PPM1K-DT	C	0.07	1.41 (1.31 to 1.52)	6.97×10^{-20}	1.26 (1.12 to 1.42)	1.21×10^{-04}	1.37 (1.28 to 1.45)	1.55×10^{-22}	0.11	61.62
rs28793136	4	89 216 768	PPM1K-DT	C	0.08	1.35 (1.26 to 1.45)	8.19×10^{-17}	1.26 (1.12 to 1.40)	6.29×10^{-05}	1.32 (1.25 to 1.40)	4.79×10^{-20}	0.27	19.60
rs1545207	4	89 239 492	PPM1K-DT	A	0.28	1.14 (1.09 to 1.20)	6.82×10^{-09}	1.12 (1.05 to 1.20)	1.07×10^{-03}	1.14 (1.09 to 1.18)	3.38×10^{-11}	0.66	0
rs16890979	4	9 922 167	SLC2A9	T	0.17	0.79 (0.74 to 0.83)	3.19×10^{-16}	0.73 (0.67 to 0.80)	1.05×10^{-11}	0.77 (0.74 to 0.81)	5.45×10^{-26}	0.19	42.09
rs16891234	4	9 946 163	SLC2A9	C	0.24	1.16 (1.11 to 1.22)	6.06×10^{-10}	1.13 (1.05 to 1.22)	8.86×10^{-04}	1.15 (1.11 to 1.20)	2.72×10^{-12}	0.57	0
rs1229984	4	100 239 319	ADH1B	T	0.03	1.51 (1.34 to 1.69)	5.00×10^{-12}	1.44 (1.21 to 1.72)	4.77×10^{-05}	1.49 (1.35 to 1.64)	1.15×10^{-15}	0.68	0
rs114791459	4	88 591 554	LOC105377323	A	0.02	1.42 (1.26 to 1.60)	7.99×10^{-09}	1.47 (1.22 to 1.77)	5.47×10^{-05}	1.43 (1.30 to 1.59)	2.01×10^{-12}	0.76	0
rs114580333	4	88 790 118	MEPE	A	0.02	1.44 (1.26 to 1.63)	3.01×10^{-08}	1.39 (1.15 to 1.69)	9.18×10^{-04}	1.42 (1.28 to 1.59)	1.10×10^{-10}	0.79	0
rs2078267	11	64 334 114	SLC22A11	C	0.47	1.16 (1.11 to 1.21)	1.72×10^{-12}	1.14 (1.07 to 1.22)	6.27×10^{-05}	1.15 (1.11 to 1.20)	6.65×10^{-16}	0.62	0

*Fixed-effects meta-analysis of the discovery GWAS and the replication analysis.
†Adjusted for age, sex and 10 first PCs.
A1, allele 1/effect allele; aOR, adjusted OR; bp, base pair position; Chr, chromosome; Freq, frequency; GWAS, genome-wide association study; PC, principal component; SNP, single nucleotide polymorphism; SU, serum urate.

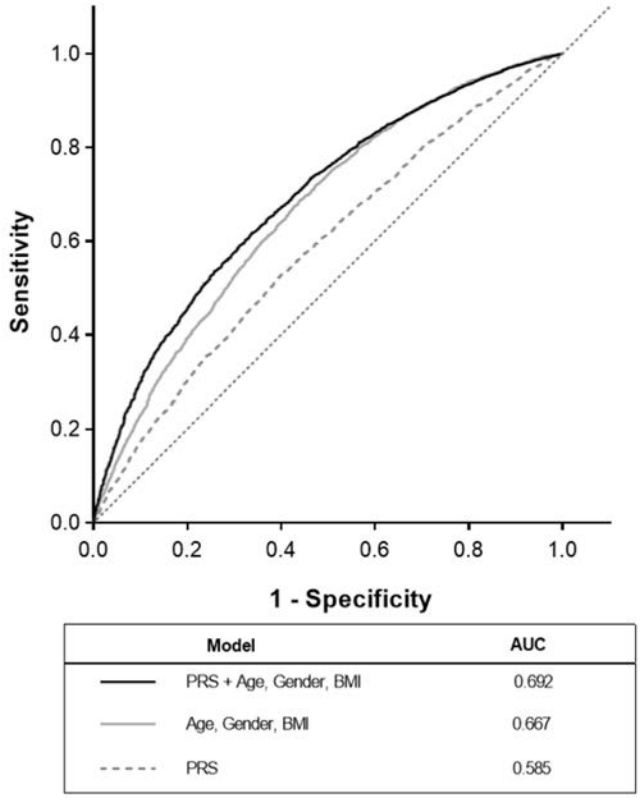

Figure 3 Area under the receiver operating characteristics (AUROC) curve for the polygenic risk score (PRS) model, demographics model and combined (demographics+PRS) model. BMI, body mass index.

58.5% for genetic factors alone, which increased to just under 70% when demographic factors were added. This is lower than the AUC of 67.2% from genetic factors alone in the study by Tin *et al* and is likely to be due to lower genetic variance due to selection of a high SU control group.[25] A smaller study using

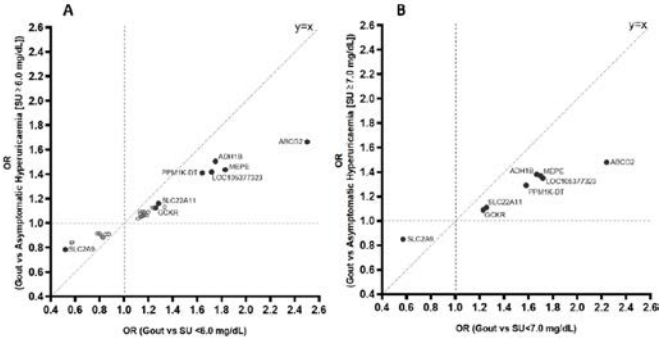

Figure 4 Scatter plot. (A) Comparison of the ORs of lead single nucleotide polymorphisms (SNPs) for both genome-wide association study (GWAS): gout versus asymptomatic hyperuricaemia (serum urate (SU) ≥6.0 mg/dL) and gout versus SU <6.0 mg/dL. Black dots represent ORs of the common risk loci of both GWAS, while grey circles represent ORs of additional lead SNPs of the gout versus SU <6.0 mg/dL that were not significant at GWAS level in the gout versus asymptomatic hyperuricaemia GWAS. (B) Comparison of the ORs of the 13 lead SNPs for gout versus asymptomatic hyperuricaemia (defined as SU ≥7.0 mg/dL), compared with the ORs in the GWAS for gout versus SU <7.0 mg/dL. Where several SNPs were present in the same gene, only that with the smallest p value was plotted in this graph. See online supplemental material for names of genes not annotated in the figure.

candidate gene hypothesis reported nominal association for ABCG2 polymorphism and gout versus hyperuricaemia.[27] The only previous gout versus hyperuricaemia GWAS was conducted in a Japanese population and reported rs7927466 in CNTN5, rs9952962 in MIR302F and a suggestive locus rs12980365 in ZNF724 that do not affect SU.[10] Although rs7927466 is not included in the UK Biobank genotype platform, it is covered by its proxy SNP rs7942264 ($r^2=1$) that did not show an association with gout; neither did rs12980365. MIR302F was not included in UK Biobank and further research on this gene is needed.

ADH1B was identified as a risk variant for gout versus AH. It has never previously been associated with gout in a GWAS— even when compared with general population. ADH1B mediates the oxidation of ethanol into acetaldehyde.[28] The SNP rs1229984 in ADH1B causes a change of an arginine to histidine, increases ethanol clearance in liver, facilitates its conversion to highly reactive acetaldehyde,[29] increases the NADH/NAD ratio that results in high lactic acid levels and increased urate reabsorption via URAT1.[30] The risk allele of rs1229984 also promotes a 'flush response' to alcohol and reduces the amount of alcohol consumed.[31] Thus, the association between this polymorphism and gout may be due to increased production and reabsorption of urate from per unit alcohol consumed. This is consistent with the observation by Yokoyama *et al* in which rs1229984 associated with SU≥7 mg/dL (OR (95% CI) 2.04 (1.58 to 2.65)), while the daily alcohol intake was comparable across variants.[32] In agreement with our study, Sakiyama *et al* (n=1048 gout cases and 1334 male controls) evaluated the effect of rs1229984 in ADH1B gene on gout. They reported an increased risk for gout with OR of 1.69 and 1.80 for His/Arg and His/His genotypes, respectively, which remained significant after correcting for alcohol consumption.[33] However, in their study patients with gout and rare variants of the SNP had greater alcohol consumption, suggesting an additional role for the latter.

Urate transporters ABCG2, SLC2A9 and SLC22A11 play essential roles in pathogenesis of hyperuricaemia.[34 35] SLC2A9 has the strongest effect on SU, accounting for 2%–3% of variance, followed by ABCG2 that explains 1% of SU variation.[35] Although both loci have also been associated with gout, GWAS of gout cases versus controls have shown a greater effect of ABCG2 than SLC2A9.[7] In this study, rs2231142 in ABCG2 had larger effect size on gout status compared with AH-control than that of rs16890979 in SLC2A9 (which is in tight LD with the GWAS hit rs12498742) and also twice as much effect on SU than the latter. This supports the hypothesis that ABCG2 plays a causal role in transition from hyperuricaemia to gout via its effect on SU. However, additional mechanisms such as defects in ABCG2 causing deficient autophagy may also operate.[27 36]

Our GWAS comparing gout cases with normouricaemic controls did not identify any inflammatory genes. A large number of lead SNPs were identified at genome-wide significance level. Most have been associated with gout or SU previously.[5 7 25 26 37] However, we identified three novel SNPs associated with gout compared with SU<6.0 mg/dL. Of these, rs11227299 (AP5B1) is associated with reduced estimated glomerular filtration rate and may cause gout by resulting hyperuricaemia.[38] The variants in MTX1 and PRSS16 genes associated with gout compared with SU<6.0 mg/dL, also associate with Parkinson's disease and schizophrenia.[39 40] This is consistent with the negative associations between Parkinson's disease and gout, and schizophrenia and elevated SU.[41 42]

This is the first GWAS to examine transition from AH to gout in Caucasians. Other strengths include a large sample size, and assessment of transition from AH or normouricaemia to gout in the same source population. However, there are several caveats to this study. First, gout definition was not based on American College of Rheumatology/European League Against Rheumatism classification criteria, but was ascertained via self-report of physician diagnosis, hospital diagnoses and ULT prescriptions. However, the UK Biobank data collection predates the classification criteria. In addition, the classification of AH controls was based on a single SU measurement, which could have been affected by diet during the previous days. Additionally, the use of non-imputed data limited the discovery power of the GWAS and PRS.

In conclusion, this study identified 13 GWAS significant risk loci, 12 of which have never previously been associated with the transition from AH to gout at GWAS level. The preponderance of urate transporters and metabolic genes that affect SU levels support the central role of hyperuricaemia in the pathogenesis of gout. Larger GWAS are required to identify if variants in inflammatory pathways also contribute to this transition.

Twitter Gabriela Sandoval-Plata @gabylusp

Acknowledgements This research was conducted using the UK Biobank resource (Project ID 45987). The authors would like to thank all participants.

Contributors Study concept and design: AA, GS-P, KM. Data analysis: GS-P. Manuscript preparation: GS-P, AA, KM. Manuscript revision for important intellectual content: all authors.

Funding Miss Sandoval-Plata's work is supported by a PhD scholarship from The Mexican National Council for Science and Technology (CONACYT) (Grant number 472 298).

Competing interests Professor AA has received departmental research grants from AstraZeneca and Oxford Immunotec, speaker bureau fees from Menarini, scientific meeting support from Pfizer, author royalties from UpToDate and Springer and has consulted for Inflazome unrelated to this work.

Patient consent for publication Not required.

Ethics approval UK Biobank has approval from the North West Multi-Centre Research Ethics Committee (REC reference 16/NW/0274). This study did not involve recontacting participants and no separate ethics approval was required.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data may be obtained from a third party and are not publicly available. Raw data used for this study are available from the UK Biobank resource.

ORCID iDs
Gabriela Sandoval-Plata http://orcid.org/0000-0002-7809-7864
Abhishek Abhishek http://orcid.org/0000-0003-0121-4919

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EPIDEMIOLOGICAL SCIENCE

Genome-wide association study identifies *RNF123* locus as associated with chronic widespread musculoskeletal pain

Md Shafiqur Rahman ,[1] Bendik S Winsvold,[2,3,4] Sergio O Chavez Chavez,[5] Sigrid Børte,[4,6,7] Yakov A Tsepilov,[8,9,10] Sodbo Zh Sharapov,[8,10] HUNT All-In Pain, Yurii S Aulchenko,[8,9] Knut Hagen,[11,12] Egil A Fors,[13] Kristian Hveem,[4,14] John Anker Zwart,[2,4,7] Joyce B van Meurs,[5] Maxim B Freidin,[1] Frances MK Williams[1]

Handling editor Josef S Smolen

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2020-219624).

For numbered affiliations see end of article.

Correspondence to
Professor Frances MK Williams, Twin Research and Genetic Epidemiology Depart, King's College London, London, London, UK;
frances.williams@kcl.ac.uk

Received 30 November 2020
Revised 23 February 2021
Accepted 25 February 2021
Published Online First 29 April 2021

ABSTRACT

Background and objectives Chronic widespread musculoskeletal pain (CWP) is a symptom of fibromyalgia and a complex trait with poorly understood pathogenesis. CWP is heritable (48%–54%), but its genetic architecture is unknown and candidate gene studies have produced inconsistent results. We conducted a genome-wide association study to get insight into the genetic background of CWP.

Methods Northern Europeans from UK Biobank comprising 6914 cases reporting pain all over the body lasting >3 months and 242 929 controls were studied. Replication of three independent genome-wide significant single nucleotide polymorphisms was attempted in six independent European cohorts (n=43 080; cases=14 177). Genetic correlations with risk factors, tissue specificity and colocalisation were examined.

Results Three genome-wide significant loci were identified (*rs1491985, rs10490825, rs165599*) residing within the genes *Ring Finger Protein 123* (*RNF123*), *ATPase secretory pathway Ca^{2+} transporting 1* (*ATP2C1*) and *catechol-O-methyltransferase* (*COMT*). The *RNF123* locus was replicated (meta-analysis p=0.0002), the *ATP2C1* locus showed suggestive association (p=0.0227) and the *COMT* locus was not replicated. Partial genetic correlation between CWP and depressive symptoms, body mass index, age of first birth and years of schooling were identified. Tissue specificity and colocalisation analysis highlight the relevance of skeletal muscle in CWP.

Conclusions We report a novel association of *RNF123* locus and a suggestive association of *ATP2C1* locus with CWP. Both loci are consistent with a role of calcium regulation in CWP. The association with *COMT*, one of the most studied genes in chronic pain field, was not confirmed in the replication analysis.

Key messages

What is already known about this subject?
- Chronic widespread musculoskeletal pain (CWP) is a primary diagnostic feature of fibromyalgia.
- CWP is moderately heritable, but precise genes involved in the pathogenesis of CWP are yet to be identified.

What does this study add?
- This is the largest genetic study conducted on CWP to date and identified novel genetic risk loci (*Ring Finger Protein 123* and *ATPase secretory pathway Ca^{2+} transporting 1*).
- The genetic signal points to peripheral pain mechanisms in CWP, and shows genetic correlation with other traits, including body mass index and depression.

How might this impact on clinical practice or future developments?
- The findings add to aetiological basis of CWP.

INTRODUCTION

Chronic widespread musculoskeletal pain (CWP) is a common complex trait influenced by genetic and environmental factors, most of which have yet to be determined.[1] CWP and fibromyalgia syndrome are sometimes used interchangeably, although the latter is generally more severe and includes other features such as sleep disturbance, fatigue and depression.[2] It is thought to represent a subgroup at the more severe end of the spectrum of CWP.[3] The prevalence of CWP is 10.6% in the world population and 14.2% in the UK population.[4 5] It is associated with high societal cost.[6] CWP is responsible for excess mortality,[7] which is thought to be attributable to cardiovascular disease, respiratory disease and cancer. Females are more affected by CWP than males,[4] and the prevalence rises with age.[5] In addition to age and sex, a number of exposures have been proposed as risk factors for CWP,[8 9] but only increased body mass index (BMI) has been consistently reported across studies, including longitudinal studies.[10–12]

Broad-sense heritability estimates for CWP range between 48% and 54%, indicating a substantial genetic contribution.[13] To date, the candidate gene approach has been extensively applied to identify genetic factors in CWP,[14] but few agnostic studies have been published.[15] The only genome-wide association study (GWAS) meta-analysis combining 14 studies identified a locus lying on chromosome 5 intergenic to *CCT5* and *FAM173B*.[15] *CCT5* has previously been implicated in neuropathy[16] and

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To cite: Rahman MS, Winsvold BS, Chavez Chavez SO, *et al*. *Ann Rheum Dis* 2021;**80**:1227–1235.

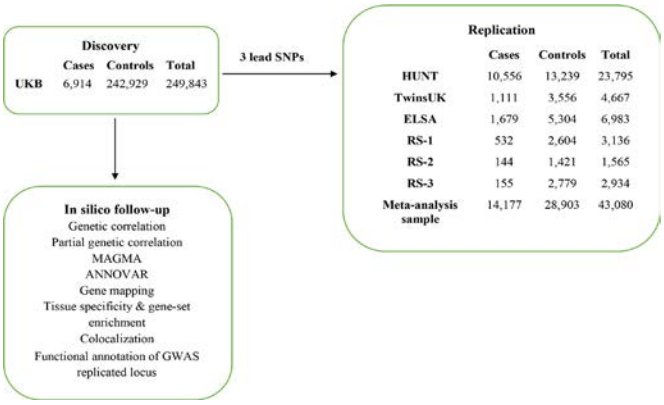

Figure 1 Overview of study design.

there is increasing evidence that small fibre neuropathy underlies a subset of fibromyalgia.[17]

Genetic factors are known to be shared by chronic pain conditions.[18 19] One of the most extensively studied chronic pain-associated genes encodes *catechol-O-methyltransferase* (*COMT*), an enzyme which regulates the production of catecholamines that act as neurotransmitters in the central nervous system (CNS) pain tract. A non-synonymous change of A to G encoding a valine (Val) to methionine (Met) substitution at codon 158 (*Val158Met*; *rs4680*) reduces the enzymatic activity of *COMT*. This single nucleotide polymorphism (SNP) has been reported to be associated with CWP in a small study of 122 participants,[20] but a subsequent association study of 3017 participants did not confirm earlier findings.[21] An inconclusive role of *COMT* was observed for temporomandibular disorders (TMD) as well.[22 23] Further investigation is required to identify genetic variants underlying CWP, which will shed light on the pathophysiological mechanisms underlying the development of chronic pain and may reveal therapeutic targets.

MATERIALS AND METHODS

An overview of study design is presented in figure 1.

Participant selection

For the discovery analysis, we performed a GWAS of CWP using UK Biobank (UKB) comprising 249 843 participants of European descent (6914 CWP cases and 242 929 controls). Independent SNPs passing a threshold p<5.0E-08 were submitted for replication in 43 080 individuals of European ancestry (14 177 CWP cases and 28 903 controls) from six independent cohorts originating in the UK (TwinsUK and The English Longitudinal Study of Ageing (ELSA)), the Netherlands (The Rotterdam Study 1, 2 and 3 (RS-1, RS-2 and RS-3)) and Norway (The Nord-Trøndelag Health Survey (HUNT)). The UKB dataset was used under project #18219. Description of each study cohort is presented in online supplemental text.

Phenotype

In UKB, CWP cases were defined by combining self-reported diagnosis of pain all over the body lasting for >3 months; simultaneous pain in the knee, shoulder, hip and back lasting 3+ months and fibromyalgia. Controls comprised those who reported no pain in the last month or reported pain all over the body in the previous month that did not last for 3 months or reported only ≥3 months of non-musculoskeletal pain (headache, facial and abdominal pain). Those reporting a self-reported diagnosis of rheumatoid arthritis, polymyalgia rheumatica, arthritis not otherwise specified, systemic lupus erythematosus, ankylosing spondylitis and myopathy were excluded from the study (online supplemental figure S1). Further phenotype details for UKB and replication cohorts are provided in online supplemental text.

Table 1 Sample characteristics stratified by case/control status for discovery and replication cohorts

	Cases	Controls	P value
Discovery cohort (UK Biobank)			
Female	4470 (64.7%)	128 599 (47.1%)	<0.0001
Male	2444 (35.3%)	114 330 (52.9%)	
Age (mean±SD)	57.8±7.45	57.0±8.09	<0.0001
BMI (mean±SD)	30.02±5.97	26.83±4.40	<0.0001
Replication cohorts			
TwinsUK			
Female	1041 (93.7%)	3116 (87.6%)	<0.0001
Male	70 (6.3%)	440 (12.4%)	
Age (mean±SD)	54.78±10.48	50.12±13.21	<0.0001
BMI (mean±SD)	27.39±5.11	25.74±4.57	<0.0001
HUNT			
Female	6315	5836	<0.0001
Male	4241	7403	
Age (mean±SD)	55.95±9.48	54.82±10.31	<0.0001
BMI (mean±SD)	27.37±4.33	26.52±3.88	<0.0001
ELSA			
Female	1090 (64.9%)	2660 (50.2%)	<0.001
Male	589 (35.1%)	2644 (49.8%)	
Age (mean±SD)	68.10±9.49	66.55±9.98	<0.0001
BMI (mean±SD)	28.60±4.98	27.08±4.22	<0.0001
RS-1			
Female	422	1323	<0.0001
Male	110	1281	
Age (mean±SD)	64.49±5.30	64.60±5.24	0.6660
BMI (mean±SD)	26.98±3.91	26.14±3.54	<0.0001
RS-2			
Female	106	745	<0.0001
Male	38	676	
Age (mean±SD)	61.59±4.59	61.93±4.72	0.2651
BMI (mean±SD)	28.54±4.73	27.77±3.91	0.0363
RS-3			
Female	128	1516	<0.0001
Male	27	1263	
Age (mean±SD)	56.28±5.77	56.32±5.46	0.0348
BMI (mean±SD)	28.54±4.86)	27.71±4.62	0.0827

BMI, body mass index; ELSA, The English Longitudinal Study of Ageing; HUNT, The Nord-Trøndelag Health Survey; RS-1, RS-2 and RS-3, The Rotterdam Study 1, 2 and 3; SD, Standard deviation .

Genotyping and imputation

Genotyping and imputation methods across cohorts are summarised in online supplemental table S1 (online supplemental text).

Statistical analysis and in silico follow-up

The details of statistical analysis, and in silico follow-up are described in online supplemental text. In brief, GWAS in the discovery sample was performed using linear mixed-effects model implemented in BOLT-LMM (V.2.3.2).[24] An additive

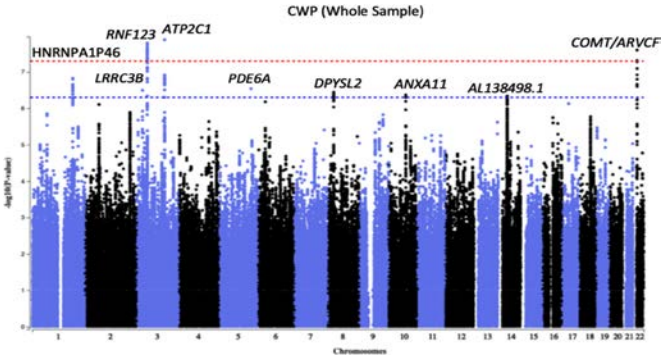

Figure 2 Manhattan plot of a genome-wide association analysis of chronic widespread musculoskeletal pain (CWP). Each circle in the plot represents a single nucleotide polymorphism (SNP), which was positioned following genomic build GRCh37. The y-axis shows the corresponding –log10 p values and the x-axis shows chromosome position along with SNPs. The horizontal red dotted line indicates genome-wide significance threshold at $p=5.0\times10^{-8}$. The horizontal blue dotted line indicates suggestive genome-wide significance threshold at $p=5.0\times10^{-7}$. Gene labels represent nearest genes to independent SNPs located at loci associated with $p<5.0\times10^{-7}$.

genetic model for SNP effect on CWP was adjusted for age, sex, genotyping platform and the first 10 genetic principal components provided by UKB. A sensitivity GWAS (controls: 223 606 and CWP cases: 6914) was performed excluding participants with chronic non-musculoskeletal pain such as headache, facial and abdominal pain from the controls. Independent SNPs at GWAS significant loci were identified using Conditional and Joint[25] analysis and submitted for replication. Independent SNPs across all replication cohorts were meta-analysed using fixed-effects model with both sample size, and inverse-variance weighting implemented in METAL.[26] SNP heritability was estimated using BOLT-REML[24] and converted to liability scale. Linkage disequilibrium score regression (LDSR)[27] was used to estimate inflation in test statistics and genetic correlations. We also estimated partial genetic correlations.[28] We used Functional Mapping and Annotation (FUMA) webtool[29] for the annotation of functional consequences of CWP-associated SNPs, gene mapping, tissue specificity and gene-set enrichment. Differential expression of replicated independent SNP was assessed using the GTEx V.8 tissues.[30] Colocalisation of GWAS-independent SNPs in human skeletal muscle and dorsal root ganglion (DRG) tissues was assessed using publicly available data.[30 31] Functional annotation of GWAS-replicated locus was performed using Open Targets Platform.[32]

RESULTS

Details of the discovery and replication cohorts are presented in table 1. Cases were enriched for females compared with controls in all cohorts ($p<0.001$) and were on average older in the discovery, and in three replication cohorts ($p<0.05$). In all cohorts, BMI was significantly higher in cases than controls ($p<0.0001$) except for RS-3 where a similar but non-significant trend was observed ($p=0.0827$).

Discovery genome-wide association study

Three genomic loci tagged by *rs1491985*, *rs10490825* and *rs165599* passed genome-wide significance threshold of $p<5E-08$ (figure 2). Observed inflation in test statistics ($\lambda_{GC}=1.146$, online supplemental figure S2) was due to polygenicity (LDSR intercept$=1.002\pm0.0085$, LDSR ratio$=0.0118\pm0.0497$) rather

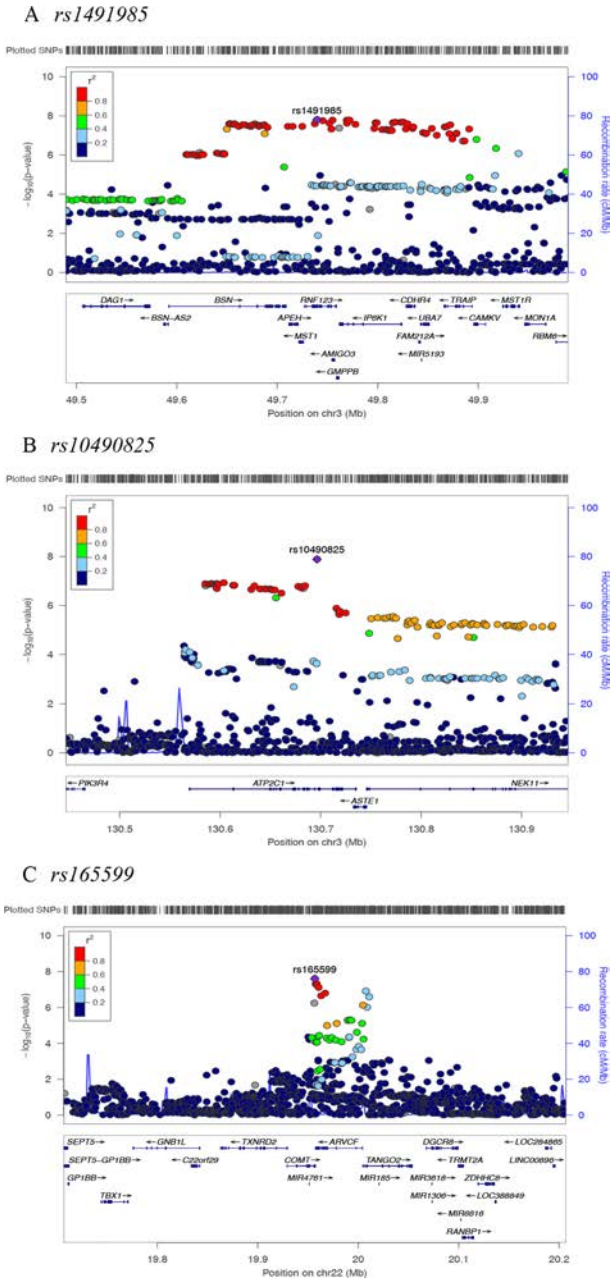

Figure 3 Regional plots for three independent chronic widespread musculoskeletal pain associated single nucleotide polymorphisms (SNPs). Independent SNPs are coloured in purple. Other coloured circles indicate pairwise linkage disequilibrium (LD). The strength of LD (r^2) presented in the upper left corner of each plot.

than population stratification. SNP heritability of CWP was 0.05 ± 0.003 on the observed scale, and 0.33 ± 0.0004 on the liability scale meaning that the observed SNPs explain approximately 33% of the variance in CWP risk. Independent SNPs were located in the gene *Ring Finger Protein 123* (*RNF123*) (chromosome 3, *rs1491985*, intronic variant, p=1.60E-08), *ATPase secretory pathway Ca^{2+} transporting 1* (*ATP2C1*) (chromosome 3, *rs10490825*, intronic variant, p=1.30E-08) and *COMT* (chromosome 22, rs165599, 3'-untranslated region (3'-UTR) variant, p=2.50E-08), respectively (figure 3A–C; online supplemental table S2). Six additional loci near or within genes *HNRNPA1P46*, *LRRC3B*, *PDE6A*, *DPYSL2*, *ANXA11* and *AL138498*.1 were identified at suggestive GWAS threshold of $p<5E-07$. Sensitivity GWAS excluding participants with chronic

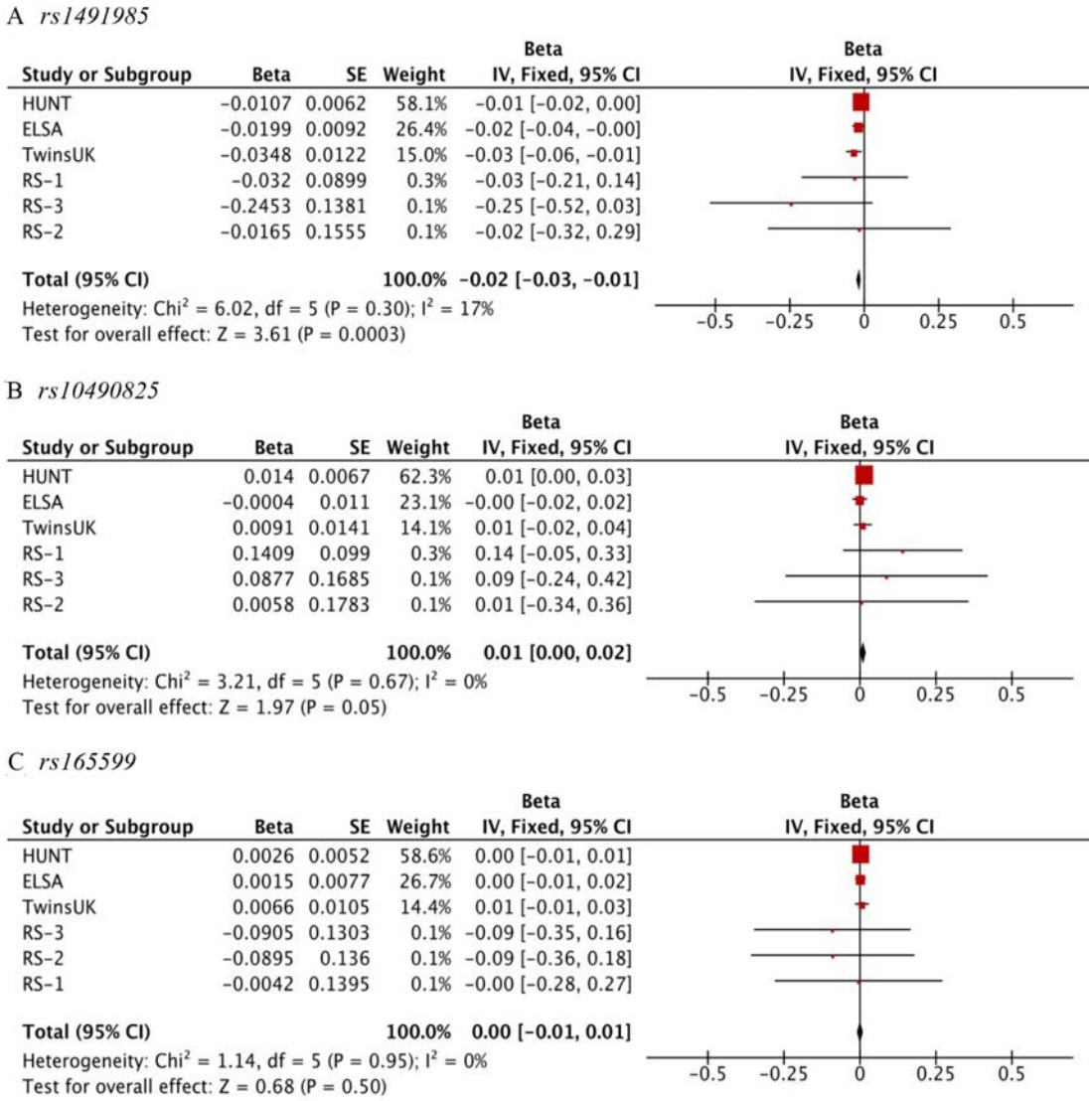

Figure 4 Forest plot for the association of (A) *rs1491985*, (B) *rs10490825,* and (C) *rs165599* with chronic widespread musculoskeletal pain. X-axis shows effect size measures are presented as beta value. The red square with horizontal black line represents the cohort-specific effect with a corresponding CI for the single nucleotide polymorphism (SNP) of interest. Size of the square indicates the weight of the study and reflects sample size. The vertical black line indicates 'line of no effect'. Overall effect is presented as a black diamond. Test statistics for each cohort, meta-analysis and heterogeneity are available on the left-hand side. The *rs1491985* and *rs10490825* were not present in The English Longitudinal Study of Ageing (ELSA); therefore *rs9870858* and *rs17329848* were used as proxy SNPs, respectively (online supplemental text).

non-musculoskeletal pain provided similar findings except that *COMT* locus now became suggestively significant (p=5.3E-08) (online supplemental figure S3).

Replication results and meta-analysis

Results are presented in online supplemental table S3, with meta-analysis of the six replication samples as shown in figure 4 (online supplemental tables S4, S5). Given the significance threshold for replication: 0.05/3=0.017, association between CWP and *rs1491985* was considered replicated (sample-size based p=0.0002; standard-error based p=0.0003). *Rs10490825* showed suggestive association with CWP (sample-size based p=0.0227; standard-error based p=0.0490) and demonstrated a consistent direction of effect in five of the six replication samples. *Rs165599* did not replicate (sample-size based p=0.7300; standard-error based p=0.5000) and the direction of effect was not consistent across cohorts: in three cohorts, allele A was protective, while in the other three it was the risk allele. None of the three SNPs displayed statistically significant heterogeneity in the replication cohorts.

CWP shares genetic components with BMI, depression, age at first birth and years of schooling

Two hundred and nine traits from LD-hub (online supplemental text) were examined for genetic correlation with CWP. We selected traits for which the absolute value of the correlation coefficient (r_g) was >0.2, and for which the Bonferroni-corrected p was <0.01/209=4.78E-05. Twenty-three traits fulfilled these criteria (online supplemental figure S4). The highest positive genetic correlation was observed for depressive symptoms (r_g=0.65) and the highest negative correlation was observed for college completion (r_g=−0.61). Many of the 23 genetically correlated traits were correlated with each other raising concerns about their independency of correlations with CWP. We therefore calculated partial genetic correlations

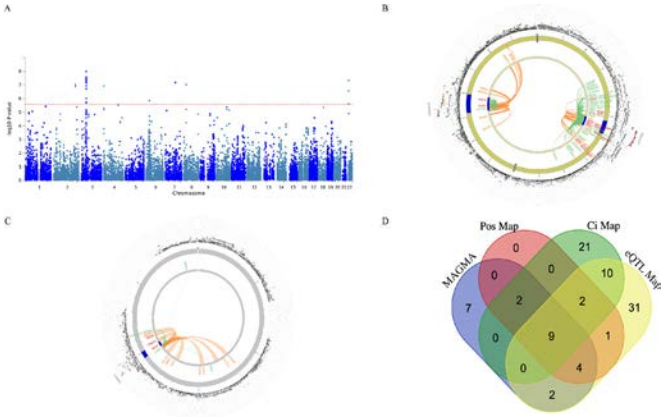

Figure 5 (A) Manhattan plot of the genome-wide gene-based associationanalysis, (B) & (C) The circus plot displaying chromatininteractions (Ci) and expression quantitative trait loci (eQTLs) onchromosomes 3 and chromosomes 22, respectively, (D) Venn diagramshowing overlap of genes implicated by genome-wide gene-basedanalysis implemented in MAGMA, positional mapping (Pos Map),chromatin interaction mapping (Ci Map), and expression quantitativetrait locus mapping (eQTL Map). (A) The y-axis shows the —log10transformed two-tailed p-value of each gene from a linear model andthe chromosomal position on the x-axis. The red dotted line indicatesthe Bonferroni-corrected threshold for genome-wide significance ofthe gene-based test. (B, C) The most outer layer of the circus plotdisplaying Manhattan plot with $-\log_{10}$ p-values forchronic widespread musculoskeletal pain associated independent singlenucleotide polymorphisms (SNPs). Each SNP is presented with rsID.Linkage disequilibrium (LD) relationship between independent SNPs atthe locus and their proxies are indicated with red (r^2 >0.8) and orange (r^2 > 0.6). Grey SNPs indicate minimalLD with r^2 ≤0.20.The outer circle represents chromosome with genomic risk loci arehighlighted in blue. Either Ci- or eQTL mapped genes are displayed onthe inner circle. Ci- and eQTL mapped genes are presented in orangeor green color, respectively. Genes mapped with both approaches arecolored red.

conditionally independent of each other. Using hierarchical clustering of genetic correlations we identified seven clusters (online supplemental figure S5A), with seven traits selected to represent each cluster (BMI, triglycerides, depressive symptoms, coronary artery disease, smoking, age of first birth and years of schooling) to quantify partial genetic correlation with CWP. We found depressive symptoms (r_g=0.59), BMI (r_g=0.20), age of first birth (r_g=−0.26) and years of schooling (r_g=−0.17) independently correlated with CWP (online supplemental figure S5B and table S6).

Tissue-specific expression of CWP mapped gene sets

The results of functional consequences of GWAS-independent SNPs and their proxies are presented in online supplemental figure S6 (online supplemental text). Four different gene mapping strategies were implemented in FUMA (genome-wide gene-based association analysis, positional, expression quantitative trait locus (eQTL) and chromatin interaction mapping) linking annotated SNPs to 89 genes of which *MST1, GMPPB, APEH, RNF123, ARVCF, AMIGO3, IP6K1, TANGO2* and *TRAIP* were identified using all four methods (figure 5A–D).[33] Mapped genes were investigated for tissue-specific gene expression and gene-set enrichment. In 54 specific GTEx tissues types, differentially expressed gene sets enriched for skeletal muscle, several brain tissues, heart, whole blood, pancreas and transverse colon (figure 6A, online supplemental table S7). In 30 general GTEx tissue types, differentially expressed gene sets enriched for skeletal muscle, pancreas, heart, blood and brain (figure 6B, online supplemental table S8). In both sets of GTEx tissues, overall enrichment for differentially expressed gene sets containing *RNF123* and *ATP2C1* genes were stronger for skeletal muscle than other tissues. *RNF123* was found to be highly expressed in skeletal muscle compared with other tissue types (figure 6C). None of the hallmark gene sets available in the molecular signature database was identified in the analysis.

Putative causal genes in *RNF123* locus

Colocalisation analysis identified a 93% probability of shared eQTL variant *rs6809879*, which controls *Cadherin Related Family Member 4* (*CDHR4*) expression in the skeletal muscle and CWP association signal near the *RNF123* locus (online supplemental table S9, online supplemental figure S7A). Additionally, significant colocalisation was found for *rs13093525*, which controls *APEH* expression in DRG at exon level (72% probability of shared variant with *RNF123* locus). Both *rs6809879* and *rs13093525* were in complete LD with independent SNP *rs1491985* (R^2=1) (online supplemental table S10, online supplemental figure S7B). No evidence of skeletal muscle or DRG eQTL colocalisation was observed for *ATP2C1* and *COMT* loci. Functional annotation of *RNF123* locus identified nine genes (SLC25A20, *NDUFAF3*, *DAG1*, *HYAL1, GMPPB, TRAIP, RHOA, CACNA2D2* and *IMPDH2*) specific to musculoskeletal system diseases, of which *CACNA2D2*, *NDUFAF3* and *IMPDH2* enriched as druggable targets (online supplemental figure S8).

DISCUSSION

CWP is a prevalent condition with moderate heritability and serves as a cardinal diagnostic feature of fibromyalgia. Therefore, our findings are of importance for better understanding the genetic basis of fibromyalgia. We report here the largest GWAS of CWP to date using 249 843 participants from the UKB, identifying 3 genome-wide significant loci implicating *RNF123*, *ATP2C1* and *COMT*. The association in *RNF123* was replicated, whereas *ATP2C1* showed a suggestive association, and the *COMT* locus did not replicate in 43 080 individuals from independent cohorts.

RNF123 gene encodes E3 ubiquitin-protein ligase, has a role in cell cycle progression, metabolism of proteins and innate immunity.[34 35] This gene is highly expressed in skeletal muscle than other tissues. Recent studies involving UKB samples also associated the locus with musculoskeletal pain.[19 36] However, it is not clear how *RNF123* may contribute to CWP. Using in silico follow-up, we identified *CDHR4*, *APEH*, *SLC25A20*, *NDUFAF3*, *DAG1*, *HYAL1*, *GMPPB*, *TRAIP*, *RHOA*, *CACNA2D2* and *IMPDH2* genes as putative causal candidates at the locus, of which *CACNA2D2*, *NDUFAF3* and *IMPDH2* can be targeted using known drugs.[37–39] Notably, *CACNA2D2* encodes the alpha-2/delta subunit of the voltage-dependent calcium channel complex, which is a receptor for gabapentinoids,[40] used by some in the management of fibromyalgia.[41 42] Another prioritised gene *CDHR4* belongs to cadherin superfamily has a role in calcium-ion binding to facilitate cadherin-mediated cell-cell interaction.[43 44]

Additionally, the *ATP2C1* locus demonstrated suggestive association in replication (p=0.0227). There was a consistent direction of effect for *ATP2C1* locus in six replication cohorts but not ELSA, where we used a proxy SNP, which had close to zero effect size (beta=−0.0004±0.0110). This is the first study to implicate *ATP2C1* with musculoskeletal

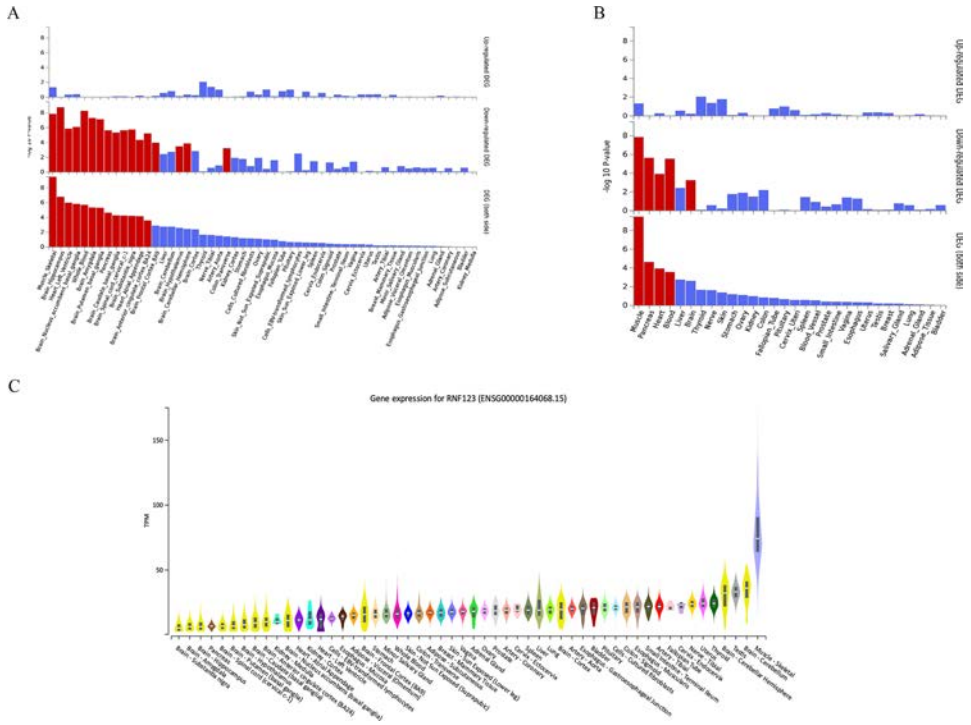

Figure 6 (A) Differentially expressed gene (DEG) plots for chronic widespread musculoskeletal pain (CWP) in 54 tissue types from GTEX v8, (B) DEG plots for CWP in 30 general tissue types from GTEX v8 and (C) Differential expression of *RNF123* gene across tissue types from GTEX v8. (A, B) In both plots, the y-axis represents the —log10 transformed two-tailed p value of the hypergeometric test. Significantly enriched DEG sets (Bonferroni-corrected p value <0.05) are highlighted in red. (C) Y-axis represents transcripts per million (TPM) and x-axis represents the GTEx (V.8) tissues. The figure was adapted from GTEx portal (https://www.gtexportal.org/home/gene/ENSG00000164068).

pain using an agnostic approach. The *ATP2C1* gene encodes for the ATP-powered magnesium-dependent calcium pump protein hSPCA1, which mediates Golgi uptake of cytosolic Ca(2+) and Mg(2+).[45] A loss of function mutation in the *ATP2C1* leads to Hailey-Hailey disease (HHD), an autosomal dominant skin condition characterised by blistering and erosion of the epidermis.[46] Interestingly, HHD may be treated successfully with low-dose naltrexone, an opioid receptor antagonist, which has also been used in the management of fibromyalgia.[47 48] A recent study showed that naltrexone is capable of restoring calcium homeostasis in natural killer cells of patients with chronic fatigue syndrome.[49] Additionally, the role of calcium regulation in pain processing is well known.[50–52] Taken together, our findings suggest a role in the regulation of calcium influencing CWP/fibromyalgia.

COMT is one of the most studied genes in human pain.[53] Almost 30 SNPs and 3 haploblocks of the *COMT* gene have been studied in acute clinical, experimental and chronic pain. *Rs4680* of the *COMT* gene is extensively studied in many pain phenotypes such as pain sensitivity, TMD and fibromyalgia.[54] Across multiple ethnic populations, *rs4680* was implicated with fibromyalgia.[55] However, a meta-analysis of 8 case-control studies (589 fibromyalgia cases and 527 controls) did not confirm earlier association.[56] To date, the largest study that assessed the association between *COMT* haplotypes (*rs4680, rs4818, rs4633* and *rs6269*) and fibromyalgia included 60 367 participants (2713 ICD-9 diagnosed fibromyalgia) and found no association.[57] They have also been refuted in other European CWP samples[21 58] and a large candidate gene study of fibromyalgia.[59] However, we identified *rs165599*, located at 3'-UTR of *COMT*, associated with CWP in the discovery sample but not in the meta-analysis or any of the replication cohorts. This variant is not in LD with previously studied *COMT* SNPs *rs4680, rs4818, rs4633* and *rs6269*, and was found not to be associated with chronic musculoskeletal pain including CWP neither when studied as a single SNP nor as a part of a haploblock.[60–62] Several explanations of our non-replication of *COMT* locus are possible. First, there was lower power pertaining to overall meta-analysis, which was estimated at 48% based on the effect size observed in the discovery sample (n=249 843), replication sample size (n=43 080) and the number of tests conducted (n=3). Our meta-analysis did have 90% power to detect a relative risk as small as 1.04 but the estimated *COMT* effect was only 1.012 (beta=0.0027±0.004; OR=1.012, 95% CI=0.97 to 1.05). However, our replication sample size was larger than many of the earlier studies that reported the association between *COMT* and CWP.[20 63] Second, we observed a tendency towards non-significance for the *COMT* locus in the sensitivity GWAS due to the exclusion of participants with non-musculoskeletal pain from the control group suggesting that *COMT* predisposes to chronic pain in general. Finally, genetic factors underlying chronic pain and psychiatric comorbidity (e.g. depression and neuroticism) are known to be shared.[64] However, previous GWAS on chronic pain,[28 65 66] depression[67] and neuroticism[68] have failed to detect an association with *COMT*. Thus, if there is a role of *COMT* in CWP, it is likely minimal.

Epidemiological studies have consistently reported higher BMI to be associated with an increased risk of CWP.[10 11 69] Our analysis showed significantly higher BMI in CWP cases compared with controls (p<0.0001) in all cohorts except RS-3. In line with this, we observed a positive genetic overlap between BMI and CWP independent of genetic confounders. Similarly, genetically independent pairwise

genetic correlation for depressive symptoms, age of first birth and years of schooling was seen with CWP. These findings indicate the presence of shared molecular pathways underlying these traits.

Functional analysis showed that FUMA mapped genes differentially expressed in skeletal muscle, several areas of the CNS, pancreas, whole blood and heart tissues. These findings suggest the involvement of nervous, musculoskeletal and neuroendocrine systems in CWP. These physiological systems have been implicated in fibromyalgia by previous studies.[70–72] Evidence suggests that both peripheral and central pain mechanisms influence CWP.[73 74] We observed overall stronger enrichment for differentially expressed gene sets in skeletal muscle than other GTEx tissues. Also, skeletal muscle and DRG eQTLs colocalise with the *RNF123* locus. These findings suggest a substantial involvement of peripheral pain mechanisms in CWP.

The study has limitations. The case definition of CWP depends on self-report together with exclusion of other conditions with symptoms leading to chronic pain.[75] A clinical diagnosis of CWP would have been infeasible in a sample this large. Also, we used common SNPs to estimate the heritability of CWP, so the contribution of other variants in the heritability estimated remains unknown. The phenotype definition used in this study to estimate SNP heritability has differed from the Kato *et al*[13] study, where a modulated American College of Rheumatology[76] criteria based on self-report was used to estimate broad-sense heritability. However, using UKB samples, a study reported the SNP heritability of pain all over the body, regardless of chronicity, on the liability scale was 0.31±0.072.[64] We found a similar but slightly higher estimate for CWP (0.33±0.0004), suggesting our definition is meaningful and CWP is a trait of high genetic influence. Finally, our findings cannot be generalisable to ancestry other than northern Europeans (online supplemental text).

In summary, this study identified a novel association for CWP in the *RNF123* locus and suggested the role of calcium regulation, by the involvement of the *CDHR4, CACNA2D2* and *ATP2C1* genes. The association of the *COMT* locus with CWP was not replicated, suggesting a small influence, if any. We found evidence that the epidemiological association of BMI and CWP is at least in part genetically mediated. Finally, our results suggest a profound role of peripheral mechanisms in the pathogenesis of CWP.

Author affiliations
[1]Department of Twin Research and Genetic Epidemiology, School of Life Course Sciences, King's College London, London, UK
[2]Department of Research, Innovation and Education,Division of Clinical Neuroscience, Oslo University Hospital, Oslo, Norway
[3]Department of Neurology, Oslo universitetssykehus Ullevål, Oslo, Norway
[4]K. G. Jebsen Center for Genetic Epidemiology, Department of Public Health and Nursing, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology, Trondheim, Norway
[5]Department of Internal Medicine, Erasmus Medical Center, Rotterdam, Zuid-Holland, The Netherlands
[6]Research and Communication Unit for Musculoskeletal Health (FORMI), Department of Research, Innovation and Education, Division of Clinical Neuroscience, Oslo University Hospital, Oslo, Norway
[7]Institute of Clinical Medicine,Faculty of Medicine, University of Oslo, Oslo, Norway
[8]Laboratory of Theoretical and Applied Functional Genomics, Novosibirsk State University, Novosibirsk, 630090, Novosibirskaâ, Russia
[9]PolyOmica, 's-Hertogenbosch, PA, The Netherlands
[10]Laboratory of Recombination and Segregation Analysis, Institute of Cytology and Genetics, 10 Lavrentiev Avenue, Novosibirsk, 630090, Russia
[11]Department of Neuromedicine and Movement Science, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology, Trondheim, Norway
[12]Clinical Research Unit Central Norway, St Olavs University Hospital, Trondheim, Norway
[13]Department of Public Health and Nursing, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology, Trondheim, Norway
[14]HUNT Research Center, Department of Public Health and Nursing, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology, Trondheim, Norway

Acknowledgements The authors would like to thank all the participants of UK Biobank, The Nord-Trøndelag Health Survey, English Longitudinal Study of Aging, TwinsUK and Rotterdam study I, II and III. The contribution of inhabitants, general practitioners and pharmacists of the Ommoord district to the Rotterdam Study is gratefully acknowledged. The UK Biobank study was approved by the National Health Service National Research Ethics Service (ref. 11/NW/0382) and all participants provided written informed consent. Genome-wide association analysis was performed using the UK Biobank resource under project number 18219. The English Longitudinal Study of Ageing is jointly run by University College London, Institute for Fiscal Studies, University of Manchester and National Centre for Social Research. Genetic analyses have been carried out by UCL Genomics and funded by the Economic and Social Research Council and the National Institute on Aging. Data governance was provided by the METADAC data access committee, funded by ESRC, Welcome and MRC (2015-2018: Grant Number MR/N01104X/1 2018-2020: Grant Number ES/S008349/1). TwinsUK is funded by the Wellcome Trust, Medical Research Council, European Union, Chronic Disease Research Foundation (CDRF), Zoe Global Ltd and the National Institute for Health Research (NIHR)-funded BioResource, Clinical Research Facility and Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust in partnership with King's College London. The Nord-Trøndelag Health Study (The HUNT Study) is a collaboration between HUNT Research Centre (Faculty of Medicine and Health Sciences, NTNU, Norwegian University of Science and Technology), Trøndelag County Council, Central Norway Regional Health Authority and the Norwegian Institute of Public Health. The genotyping was financed by the National Institute of Health (NIH), University of Michigan, The Norwegian Research Council and Central Norway Regional Health Authority and the Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology (NTNU). The genotype quality control and imputation has been conducted by the K.G. Jebsen Center for Genetic Epidemiology, Department of Public Health and Nursing, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology (NTNU). The Rotterdam Study is supported by the Erasmus MC University Medical Center and Erasmus University Rotterdam; The Netherlands Organization for Scientific Research (NWO); The Netherlands Organization for Health Research and Development (ZonMw); the Research Institute for Diseases in the Elderly (RIDE); The Netherlands Genomics Initiative (NGI); the Ministry of Education, Culture and Science; the Ministry of Health, Welfare and Sports; the European Commission (DG XII) and the Municipality of Rotterdam.

Collaborators Members of HUNT All-In Pain: Amy E Martinsen (1. K. G. Jebsen Center for Genetic Epidemiology, Department of Public Health and Nursing, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology, Trondheim, Norway; 2. Department of Research, Innovation and Education, Division of Clinical Neuroscience, Oslo University Hospital, Oslo, Norway; 3. Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo, Norway.), Anne Heidi Skogholt (K. G. Jebsen Center for Genetic Epidemiology, Department of Public Health and Nursing, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology, Trondheim, Norway.), Ben Brumpton (K. G. Jebsen Center for Genetic Epidemiology, Department of Public Health and Nursing, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology, Trondheim, Norway.), Cristen Willer (Department of Internal Medicine, Division of Cardiovascular Medicine, University of Michigan, Ann Arbor, 48109, MI, USA.), Ingrid Heuch (Department of Research, Innovation and Education, Division of Clinical Neuroscience, Oslo University Hospital, Oslo, Norway.), Ingunn Mundal (Department of Health Science, Molde University College, Molde, Norway.), Jonas Bille Nielsen (1. K. G. Jebsen Center for Genetic Epidemiology, Department of Public Health and Nursing, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology, Trondheim, Norway; 2. Department of Internal Medicine, Division of Cardiovascular Medicine, University of Michigan, Ann Arbor, 48109, MI, USA; 3. Department of Epidemiology Research, Statens Serum Institut, Copenhagen, Denmark.), Kjersti Storheim (1. Research and Communication Unit for Musculoskeletal Health (FORMI), Department of Research, Innovation and Education, Division of Clinical Neuroscience, Oslo University Hospital, Oslo, Norway; 2. Faculty of Health Sciences, Department of physiotherapy, Oslo Metropolitan University, Oslo, Norway.), Kristian Bernhard Nilsen (1. Department of Neuromedicine and Movement Science, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology (NTNU), Trondheim, Norway. 2. Department of Neurology, Oslo University Hospital, Oslo, Norway.), Lars Fritsche (Center for Statistical Genetics, Department of Biostatistics, University of Michigan, Ann Arbor, 48109, MI, USA.), Laurent F. Thomas (1. K. G. Jebsen Center for Genetic Epidemiology, Department

of Public Health and Nursing, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology, Trondheim, Norway; 2. Department of Clinical and Molecular Medicine, Norwegian University of Science and Technology, Trondheim, Norway.), Linda M Pedersen (Department of Research, Innovation and Education, Division of Clinical Neuroscience, Oslo University Hospital, Oslo, Norway.), Maiken E Gabrielsen (K. G. Jebsen Center for Genetic Epidemiology, Department of Public Health and Nursing, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology, Trondheim, Norway.), Marianne Bakke Johnsen (1. K. G. Jebsen Center for Genetic Epidemiology, Department of Public Health and Nursing, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology, Trondheim, Norway; 2. Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo, Norway; 3. Research and Communication Unit for Musculoskeletal Health (FORMI), Department of Research, Innovation and Education, Division of Clinical Neuroscience, Oslo University Hospital, Oslo, Norway.), Marie Udnesseter Lie (1. Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo, Norway; 2. Research and Communication Unit for Musculoskeletal Health (FORMI), Department of Research, Innovation and Education, Division of Clinical Neuroscience, Oslo University Hospital, Oslo, Norway.), Oddgeir Holmen (HUNT Research Center, Department of Public Health and Nursing, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology, Trondheim, Norway.), Synne Øien Stensland (1. Research and Communication Unit for Musculoskeletal Health (FORMI), Department of Research, Innovation and Education, Division of Clinical Neuroscience, Oslo University Hospital, Oslo, Norway; 2. NKVTS, Norwegian Centre for Violence and Traumatic Stress Studies.), Wei Zhou (1. Department of Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, MI, USA. 2. Analytic and Translational Genetics Unit, Massachusetts General Hospital, Boston, Massachusetts, USA.)

Contributors MSR prepared the manuscript. Discovery GWAS: MSR, MBF and FMKW designed the study. MSR, YAT, MBF and FMKW developed phenotype definition. MSR performed data management and GWAS in the discovery cohorts. Replication: MSR, MBF and FMKW developed replication protocol and coordinated the study; MSR, BSW, JAW, MBF and FMKW designed the study; BSW, SB, KHa, EAF, KHv, JAZ, HUNT All-in Pain authors, SOCC, JBvM and FMKW collected replication cohort's data; MSR, BSW, SB, KHv, JAZ, SOCC and JBvM were involved data management; MSR, BSW, SB, KHa, EAF, JAZ, HUNT All-in Pain authors, MBF and FMKW developed phenotype definition; MSR, BSW, SB, SOCC and JBvM analysed the data. MSR performed replication meta-analysis. Post-GWAS bioinformatics: MSR, YAT and SZS performed post-GWAS bioinformatics analysis. MBF, YSA and FMKW provided the statistical and bioinformatics consultation in the project. MSR, KHa, SOCC, YAT, SZS, YSA, JBvM, MBF and FMKW interpreted the findings. FMKW was responsible for the overall supervision of the project. All authors commented on the manuscript and agreed on the final version to be published.

Funding MSR received funding from the European Union's Horizon 2020 research and innovation program IMforFUTURE, under H2020-MSCA-ITN grant agreement number 721815. The work of YAT was supported by PolyOmica and by the Russian Foundation for Basic Research (project 19-015-00151). The work of SZS was supported by the Russian Ministry of Science and Education under the 5-100 Excellence Programme and by the Ministry of Education and Science of the RF via the Institute of Cytology and Genetics SB RAS (project number 0259-2021-0009/AAAA-A17-117092070032-4). The work of YSA was supported by PolyOmica.

Competing interests YSA is co-owner of Maatschap PolyOmica and PolyKnomics BV, private organisations, providing services, research and development in the field of computational and statistical, quantitative and computational (gen)omics.

Patient consent for publication Not required.

Ethics approval Study-specific ethical approval was provided by the local ethics committees. All study participants provided written informed consent. Details are provided in the online supplementary material.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Summary statistics from our discovery and sensitivity GWAS was deposited at Zenodo (https://doi.org/10.5281/zenodo.4459546). Other data relevant to the study are included in the article or uploaded as online supplementary information.

ORCID iD

Md Shafiqur Rahman http://orcid.org/0000-0003-4068-6775

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CLINICAL SCIENCE

Anticardiolipin and other antiphospholipid antibodies in critically ill COVID-19 positive and negative patients

Uriel Trahtemberg ,[1] Robert Rottapel,[2,3] Claudia C Dos Santos,[1,4,5] Arthur S Slutsky,[4,5] Andrew Baker,[1,4,5] Marvin J Fritzler [6]

Handling editor Josef S Smolen

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2021-220206).

For numbered affiliations see end of article.

Correspondence to
Professor Marvin J Fritzler, Medicine, University of Calgary Cumming School of Medicine, Calgary, Canada; fritzler@ucalgary.ca

Received 20 February 2021
Revised 16 March 2021
Accepted 7 April 2021
Published Online First 26 April 2021

ABSTRACT

Background Reports of severe COVID-19 being associated with thrombosis, antiphospholipid antibodies (APLA), and antiphospholipid syndrome have yielded disparate conclusions. Studies comparing patients with COVID-19 with contemporaneous controls of similar severity are lacking.

Methods 22 COVID-19$^+$ and 20 COVID-19$^-$ patients with respiratory failure admitted to intensive care were studied longitudinally. Demographic and clinical data were obtained from the day of admission. APLA testing included anticardiolipin (aCL), anti-β2glycoprotien 1 (β2GP1), antidomain 1 β2GP1 and antiphosphatidyl serine/prothrombin complex. Antinuclear antibodies (ANAs) were detected by immunofluorescence and antibodies to cytokines by a commercially available multiplexed array. Analysis of variance was used for continuous variables and Fisher's exact test was used for categorical variables with α=0.05 and the false discovery rate at q=0.05.

Results APLAs were predominantly IgG aCL (48%), followed by IgM (21%) in all patients, with a tendency towards higher frequency among the COVID-19$^+$. aCL was not associated with surrogate markers of thrombosis but IgG aCL was strongly associated with worse disease severity and higher ANA titres regardless of COVID-19 status. An association between aCL and anticytokine autoantibodies tended to be higher among the COVID-19$^+$.

Conclusions Positive APLA serology was associated with more severe disease regardless of COVID-19 status.

Trial registration number NCT04747782

Key messages

What is already known about this subject?

- COVID-19 is associated with coagulopathy and high morbidity and mortality.
- COVID-19 shares some of these clinical features with antiphospholipid syndrome.
- Reports of an association of antiphospholipid antibodies with high-risk COVID-19 have yielded disparate conclusions, but they lacked longitudinal follow-up and control groups of similar severity.

What does this study add?

- Antiphospholipid syndrome serology assessed longitudinally was predominantly anticardiolipin IgG autoantibodies, in 48% of patients.
- Anticardiolipin serology was associated with worse disease severity in both COVID-19 positive and negative patients.

How might this impact on clinical practice or future developments?

- The use of antiphospholipid antibodies tests in the COVID-19 clinical setting needs to be taken in context; whereas they are associated with more serve disease, they do not discriminate between COVID-19 positive and negative patients.

INTRODUCTION

Antiphospholipid antibodies (APLAs) are biomarkers of a spectrum of clinical features observed in antiphospholipid syndrome (APS).[1] Features of APS include venous and arterial thrombosis involving multiple organs and having various presentations.[1] APLAs that are components of APS criteria include IgG and/or IgM anticardiolipin (aCL), anti-β2-glycoprotein1 (anti-β2GP1) and the 'lupus anticoagulant' (LAC).[2] Other non-criteria APLA such as antiphosphatidylserine/prothrombin (PS/PT) complex, anti-PT and antidomain 1 of β2-GP1 have also found a diagnostic niche in APS.[3 4]

One of the salient features of COVID-19 is the development of thrombotic events associated with severe morbidity and mortality.[5–8] In the context of systemic inflammation and dysregulated immunity,[9] some reports have linked APLA to these thromboses,[10 11] severe COVID-19[6 12] and release of neutrophil extracellular traps.[6] However, APLAs are also described in a variety of other infectious diseases[13] and critically ill patients have high rates of thromboembolism that were not linked to APS or APLA[14] (critically reviewed in ref. 15). Therefore, the association of COVID-19 with APLA and their potential pathogenic role[16] has not been clearly demonstrated due to the lack of contemporaneous, COVID-19 negative controls. Here, we compare the prevalence and clinical correlations of APLA in patients with severe COVID-19 as compared with contemporaneous non-COVID19 patients with similar clinical characteristics.

METHODS

Informed consent was obtained from all patients or their legal surrogates. Inclusion criteria were age ≥18 years, admission to intensive care unit (ICU)

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To cite: Trahtemberg U, Rottapel R, Dos Santos CC, *et al*. *Ann Rheum Dis* 2021;**80**:1236–1240.

Table 1 Patient demographics, clinical and autoantibody status

	Cohort	All	COVID$^+$	COVID$^-$
	N	42	22	20
Age	Mean (CI)	58.2 (62.7 to 54.1)	60.9 (66.6 to 55.3)	55.7 (62 to 48.7)
Sex	N male (%)	29/42 (69)	17/22 (77)	12/20 (60)
Censored?	N (%)	5/42 (12)	4/22 (18)	1/20 (5)
No of days before censoring	Mean (CI)	39.4 (59.4 to 19.4)	44.3 (66.2 to 22.3)	20 (NA)
Days from symptom onset to ICU	Mean (CI)	6 (8.3 to 3.7)	7.5 (9.9 to 5.2)	4.2 (8.5 to 0)
APACHE II on ICU admission	Mean (CI)	25.3 (27.6 to 22.9)	23.7 (27 to 20.4)	27 (30.5 to 23.5)
Mean of SOFA score for first 3 days	Mean (CI)	9.6 (10.7 to 8.5)	9.3 (11 to 7.7)	9.9 (11.6 to 8.3)
Mean of SOFA score for first 7 days	Mean (CI)	8.9 (10.1 to 7.8)	9.1 (11 to 7.3)	8.7 (10.3 to 7.2)
ICU days (censored)	Mean (CI)	14.1 (17.3 to 10.8)	14.2 (20.5 to 7.8)	14 (16.9 to 11.1)
Death in ICU	N (%)	13/42 (31)	7/22 (32)	6/20 (30)
Mechanical ventilation days (censored)	Mean (CI)	14.4 (18.9 to 10)	16.8 (25.1 to 8.6)	11.8 (14.9 to 8.7)
Total days of ventilation rescue measures	Mean (CI)	2.9 (4.3 to 1.4)	4.4 (7 to 1.8)	1.2 (2 to 0.4)
Therapeutic anticoagulation used	N (%)	8/42 (19)	3/22 (14)	5/20 (25)
Mean platelet count	Mean (CI)	239 (269 to 209)	264 (313 to 214)	212 (245 to 179)
Mean platelet to neutrophil ratio	Mean (CI)	35.2 (42 to 28.4)	38.7 (48.4 to 29)	31.4 (41.6 to 21.2)
aCL IgG	N (%)	20/42 (48)	13/22 (59)	7/20 (35)
aCL IgM	N (%)	9/42 (21)	7/22 (32)	2/20 (10)
Anti-β2GPI IgG	N (%)	0	0	0
Anti-β2GPI IgM	N (%)	0	0	0
Anti-domain 1 β2GP1 IgG	N (%)	0	0	0
Anti-PS/PT IgG	N (%)	0	0	0
Anti-PS/PT IgM	N (%)	1/42 (2)	1/22 (5)	0

The data were censored on 31 May 2020. Days from symptom onset were self-reported by the patients or their representatives. The SOFA score was performed daily for all patients; the average was calculated for the first 3 and 7 days in the ICU for each patient, and the mean of those averages are reported. For patients who underwent tracheostomy, mechanical ventilation days are counted until successfully weaned from ventilatory support for 24 hours. Rescue measures included use of paralytics, proning and inhaled NO (counted additively if more than one intervention used in the same day). The clinical outcomes were measured for up to 3 months. All the serologies were tested longitudinally and are reported for the first 10 days from admission to the ICU (for standardisation among patients). There was no statistically significant difference between COVID$^+$ and COVID$^-$ patients for all variables, using ANOVA for continuous variables and Fisher's exact test for categorical variables at α=0.05, followed by the false discovery rate at q=0.05.

aCL, anticardiolipin; ANOVA, analysis of variance; APACHE, Acute Physiology and Chronic Health Evaluation (score); β2GP1, beta two glycoprotein I; ICU, intensive care unit; PS/PT, phosphatidyl serine/prothrombin complex; SOFA, sequential organ failure assessment (score).

with acute respiratory failure. Exclusion criteria were inability to ascertain the primary outcome or obtain a baseline blood sample, and SARS-CoV2 infection in the 4 weeks prior to admission. COVID-19 status was determined with PCR of nasopharyngeal swabs and/or endotracheal aspirates. Follow-up was 3 months post-ICU admission or hospital discharge. Primary outcome was death in the ICU. Secondary outcomes were in hospital-death, ICU utilisation metrics, organ dysfunction measures and severity scores. Clinical data and serum samples were collected longitudinally at days 0, 1, 3, 5, 7 and 10; after day 10 or ICU discharge. aCL, anti-β2GP1 and anti-PS/PT were tested for IgG and IgM, as well as IgG anti-domain 1 β2-GP1; all by ELISA or chemiluminescence (Inova Diagnostics, San Diego, California, USA). Analysis of variance was used for continuous variables and Fisher's exact test was used for categorical variables at α=0.05, followed by a false discovery rate adjustment at q=0.05. Detailed methods are available (online supplemental file), including methods for detection of anti-nuclear autoantibodies (ANA) by HEp-2 immunofluorescence assay (IFA) (Inova Diagnostics) and antigen-specific autoantibodies (TheraDiag, Paris, France) and anticytokine autoantibodies (Millipore, Oakville, Ontario, Canada) using addressable laser bead immunoassays.

RESULTS

The demographic and clinical parameters of 22 COVID-19 positive (COVID$^+$) and 20 COVID-19 negative (COVID$^-$) patients (table 1) included an average of 14.1-day stays in ICU and 31% mortality, but no statistically significant differences between the two cohorts, including the lack of significant differences in the number of thrombotic events requiring therapeutic anticoagulation, platelet counts or platelet counts normalised to the neutrophil counts (to index for severity) (table 1). None of the patients had a history of antecedent APS, systemic lupus erythematosus (SLE) or other conditions associated with APS, nor were there significant differences in other past medical history between COVID$^+$ and COVID$^-$ patients (online supplemental table 1).

Frequency, development and distribution of aCL

Forty-eight per cent of all the ICU cohort had a positive IgG aCL test (table 1); interestingly, fewer patients had elevated titres of IgM aCL (n=9, 21%), with only two patients having IgM without IgG. Although more COVID-19$^+$ had aCL antibodies, the difference was not statistically significant (table 1); aCL titres were slightly higher among the COVID-19$^+$ (not statistically significant, (online supplemental table 2) and online supplemental figure 1). Longitudinally testing for anti-β2-GP1 and anti-PS/PT for IgG and IgM, as well as domain 1 anti-β2-GP1 IgG revealed only one patient (COVID-19$^+$) with positive serology for any of these autoantibodies. This patient seroconverted to IgM anti-PS/PT at days 5–7 of ICU hospitalisation. Table 2 shows the temporal development of the aCL IgG and IgM antibodies stratified by COVID-19 status. Late appearing (beyond 10 days after admission) aCL antibodies were not included in the statistical

Table 2 Development of ACL IgG and IgM over time

Cohort		aCL detected on admission	aCL developed within 10 days	Late appearing aCL (after 10 days)
aCL IgG positive	COVID$^+$	4	9	2
	COVID$^-$	3	4	0
aCL IgM positive	COVID$^+$	1	6	2
	COVID$^-$	1	1	1

Late aCL was not included in the statistical analyses to avoid survival and availability bias, and is shown here for qualitative assessment.
aCL, anticardiolipin antibodies.;

analyses to avoid survival and availability bias. Anti-CL were not associated with age or sex (not shown).

aCL versus disease severity, platelet counts and need for anticoagulation

Patients positive for aCL IgG demonstrated a consistent trend for worse outcomes in all the measures tested but this did not reach statistical significance after adjusting for multiple comparisons (table 3). These trends remained when analysed separately for COVID$^+$ and COVID$^-$ (not shown). aaCL IgG positive patients showed no significant differences in platelet counts, platelet to neutrophil ratio or the need for therapeutic anticoagulation (table 3).

aCL association with ANA, antigen-specific autoantibodies and anti-cytokine autoantibodies

We tested a broad range of non-APS autoantibodies to understand the autoimmune context of these patients and their potential relationship to APS autoantibodies. Although aCL IgG positivity was not associated with the presence of HEp-2 IFA ANA at a dilution of 1:160, it was significantly associated with higher ANA titres (online supplemental figure 2), p=0.03). This trend remained when analysing the COVID$^+$ and COVID$^-$ patients separately (data not shown). IgG aCL positivity was also significantly associated with anticytokine autoantibodies, both when analysed for positive or high-positive anticytokine titres (p=0.003 for both, adjusted for multiple comparisons); this was not related to any particular anticytokine autoantibody, although anti-interferon-γ, anti-IL10 and anti-IL-17f were the most prevalent (online supplemental table 3). When analysing the aCL IgG positive according to their COVID-19 status, the COVID$^+$ had significantly higher levels of anticytokine autoantibodies than the COVID$^-$ (online supplemental table 4). aCL IgG was not associated with antigen-specific autoantibodies, including SLE and myositis-related autoantibodies (not shown).

DISCUSSION

In the year since the onset of the SARS-CoV2 pandemic, there has been a remarkable surge in publications about one disease, COVID-19, chronicling the clinical onset and outcomes, and a host of biomarkers purported to have related pathophysiological significance (reviewed in references 17 18). The key observation of this study is that patients with positive IgG aCL showed a trend towards more severe disease regardless of whether they were COVID$^+$ and COVID$^-$. That is, while COVID$^+$ patients showed non-significant trends towards worse respiratory outcomes when compared with COVID$^-$, aCL status had an independent association with disease severity, and did not modulate the outcomes differentially based on COVID status. The pathological significance of aCL seropositivity is unclear since there were no major differences in platelet counts or thrombotic events in the two cohorts. Others have reported a high prevalence of aCL autoantibodies among COVID$^+$ patients, but these studies lacked contemporaneous COVID$^-$ control groups of similar disease severity.[6 15 19 20]

Although aCL tended to associate with COVID-19$^+$, they did not associate with the presence of other antigen-specific autoantibodies, although they had a strong association with certain anticytokine autoantibodies, which are reported to neutralise corresponding type I IFNs ability to block SARS-CoV-2 infection in vitro.[21] Interestingly, some patients had positive IgG aCL serology on ICU admission (table 2) in the absence of another relevant comorbidity such as APS or SLE (online supplemental table 1). These observations suggest that aCL positivity in the setting of acute severe respiratory illness may be a marker of a unique phenotype with variable temporal expression of aCL and anticytokine antibodies. The temporal dynamic is evidenced by the relatively long time frame from symptom onset to ICU

Table 3 Association between ACL IgG and disease severity, platelet counts and need for anticoagulation

	Cohort	All	aCL IgG positive	aCL IgG negative
	N	42	20	22
Age	Mean (CI)	58.2 (62.7 to 54.1)	55.9 (62.9 to 49)	60.7 (66.4 to 55)
Sex	N male (%)	29/42 (69)	13/20 (65)	16/22 (73)
Days from symptom onset to ICU	Mean (CI)	6 (8.3 to 3.7)	8.7 (12.8 to 4.6)	3.4 (5.4 to 1.5)
APACHE II on ICU admission	Mean (CI)	25.3 (27.6 to 22.9)	25.7 (28.5 to 22.9)	24.9 (28.8 to 20.9)
Mean of SOFA score for first 3 days	Mean (CI)	9.6 (10.7 to 8.5)	10.6 (12.2 to 9.1)	8.7 (10.3 to 7)
Mean of SOFA score for first 7 days	Mean (CI)	8.9 (10.1 to 7.8)	10 (11.7 to 8.4)	8 (9.5 to 6.4)
ICU days (censored)	Mean (CI)	14.1 (17.3 to 10.8)	16.6 (21.9 to 11.3)	12.1 (16.5 to 7.6)
Death in ICU	N (%)	13/42 (31)	8/20 (40)	5/22 (23)
Mechanical ventilation days (censored)	Mean (CI)	14.4 (18.9 to 10)	18.2 (25.5 to 10.8)	11.1 (16.4 to 5.7)
Total days of ventilation rescue measures	Mean (CI)	2.9 (4.3 to 1.4)	3.6 (5.6 to 1.5)	2.3 (4.4 to 0.1)
Therapeutic anticoagulation used	N (%)	8	4/20 (20)	4/22 (18)
Mean platelet count	Mean (CI)	239 (269 to 209)	268 (321 to 216)	212 (246 to 179)
Mean platelet to neutrophil ratio	Mean (CI)	35.2 (42 to 28.4)	34.8 (45.2 to 24.3)	35.6 (45.4 to 28.9)

See table 1 for details on the variables shown. There were no statistically significant differences between aCL IgG positive and aCL IgG negative patients for all variables, using ANOVA for continuous variables and Fisher's exact test for categorical variables at α=0.05, followed by the false discovery rate at q=0.05.
aCL, anticardiolipin; ANOVA, analysis of variance; APACHE, Acute Physiology and Chronic Health Evaluation (score); ICU, intensive care unit; SOFA, sequential organ failure assessment (score).

admission to the development of IgG aCL (table 3). Our findings highlight the importance of longitudinal monitoring of acutely ill patients. It seems plausible that disparate conclusions in the literature with respect to the significance of APLAs in COVID-19 may relate to arbitrary sampling times and lack of longitudinal follow-up in the setting of dynamic inflammatory diseases.

While some reports have included LAC in their analyses, we did not because LAC is known to be an unreliable biomarker in severe illnesses where C reactive protein, anticoagulant use and other factors confound its detection.[22 23] In this study, we used the anti-PS/PT test regarded by some as a surrogate for LAC (reviewed in reference 3). However, only one patient developed anti-PS/PT 5–7 days after admission. Further, our observation that no patient had antibodies to β2-GP1 (an APS criteria antibody) or to domain 1 β2-GPI (reportedly higher specificity for APS) argues against the presence of APS in our cohort. In addition, aCL in isolation and/or the depletion of β2-GPI reactivity has been associated with the loss of pathogenic thrombosis formation (reviewed in reference 3). In a study of 37 COVID+ acute respiratory disease vs 31 prepandemic (not contemporaneous) acute respiratory disease controls using a sample collected within 48 hours of admission, Frapard *et al* reported that 37 patients with COVID-19 exhibited more thrombotic events as compared with 31 prepandemic controls but the occurrence of APLA in the two groups was similar.[24] Using APLA assays similar to ours, Borghi *et al* reported a low prevalence of APLA in COVID$^+$ sera, where the most common target was IgG β2-GP1 (15.6%).[20] In addition, the primary β2GP1 antibody targets were in domains 2–4 which are less specific for APS.[20] In agreement with our study, Bertin *et al*[12] and Borghi *et al*[20] concluded that APLA were not associated with major thrombotic events.

The main limitation of our study is the small sample size, although studies using somewhat larger COVID-19 cohorts have reached similar conclusions.[12 20] The strengths of our study include its prospective, contemporaneous COVID$^-$ cohort with similar severity of disease. Importantly, we tested a broad APLA serological panel longitudinally, providing a more robust assessment of its true prevalence and incidence than in other reported studies; this is particularly relevant for such acutely ill patients with dynamic clinical courses. Finally, our use of an extensive serological panel allowed us to better characterise the broad phenotype associated with aCL.

Author affiliations
[1]Critical Care, St Michael's Hospital, Toronto, Ontario, Canada
[2]Departments of Medicine and Immunology, University of Toronto, Toronto, Ontario, Canada
[3]Division of Rheumatology, St. Michael's Hospital, Toronto, Ontario, Canada
[4]Keenan Centre for Biomedical Research, Li Ka Shing Knowledge Institute, St Michael's Hospital, Toronto, Ontario, Canada
[5]Interdepartmental Division of Critical Care Medicine, University of Toronto, Toronto, ON, Canada
[6]Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada

Acknowledgements The authors acknowledge the technical assistance of Haiyan Hou, Meifeng Zhang and Emily Walker in the MitogenDx Laboratory at the University of Calgary. We thank Marlene Santos, Gyan Sadhu, Imrana Khalid and Sebastian Duncan, the research coordinators at St Michael's Hospital Critical Care Research Unit. We are grateful to patients and families that have generously consented to the study.

Contributors This report is part of the COLOBILI study (Coronavirus longitudinal biomarkers in lung injury). AB and CDS are principal investigators; MJF, RR and AS are collaborators/coinvestigators and UT is the research lead. RR, MJF, UT and CDS conceived of the study; MJF, UT and RR wrote the manuscript drafts; AS, AB and CDS provided critique and technical guidance; UT performed the data analysis and creation of the figures. All authors edited the manuscript, through to the final version, read and approved the final submission.

Funding St Michael's Hospital Foundation, internal competitive grant to AB and CDS. Autoantibody testing was provided as a gift in kind by MitogenDx (Calgary, AB, Canada).

Competing interests MJF is the Director of MitogenDx. MJF is a consultant for and received speaking honoraria from Inova Diagnostics Inc (San Diego, California, USA) and Werfen International (Barcelona, Spain). All the other authors have no disclosures to declare.

Patient and public involvement statement Patients and public were not involved in the design of the study. During the initial phases of the study, we obtained feedback from the patients and their substitute decision makers. Their concerns, questions and preferences were incorporated into improved processes for consent and collection of biological samples. The consent forms have checkboxes with optional aspects of the study, to accommodate different patient preferences. The results of the study will be disseminated in lay versions by St. Michael's Hospital public relations and communications departments for the benefit of the public.

Patient consent for publication Not required.

Ethics approval This is an observational cohort study of patients admitted to St. Michael's Hospital (Toronto, ON, Canada), as approved by the Research Ethics Board (REB# 20–078).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as online supplemental information. The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

ORCID iDs
Uriel Trahtemberg http://orcid.org/0000-0001-9103-2494
Marvin J Fritzler http://orcid.org/0000-0003-1652-6608

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Challenges in the diagnosis of early rheumatoid arthritis in times of COVID-19

Challenges in the provision of care and patients' reluctance to access healthcare services and adhere to drug prescriptions in course of COVID-19 pandemic undermine the fundamental principles of early diagnosis and treat to target, which have revolutionised the natural history of many chronic inflammatory diseases starting from rheumatoid arthritis (RA).[1] Although most rheumatologists have the impression that the intervals between symptom onset and first consultation have increased,[2] no data currently indicate whether and to what extent the picture of early RA has changed during COVID-19.

Here, we analysed data from the Pavia Early Arthritis Clinic (EAC) inception cohort. Referral criteria have been described previously, and include the presence of signs and symptoms of suspected inflammatory arthritis for <12 months of duration.[3] By 31 December 2020, the EAC collects information on 2.508 patients. The service has undergone complete closure during the first wave of COVID-19 pandemic from 9 March to 18 May 2020. In this period, emergency visits were guaranteed through the general rheumatology outpatient clinic. For this study, baseline characteristics of patients referred in the semester following the lockdown (July to December 2020) were compared with: (1) patients referred in the semester immediately preceding the lockdown (July to December 2019); (2) patients referred in a semester of routine use of the 2010 RA criteria (July to December 2015); (3) patients referred in the semester following the dissemination of the 2010 RA criteria (July to December 2011); and (4) patients referred in the semester preceding the publication of the 2010 criteria (July to December 2009). Data were extracted from 452 patients. Overall, the access of patients with new-onset suspected inflammatory arthritis was relatively stable over the years until 2019, while COVID-19 pandemic coincided with a marked reduction in new referrals (−25.9%) and in the proportion of patients fulfilling RA criteria at presentation (36.1% vs 45.4%, p=0.19) (figure 1A). Furthermore, while the ratio between autoantibody-positive and autoantibody-negative RA was well balanced before the lockdown, and in line with the prevalence of autoantibody negativity recognised over the past decade,[4] nearly 70% of patients with RA referred in the second semester of 2020 were autoantibody positive (online supplemental table S1). As shown in figure 1B–F and in online supplemental table S2, restriction measures imposed by COVID-19 profoundly impacted on the clinical presentation of autoantibody-positive RA. The introduction and progressive consolidation of the 2010 classification criteria had indeed favoured a reduction of the diagnostic delay until 2019, with 55.7% of the patients seen within the 'window of opportunity' of 12 weeks compared with 37.5% of those classified according to the 1987 criteria in 2009 (p=0.20). Furthermore, autoantibody-positive RA had become progressively milder, with lower levels of objective parameters of inflammation. In contrast, in patients seen during the pandemic, the diagnostic delay was significantly longer, with only 5.6% of the patients captured within 12 weeks (p<0.001 vs 2019, 2015 and 2011 semesters grouped together; p=0.04 vs the 2009 semester). Overall disease activity was increased as a result of an inversion of the trend towards lower levels of inflammatory features as well as further increase in the historical trend towards worsening of patient-derived measures.[5] The proportion of erosive RA was not significantly different, likely due to the latency between disease activity and its effects on radiographic progression.[6] The introduction of the 2010 criteria had instead produced smaller changes in the pattern of presentation of autoantibody-negative RA (online supplemental table S3). Yet, COVID-19 pandemic also impacted on this autoantibody serotype by selecting fewer patients with more frequent polymyalgic involvement (50% vs 21.1% in 2019, 2015 and 2011 semesters grouped together, p=0.17) and higher inflammatory features, and more urgent need of medical advice, as expressed by the lower diagnostic delay. No significant changes neither in the rates of new referrals nor in disease characteristics were

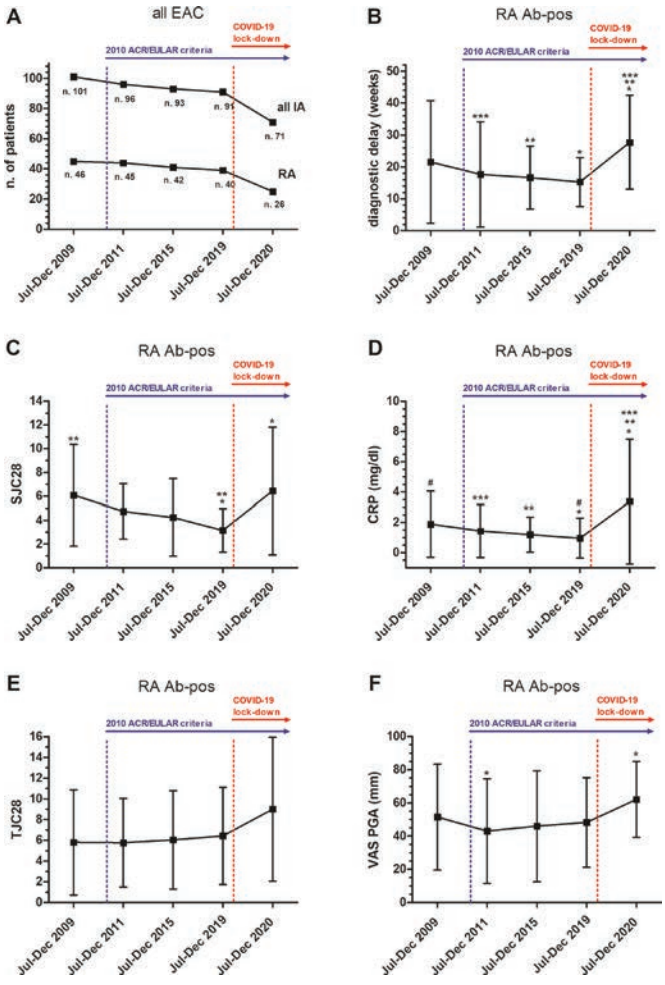

Figure 1 Frequency and characteristics at presentation of new-onset rheumatoid arthritis (RA) referred in course of COVID-19 pandemic. (A) Temporal trends of the rate of new referrals to our Early Arthritis Clinic (EAC) of patients with suspected inflammatory arthritis (IA) and patients fulfilling classification criteria for RA already at presentation. (B) Diagnostic delay (from symptom onset to diagnosis, in weeks) of patients with new-onset RA referred to the Pavia EAC during COVID-19 pandemic in comparison with reference semesters before the pandemic. (C–F) Temporal trends of objective measures of inflammation (28-joint swollen joint count, SJC28, C; levels of C-reactive protein, CRP, D) and patient-reported measures (28-tender joint count, TJC28, E; patient global assessment (PGA) of disease activity, F). Data are expressed as mean (SD) values. *, **, *** and # indicate significant differences (p<0.05) between each group by means of one-way analysis of variance (ANOVA) with Bonferroni correction for multiple comparisons. ACR, American College of Rheumatology; EULAR, European League Against Rheumatism; VAS, visual analogue scale.

observed in relation to different restriction measures imposed by the Italian government in course of 2020.

We acknowledge that the small sample sizes derived from monocentric EAC cohorts need replication. However, our data provide first evidence of how COVID-19 pandemic is changing the pattern of presentation of RA. The remarkable improvements in the outcomes of autoantibody-positive RA achieved over the past 20 years[7] risk to be rapidly vanished by a retrogression back to the diagnostic delay and the severity typical of EAC cohorts prior to the introduction of the 2010 criteria.[8] Equally important, despite the incidence of autoantibody-negative RA is increasing,[4] restrictions imposed by the pandemic, together with the erroneous but common beliefs attributing to this disease subtype a harmless course, may leave patients with less abrupt onsets underdiagnosed, with further impact on prognosis which remains per se unfavourable even in the modern treatment era.[7] The challenge is thus to keep on fighting COVID-19 without forgetting non-COVID-19 diseases.

Bernardo D'Onofrio [1,2] **Ludovico De Stefano,**[1,2] **Bianca Lucia Palermo,**[1,2] **Blerina Xoxi,**[1] **Antonio Manzo,**[1,2] **Carlomaurizio Montecucco** [1,2] **Serena Bugatti** [1,2]

[1]Division of Rheumatology, IRCCS Policlinico San Matteo Foundation, Pavia, Italy
[2]Department of Internal Medicine and Therapeutics, University of Pavia, Pavia, Italy

Correspondence to Professor Serena Bugatti, Division of Rheumatology, IRCCS Policlinico San Matteo Foundation, Pavia 27100, Italy; serena.bugatti@unipv.it

Handling editor Josef S Smolen

Contributors BDO contributed to the acquisition, analysis and interpretation of data and drafted the manuscript. LDS contributed to the analysis and interpretation of data. BLP and BX contributed to the acquisition of data. AM and CM contributed to the interpretation of data. SB conceived the work, contributed to the analysis and interpretation of data and drafted the manuscript. LDS, BLP, BX, AM and CM revised the manuscript critically for important intellectual content. All the authors provided final approval of the version to be published.

Funding This study was supported in part by fundings from the IRCCS Policlinico San Matteo Foundation, Pavia, Italy.

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval The study was approved by the local ethical committee of the IRCCS Policlinico San Matteo Foundation (08004598/b).

Provenance and peer review Not commissioned; externally peer reviewed.

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2021-220219).

To cite D'Onofrio B, De Stefano L, Palermo BL, *et al*. *Ann Rheum Dis* 2021;**80**:1242–1243.

Received 22 February 2021
Accepted 19 May 2021

Published Online First 26 May 2021

Ann Rheum Dis 2021;**80**:1242–1243. doi:10.1136/annrheumdis-2021-220219

ORCID iDs
Bernardo D'Onofrio http://orcid.org/0000-0003-4122-5057
Carlomaurizio Montecucco http://orcid.org/0000-0001-8263-3925
Serena Bugatti http://orcid.org/0000-0002-5396-7077

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Ensuring tight control in patients with rheumatoid arthritis treated with targeted therapies during the COVID-19 pandemic using a telehealth strategy

COVID-19 pandemic in its early months has deeply influenced rheumatic patients' follow-up in terms of treatment adherence, disease control achieved with treat-to-target and tight-control strategies. Nationwide mitigation strategies such as confinement, travel restrictions and inadequate access to routine visits catalysed the rapid switch to remote rheumatologic consultations as an attempt to partially compensate for the decline of in-person outpatient visits.

This observational retrospective study was conducted to establish if the hybrid of in-person and telephone tight-control approach activated by our rheumatology unit in Milan (Italy) during the first lockdown (LD) period has been effective in maintaining remission in patients with rheumatoid arthritis (RA) treated with targeted therapies and to identify potential factors associated with its maintenance.

Data were extracted from a longitudinal observational registry (Eethics Committee 138_1999) including consecutive adult patients with RA treated with biologic or targeted synthetic drugs. During the first pandemic wave, before the visit, rheumatologists provided virtual care handled by telephone to assess the clinical status and to guarantee the absence of current contraindications to therapy. After tele counselling, based on the care required, patients could choose whether to convert the next appointments to a telephone visit and receive drug home delivery or to maintain their

Table 1 Results of the final model (stepwise selection) applied to the multivariate analysis of factors that potentially could interfere with disease control in patients with CDAI remission

	Adjusted OR (95% CI)	P value
Period (LD vs pre-LD)	1.24 (0.83 to 1.85)	0.292
Period (post-LD vs pre-LD)	1.22 (0.84 to 1.77)	0.299
Gender (male vs female)	2.26 (1.15 to 4.61)	0.020
Disease duration (≥10 years vs <10 years)	0.65 (0.36 to 1.15)	0.141
Ethnicity (Hispanic/Asian vs Caucasian)	0.32 (0.12 to 0.83)	0.021
Fibromyalgia (yes vs no)	0.30 (0.11 to 0.82)	0.021

CDAI, Clinical Disease Activity Index; LD, lockdown.

standard in-person consultation. For each patient, Clinical Disease Activity Index (CDAI)[1] was collected during face-to-face visits. Moreover, difficult-to-treat (D2T) patients with RA according to EULAR definition[2] were analysed in this study.

At baseline, 502 patients with RA were eligible for this study and they were followed-up over the first wave of the pandemic. Among these, 91 patients chose drug home delivery, 52 patients failed to complete their follow-up; all the 450 patients who completed the follow-up, were included in the final analysis (online supplemental figure 1S). The median age was 59.4 years (IQR 50.7–68.4), 370 (82.22%) were women, median disease duration was 13.9 years (IQR 7.9–22.5). More details are listed in online supplemental tables 1S and 2S. The CDAI remission rate was 40.22% (n=181) and 43.78% (n=197) during pre-LD and post-LD, respectively. As for the 359 patients who choose in-person visits during LD, 43.18% (n=155) were in remission state according to CDAI (online supplemental table 3S). Although our experience cannot be generalised, these percentages are similar to those of other European cohorts.[3 4]

To evaluate the effect of LD on the percentage of patients in remission, logistic mixed-effects regression models were fitted, with CDAI remission as a response variable (see online supplemental file for the statistical analysis). The analysis did not show a statistically significant decrease in the percentage of patients fulfilling CDAI remission all along the three periods (online supplemental table 4S). Moreover, the final model (stepwise selection) applied to the multivariate analysis of factors that potentially could interfere with disease control in patients with CDAI remission showed that the probability to be in remission was significantly associated with the male gender, while Hispanic or Asian ethnicity and presence of fibromyalgia showed a decreased odds for remission (table 1). These results confirm characteristics known to be predictive for clinical remission.[5]

Finally, 52 D2T patients with RA were evaluated in a hospital setting pre-LD and post-LD. Among them, 43 choose in-person visit during LD. Median values of CDAI during pre-LD, LD and post-LD were 14.5 (IQR 12–21), 9 (IQR 5.5–16) and 11 (IQR 6–19.2), respectively (online supplemental figure 2S).

Telephone-based tight-control strategy used during the first wave of COVID-19 pandemic ensured satisfactory management of RA treated with targeted therapies, even in D2T patients. Although during normal times, the patient–physician encounter is considered fundamental for rheumatic patients,[6] telemedicine was often the only way of practicing in the times of the pandemic.

In conclusion, the current pandemic has dramatically altered patterns of healthcare delivery. Although this temporary virtual approach is currently not spurred by regulatory changes, it seems to be a feasible compensation for face-to-face visits.

Francesca Ingegnoli [1,2] **Gilberto Cincinelli**[1,2] **Angela Flavia Luppino**[1,2] **Ennio Giulio Favalli** [2] **Annalisa Orenti**[1,3] **Patrizia Boracchi**[1,3] **Roberto Caporali**[1,2]

[1]Dept. of Clinical Sciences & Community Health, Research Center for Adult and Pediatric Rheumatic Diseases, Università degli Studi di Milano, Milano, Italy
[2]Dept of Rheumatology & Clinical Sciences, Clinical Rheumatology Unit, ASST Gaetano Pini-CTO, Milano, Italy
[3]Lab of Medical Statistics, Epidemiology and Biometry GA Maccacaro, Milano, Italy

Correspondence to Professor Francesca Ingegnoli, Department of Clinical & Community Science, University of Milano, UNIMI, Milano 20122, Italy; francesca.ingegnoli@unimi.it

Handling editor Josef S Smolen

Contributors All authors contributed to study design. FI, FL, GC contributed to data collection. All authors contributed to data analysis and interpretation. PB and AO contributed to statistical analysis. All authors contributed to drafting of the manuscript.

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval ethics Committee 138_1999

Provenance and peer review Not commissioned; externally peer reviewed.

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2021-220142).

To cite Ingegnoli F, Cincinelli G, Luppino AF, *et al*. *Ann Rheum Dis* 2021;**80**:1243–1245.

Received 10 February 2021
Revised 1 April 2021
Accepted 2 April 2021
Published Online First 13 April 2021

Ann Rheum Dis 2021;**80**:1243–1245. doi:10.1136/annrheumdis-2021-220142

ORCID iDs
Francesca Ingegnoli http://orcid.org/0000-0002-6727-1273
Ennio Giulio Favalli http://orcid.org/0000-0003-1471-6467

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Use of Janus kinase inhibitors in COVID-19: a prospective observational series in 522 individuals

Janus kinase (JAK) inhibitors for the treatment of hospitalised patients with COVID-19 have been extensively studied. Initially, at the start of the pandemic outside of China, baricitinib was shown using artificial intelligence to have a potential dual anti-cytokine and antiviral effect, computer predictions that were then supported by mechanistic data.[1–3] This included kinase assays demonstrating inhibition of host numb-associated kinases, notably AP-2-associated protein kinase 1 (AAK1) and cyclin G-associated kinase (GAK), responsible for activating protein-1 (AP-1)-mediated viral propagation and super-resolution microscopy which showed inhibition of SARS-CoV-2 entry into primary human liver spheroids.[4] Based on double-blind randomised data from the Adaptive COVID-19 Treatment Trial-II (ACTT-II) under the National Institutes of Allergy and Infectious Diseases,[5] it received an Emergency Use Authorisation from the United States Food and Drug Administration in November 2020, in combination with remdesevir for the treatment of hospitalised individuals with COVID-19.

We implemented an institutional review board approved multicentre observational cohort study in four hospitals in Moscow, Russia, to both administer and collect clinical data on individuals treated with this class of drug. Data were prospectively obtained, focusing on the primary outcome of death. Secondary variables include duration of hospitalisation, severity of COVID-19 at admission, severity of pneumonia at imaging (CT0–CT4), requirement for mechanical ventilation, intensive care unit admission, thrombotic events, pulmonary emboli and secondary infectious complications. A total of 522 individuals between May and September 2020 were treated with either baricitinib or tofactinib, orally for 7–14 days. All the patients were hospitalised COVID-19 cases. Individuals with rheumatic or inflammatory bowel disease treated with JAK inhibitors were excluded.

All patients hospitalised from May to September 2020 were analysed for the purposes of the study. In those individuals treated with tofacitinib (n=320: 10 mg n=44; 20 mg n=276), 293 patients (91.6%) recovered, and 27 (8.4%) died. The mortality rate was 2.4% in patients younger than 65 years (5/210 patients) and 20% in patients of 65 years and older (22/110 patients), as shown in table 1. In those who received baricitinib (n=202: 4 mg n=52, 8 mg n=150), 193 patients (95.5%) recovered, and 9 (4.5%) died. The mortality rate measured 2.1% in patients younger than 65 years (3/146) and 10.7% in patients of 65 years and older (6/56) (table 2). With regards to imbalance in dexamethasone treatment, we may suppose that baricitinib was administered to patients with less severe disease (98% mild and moderate) than tofacitinib (84%). No tests was applied to evaluate the statistical significance of difference for 'COVID-19 severity' and 'lung involvement' because to compare baricitinib and tofacitinib treatments was not the objective of the study.

Table 1 Clinical outcomes in patients with COVID-19 treated with tofacitinib

	All cases	<65 years old	≥65 years old
Population			
Number of patents, n (%)	320 (100)	210 (66)	110 (34)
Female, %	50	46	57
Mean age (range), years	59 (22–96)	52 (22–64)	74 (65–96)
Mean treatment duration (range), days	7 (1–18)	6 (1–17)	7 (1–18)
Dexamethasone, %	30.0	30.0	30.0
Disease (on admission)			
Clinical severity, %			
Mild	4.7	3.8	6.4
Moderate	79.7	83.3	72.7
Severe	15.0	11.9	20.9
Critical	0.6	1.0	0.0
Lung involvement, %			
CT 0	0.0	0.0	0.0
CT 1	10.9	10.0	12.7
CT 2	65.0	68.6	58.2
CT 3	22.8	20.0	28.2
CT 4	1.3	1.4	0.9
C reactive protein: clinically significant abnormality, %	73	74	71
Outcomes			
Death, n (%)	27 (8.4)	5 (2.4)	22 (20.0)
Mean days from hospitalisation till death (range), days	13 (4–60)	17 (9–34)	12 (0–33)
ICU admission, n (%)	65 (20)	28 (13)	37 (34)
Mean stay in ICU (range), days	7 (1–28)	7 (1–28)	7 (1–24)
Mechanical ventilation, n (%)	28 (8.8)	11 (5.2)	17 (15.5)
Mean duration of mechanical vent. (range), days	5 (1–26)	9 (1–26)	3 (1–6)
Safety			
Thromboses, n (%)	7 (2.2)	2 (1.0)	5 (4.6)
Pulmonary embolism, n (%)	3 (0.9)	0 (0.0)	3 (2.7)
Infectious complications, n (%)	22 (6.9)	9 (4.3)	13 (11.8)

ICU, intensive care unit.

In general, we observed that JAK inhibitors were well tolerated with a low rate of complications. Clot risk during infection with SARS-CoV-2 is well described and mechanisms include activation of platelet-associated genes.[4] Concerns regarding a prothrombotic tendency based on these data and previous studies[5] appear unfounded in the context of SARS-CoV-2 infection, despite some concerns from previous trials in rheumatoid arthritis; real-world data outside the setting of COVID-19 have not suggested an increased clot incidence.[6] As these data are not randomised and lack a comparator arm, we cannot draw conclusions regarding the efficacy of these drugs, but their oral use, lack of drug–drug interactions, short half-life with excretion via the renal system largely unchanged and dosing flexibility supports the use of these medicines in resource constrained or out-patient settings. As recently highlighted,[7] drugs such as baricitinib appear to fulfil an unmet clinical need in the treatment of

Table 2 Clinical outcomes in COVID-19 patients treated with baricitinib

	All cases	<65 years old	≥65 years old
Population			
Number of patents	202	146	56
Female, %	48	47	52
Mean age (range), years	58 (25–92)	52 (25–64)	75 (65–92)
Mean treatment duration (range), days	6 (1–35)	6 (1–11)	7 (1–35)
Dexamethasone, %	7.4	7.5	7.1
Disease (on admission)			
Clinical severity, %			
Mild	3.0	3.4	1.8
Moderate	95.0	95.2	94.6
Severe	2.0	1.4	3.6
Critical	0	1.0	0
Lung involvement, %			
CT 0	0	0	0
CT 1	8.0	7.5	19.0
CT 2	71.2	68.5	78.5
CT 3	20.8	24.0	12.5
CT 4	0	0	0
C reactive protein: clinically significant abnormality, %	95	92	100
Outcomes			
Death, n (%)	9 (4.5)	3 (2.1)	6 (10.7)
Mean from hospitalisation till death (range), days	12 (2–32)	14 (2–32)	12 (5–20)
ICU admission, n (%)	19 (9.4)	10 (6.9)	9 (16.1)
Mean stay in ICU (range), days	7 (1–30)	9 (1–30)	5 (1–13)
Mechanical ventilation, n (%)	8 (4.0)	4 (2.8)	4 (7.1)
Mean duration of mechanical vent. (range), days	7 (2–22)	9 (3–22)	6 (2–13)
Safety			
Thromboses, n (%)	1 (0.5)	0 (0)	1 (1.8)
Pulmonary embolism, n (%)	1 (0.5)	0 (0)	1 (1.8)
Infectious complications, n (%)	7 (3.5)	4 (2.8)	3 (5.4)

ICU, intensive care unit.

COVID-19 pneumonia. Ongoing studies such as ACTT-IV will help delineate its role versus dexamethasone.

Oleg Melikhov ,[1] **Tatiana Kruglova,**[2] **Karine Lytkina,**[3] **Georgy Melkonyan,**[3] **Elena Prokhorovich,**[4] **Gleb Putsman,**[5] **Grigory Rodoman,**[5] **Arkady Vertkin,**[4] **Alena Zagrebneva,**[2] **Justin Stebbing**[6]

[1]Institute of Clinical Research, Moscow, Russian Federation
[2]City Clinical Hospital No 52, Moscow, Russian Federation
[3]City Clinical Hospital No 4, Moscow, Russian Federation
[4]Moscow State University of Medicine and Dentistry n.a. AI Yevdokimov, Moscow, Russian Federation
[5]City Clinical Hospital No 24, Moscow, Russian Federation
[6]Imperial College London, London, UK

Correspondence to Dr Oleg Melikhov, Institut klinicheskih issledovanij OOO, Moskva 119590, Russian Federation; melikhov.oleg@gmail.com

Handling editor Josef S Smolen

Acknowledgements We acknowledge the kind help of Ekaterina Makarova and Svetlana Elkonina.

Contributors All authors contributed to writing the manuscript and approved the final version. OM and AZ contributed to conceptualisation, methodology, formal analysis, providing the resources, funding acquisition and project administration (responsible for overall content—guarantor). KL, TK, EP, GM, GP, GR, AV and JS contributed to data collection and analysis.

Funding Pfizer provided financial support for study design. Grant ID#61 448 843. The decision to submit the article for publication was made by the authors.

Competing interests JS conflicts are disclosed here: https://www.nature.com/onc/editors.

Patient consent for publication Not required.

Ethics approval The study was approved on 19 June 2020 by Independent Interdisciplinary Ethics Committee (Leningrandsky prosp., 51, Moscow, 125468, Russian Federation).

Provenance and peer review Not commissioned; externally peer reviewed.

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2021-220049).

To cite Melikhov O, Kruglova T, Lytkina K, *et al*. *Ann Rheum Dis* 2021;**80**:1245–1246.

Received 1 February 2021
Accepted 25 May 2021
Published Online First 7 June 2021

Ann Rheum Dis 2021;**80**:1245–1246. doi:10.1136/annrheumdis-2021-220049

ORCID iD
Oleg Melikhov http://orcid.org/0000-0001-9442-7707

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Microarray evaluation of allergen-specific IgE in eosinophilic granulomatosis with polyangiitis

The pathogenesis of asthma and ear–nose–throat (ENT) manifestations in eosinophilic granulomatosis with polyangiitis (EGPA) is still poorly understood. Asthma is present in almost all patients with EGPA.[1] Severe or uncontrolled asthma occurs in more than 40% of patients and its severity correlates with serum IgE (sIgE) levels.[2] However, sIgE towards common allergens are detectable in less than one-third of patients with EGPA using conventional diagnostic tests.[3] This suggests either that atopy is not a key pathogenic mechanism in EGPA or that uncommon antigens are involved. Our study assessed IgE specificity in EGPA using microarray technologies which have higher diagnostic reliability than traditional assays and offer a wider representation of the IgE repertoire.[4 5] We measured sIgE towards 112 purified or biotechnologically produced allergenic molecules using the ImmunoCAP Immuno Solid-phase Allergen Chip (ISAC) (online supplemental methods). Results are reported in ISAC standardised units (ISU). The study population comprised 29 patients with EGPA, evaluated during active and inactive disease (patients' characteristics are reported in the online supplemental table 1), 30 patients with atopic asthma, 31 with active anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) (20 with granulomatosis with polyangiitis and 11 with microscopic polyangiitis) and 30 healthy controls (online supplemental methods). Positive IgE (ISU>0.3) in at least 5% of the whole study population were detected for 35 allergen components. We assessed for each of these 35 components the percentage of study subjects with low (0.3–0.9 ISU), moderate/high (1.0–14.9 ISU) and very high reactivity (≥15.0 ISU). The 35 allergen components were also divided based on the allergen families they belonged to, namely, food, respiratory and non-food non-respiratory ('other') allergens. The percentage of patients positive for at least one family component was calculated.

Active and inactive EGPA displayed a lower reactivity compared with the asthmatic group (figure 1A). This difference was particularly pronounced for respiratory allergens (active EGPA (55.0%) vs asthma (86.8%), p=0.01; inactive EGPA (38.0%) vs asthma (86.8%), p=0.0001) and 'other' allergens (active EGPA (13.7%) vs asthma (50.0%), p=0.004; inactive EGPA (17.0%) vs asthma (50.0%), p=0.01) (figure 1B). There were no allergen components towards which sIgE were higher in EGPA than in asthmatics. Active and inactive EGPA displayed a similar reactivity when compared with one another or to healthy controls. Patients with AAV showed a markedly lower IgE reactivity compared with patients with EGPA and asthma (figure 1A,B). This reactivity pattern did not change when we considered a threshold of 2% rather than 5% (data not shown).

No significant differences in allergen-specific sIgE positivity were found between ANCA-positive and ANCA-negative patients. Additionally, none of the EGPA symptoms typically considered 'allergic' (eg, ENT and lung involvement, urticaria) were associated with sIgE positivity (either for food, respiratory or other allergens).

Our results, obtained using a sensitive microarray technology able to detect sIgE specific for a large allergen component panel, show that patients with EGPA have a lower reactivity towards common allergens when compared with asthmatic subjects. This is apparently in contrast with the almost universal presence of asthma and 'allergic' manifestations in EGPA. However, this corroborates previous findings obtained with traditional allergy tests, which showed that the

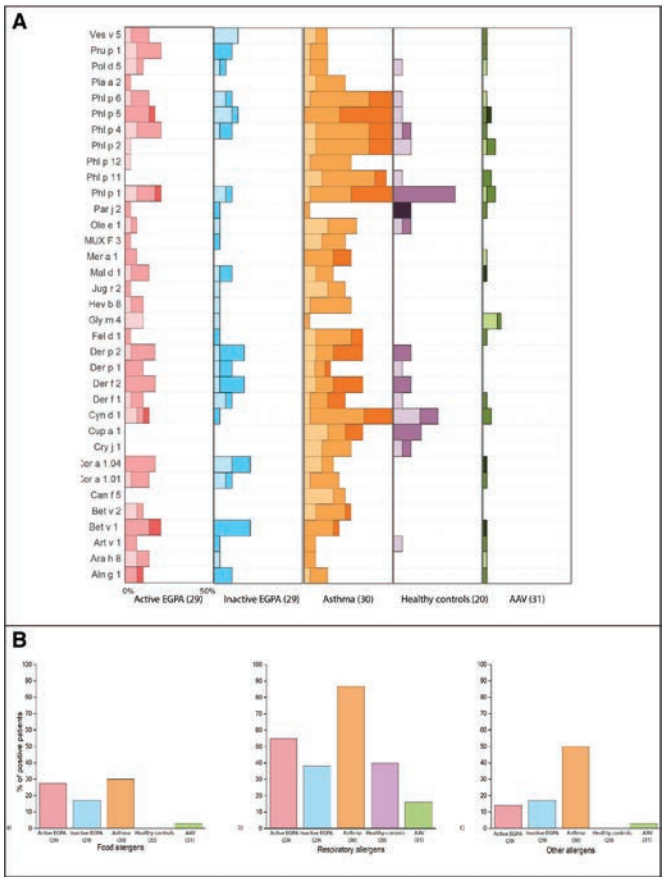

Figure 1 IgE towards common allergens in eosinophilic granulomatosis with polyangiitis (EGPA) and control groups. (A) On the Y axis, the 35 allergens for which serum IgE (sIgE) were positive in at least 5% of the study population are shown. The three shades of each colour correspond to the levels of reactivity in each study subgroup, that is, low (ISU-E 0.3–0.9), moderate high (ISU-E 1.0–14.9) and very high (ISU-E≥15.0). On the X axis, the percentages for every subgroup are expressed on a scale ranging from 0% to 50%. Allergen abbreviations are expanded in the online supplemental table 2. The number of patients per group is reported in parentheses on the X axis labels. (B) Reactivity for the different allergen families (food allergens, panel a; respiratory allergens, panel b; other allergens, panel c) in active and inactive EGPA patients (rose and blue colour, respectively) and in the control groups (orange for asthmatic patients, violet for healthy controls and green for AAV patients). Both active and inactive EGPA patients displayed lower reactivity compared with asthmatic subjects, particularly for respiratory allergens (active EGPA 55.0% vs asthma 86.8%, p=0.01; inactive EGPA 38.0% vs asthma 86.8%, p=0.0001) and 'other' allergens (active EGPA 13.7% vs asthma 50.0%, p=0.004; inactive EGPA 17.0% vs asthma 50.0%, p=0.01). Active, but not inactive, EGPA displayed higher reactivity than the other anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV), particularly towards respiratory and food allergens (respiratory: active EGPA 55.0% vs AAV 16.0%, p=0.002; food: active EGPA vs AAV 27.5% vs 3.0%, p=0.01). No significant differences were found between EGPA (active and inactive) and healthy controls in terms of allergen reactivity. The number of patients per group is reported in parentheses on the X axis labels. ISU, ISAC standardised units.

prevalence of atopy is significantly lower in EGPA than in asthmatics. Also, patients with EGPA do not suffer from seasonal allergies as do atopic asthmatics.[3] This may reflect different pathogenic mechanisms underlying atopic asthma and asthma occurring in EGPA. In line with this, omalizumab (anti-IgE monoclonal antibody) demonstrated only limited efficacy in EGPA.[6] To conclude, common allergen-induced IgE responses seem to have a marginal role in EGPA pathogenesis; therefore, further studies are needed to dissect the immunological circuits that drive 'allergic' manifestations in EGPA.

Federica Bello ,[1] **Federica Maritati,**[2] **Antonella Radice,**[3] **Renato Alberto Sinico,**[4,5] **Giacomo Emmi** ,[6] **Augusto Vaglio** [1]

[1]Biomedical Experimental and Clinical Sciences, University of Florence, Firenze, Italy
[2]Nephrology, Sant'Orsola Hospital, Bologna, Italy
[3]Microbiology Institute, ASST Santi Paolo e Carlo, Milan, Italy
[4]Medicine and Surgery, Universita degli Studi di Milano-Bicocca Scuola di Medicina e Chirurgia, Monza, Italy
[5]Clinical Nephrology, Azienda Socio Sanitaria Territoriale di Monza, Monza, Italy
[6]Department of Experimental and Clinical Medicine, University of Florence, Firenze, Italy

Correspondence to Dr Augusto Vaglio, University of Florence, Firenze 50139, Italy; augusto.vaglio@virgilio.it

Handling editor Josef S Smolen

Contributors FB analysed the data, created the figure and tables, and drafted the manuscript. FM contributed to data analysis and patient recruitment. AR contributed to data analysis and patient recruitment. RAS contributed to data analysis and patient recruitment. GE contributed to data analysis and to manuscript revision. AV designed the study, analysed the data, contributed to patient recruitment and revised the manuscript.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval The study was approved by the Ethics Committee of the University of Florence, protocol #120/2019.

Provenance and peer review Not commissioned; externally peer reviewed.

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2021-220111).

To cite Bello F, Maritati F, Radice A, *et al*. *Ann Rheum Dis* 2021;**80**:1247–1248.

Received 7 February 2021
Revised 3 April 2021
Accepted 6 April 2021
Published Online First 21 April 2021

Ann Rheum Dis 2021;**80**:1247–1248. doi:10.1136/annrheumdis-2021-220111

ORCID iDs
Federica Bello http://orcid.org/0000-0003-2878-2254
Giacomo Emmi http://orcid.org/0000-0002-3394-1451
Augusto Vaglio http://orcid.org/0000-0002-3814-9172

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Medications associated with fracture risk in patients with rheumatoid arthritis

Statins have been widely used to control dyslipidaemia, and there is strong evidence for beneficial effects for patients at risk for cardiovascular diseases.[1 2] In particular, statins may influence bone metabolism by increasing bone formation.[3 4] Recently, Ozen *et al*[5] reported that medication with opioids, selective serotonin reuptake inhibitors, and glucocorticoids were associated with an increased risk of osteoporosis-related site fractures (vertebra, hip, forearm and humerus) in patients with rheumatoid arthritis, whereas statins and tumour necrosis factor-α inhibitors were associated with a decreased risk of vertebral fractures. These findings were published in the August 2019 issue of the *Annals of the Rheumatic Diseases*. Certainly, the findings of Ozen *et al*[5] will be significant for clinicians; however, four points remain unaddressed that we would like to communicate with the authors.

First, the prevalence of osteoporotic fracture has been reported to be significantly higher in patients with chronic diseases compared with healthy subjects, particularly in women.[6–8] The association between chronic diseases and osteoporosis-related fracture (OF) has been reported in many studies. For example, a cross-sectional study by Watanabe *et al*[8] reported that the prevalence of osteoporotic vertebral fracture was as high as 79.4% in Japanese men with chronic obstructive pulmonary diseases (COPD). Similarly, Reyes *et al*[9] reported an independent association between COPD and an increased risk of hip OF in Catalonians. The results of the Ozen *et al*[5] study are in direct contrast with these other studies and demonstrated that the comorbidity of rheumatoid arthritis in statin-treated patients with dyslipidaemia affected the risk of OF. The differences between Ozen *et al*[5] findings and those of previous studies[6–9] may be attributed to the differences in baseline patient characteristics and the effects of the statin.

Second, statins are effective agents that control dyslipidaemia and are widely used in the prevention of cardiovascular diseases.[10] In addition, statins may influence bone metabolism by increasing bone formation.[11] However, the risk reduction among statin users might be that a high dose–response effect on OF risk was observed in Ozen *et al*'[5] s study. In the past, we have demonstrated that high exposure to statins has the dose–response effect of lowering new-onset dementia risk.[12]

Furthermore, in another study, we reported a beneficial effect of statin use with regard to OF risk, but not all statins.[13] The patients who took atorvastatin or rosuvastatin were at a lower risk of OF, whereas the use of lovastatin was associated with a significantly increased risk of developing new-onset OF (NOF) during the 10-year follow-up. It was also highlighted that a lower risk of NOF was associated with the more commonly prescribed high-potency statins.[13]

Third, it should be noted that OF may result from accidental occurrences, such as falls.[14] Patients with chronic disease experience muscle weakness, mobility impairment and exercise intolerance, and are prone to falls. An observational cohort study reported that COPD was associated with the increased risk of falls (OR, 1.6) compared with patients without COPD.[15] However, in this study have examined only the association between osteoporosis-related site fractures (vertebra, hip, forearm and humerus) and the medications taken by patients with rheumatoid arthritis.[5] Thus, the observed OF and associations may be underestimated in this study.

Fourth, many medications were associated with OF and included statins, antidepressants, proton-pump inhibitors, opioids, non-steroidal anti-inflammatory drugs, anticonvulsants, antipsychotics, benzodiazepines and antihypertensives.[16–18] Previous observational studies have shown that antihypertensives use has a positive or negative effect on emerging OF.[18 19] In a case–control study from Denmark[19] that evaluated 124 655 cases and 373 962 controls with hypertension, the investigators found that the risk of OF was lower among users of calcium channel blockers (OR, 0.94; 95% CI, 0.91 to 0.96) than among non-users. On the other hand, patients who took ACE inhibitors (OR, 1.64; 95% CI, 1.01 to 2.66) were at a higher risk of developing OF than non-users in our previous study.[20] Since the data for antihypertensives, such as diuretics, beta-blockers, calcium channel blockers, alpha-blockers, ACE inhibitors, and angiotensin receptor blockers were not available from this study,[5] there might be residual confounding bias because of the unmeasured factors.

Yi-Sheng Liou,[1,2] Tsung-Kun Lin,[3,4] Hung-Yi Chen,[5,6] Gwo-Ping Jong [7,8]

[1]Department of Family Medicine and Geriatrics, Taichung Veterans General Hospital, Taichung, Taiwan
[2]School of Public Health, National Defense Medical Center, Taipei, Taiwan
[3]Department of Pharmacy, Taoyuan Armed Forces General Hospital, Lungtan, Taiwan
[4]School of Pharmacy, National Defense Medical Center, Taipei, Taiwan
[5]Department of Pharmacy, China Medical University, Taichung, Taiwan
[6]Department of Pharmacy, China Medical University Beigang Hospital, Beigang, Taiwan
[7]Department of Internal Medicine, Chung Shan Medical University Hospital, Taichung, Taiwan
[8]Department of Medicine, Chung Shan Medical University, Taichung, Taiwan

Correspondence to Dr Gwo-Ping Jong, Department of Internal Medicine, Chung Shan Medical University Hospital, Taichung 40201, Taiwan; cgp8009@yahoo.com.tw

Contributors All authors were involved in the study conception, manuscript drafting and revising and final approval of the submitted version.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; internally peer reviewed.

Y-SL and H-YC contributed equally.

To cite Liou Y-S, Lin T-K, Chen H-Y, *et al*. *Ann Rheum Dis* 2021;**80**:e140.

Received 4 September 2019
Accepted 8 September 2019
Published Online First 17 September 2019

► http://dx.doi.org/10.1136/annrheumdis-2019-216293

Ann Rheum Dis 2021;**80**:e140. doi:10.1136/annrheumdis-2019-216262

ORCID iD
Gwo-Ping Jong http://orcid.org/0000-0002-7786-5497

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Response to: 'Medications associated with fracture risk in patients with rheumatoid arthritis' by Chen *et al*

We appreciate the interest shown by Dr Chen[1] concerning our recent study on medications associated with fracture risk in patients with rheumatoid arthritis (RA).[2] We would like to clarify some issues Chen *et al* indicated.

First, we agree that osteoporotic fractures are more common in patients with inflammatory diseases. However, in our study, we excluded all patients with prevalent fractures to estimate the risk related to new fractures. Our study only shows the osteoporosis site fracture incidence in patients with RA. As we indicated in our manuscript, the incidence rate of osteoporotic fractures in our cohort is similar to a recent meta-analysis of 25 cohort studies in patients with RA.[3]

Second, it is possible that high-intensity statin regimens may prevent fractures better than moderate-intensity ones especially if the protective effects are assumed to be due to anti-inflammatory properties of statins. Moreover, as Chen *et al* indicate, some antihypertensives may have protective effects on osteoporotic fracture risk. However, the only randomised controlled trial for this association is with thiazide diuretics[4 5] of which effect is also more biologically plausible compared with other antihypertensives.[6] The fracture risk change with high-intensity statins and antihypertensives should be investigated further in patients with RA.

Lastly, we appreciate the comment about our outcome-only osteoporosis site fractures. It is well-known that patients with RA have increased fall risk, which can also cause fractures. Unfortunately, we do not have any data for the level of trauma and fall risk other than disability scores. Regardless, we believe that including all types of fractures would make our outcome very heterogeneous. A significant trauma could cause a fracture in any patient even in the absence of risk factors. We are planning to collect more data regarding fall risk to better assess the fracture risk in our cohort.

Gulsen Ozen [1], **Kaleb Michaud** [1,2]

[1]Internal Medicine, University of Nebraska Medical Center, Omaha, Nebraska, USA

[2]FORWARD, The National Databank for Rheumatic Diseases, Wichita, Kansas, USA

Correspondence to Dr Kaleb Michaud, National Data Bank for Rheumatic Diseases, Wichita, KS 67214, USA; kmichaud@unmc.edu

Handling editor Josef S Smolen

Twitter Kaleb Michaud @Dr_K

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Commissioned; internally peer reviewed.

To cite Ozen G, Michaud K. *Ann Rheum Dis* 2021;**80**:e141.

Received 1 October 2019
Accepted 1 October 2019
Published Online First 10 October 2019

Linked

► http://dx.doi.org/10.1136/annrheumdis-2019-216262

Ann Rheum Dis 2021;**80**:e141. doi:10.1136/annrheumdis-2019-216293

ORCID iDs
Gulsen Ozen http://orcid.org/0000-0002-5423-393X
Kaleb Michaud http://orcid.org/0000-0002-5350-3934

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Diabetes mellitus in ankylosing spondylitis

We read with interest the article by Liao *et al*[1] regarding diabetes mellitus in ankylosing spondylitis and the meta-analysis by Mathieu *et al*. This nationwide cohort study demonstrated that the overall incidence of diabetes mellitus was 1.21-fold higher in the ankylosing spondylitis group than in the non-ankylosing spondylitis group,[1] and Mathieu *et al*'s research showed an increased cardiovascular events in ankylosing spondylitis.[2] The results of Liao *et al* are in concordance with those of a previous cohort study by Chen *et al* which showed that the incidence of diabetes mellitus was 1.17-fold higher in the ankylosing spondylitis cohort than in the non-ankylosing spondylitis cohort, with an adjusted HR of 1.16.[3] However, some methodological issues must be discussed. First, the definition of the control group used in this cohort study is too broad. What does the non-ankylosing spondylitis group mean? Second, the statistical robustness of cohort studies is lower than that of randomised trials because of potential biases related to adjustments for confounding variables. Many unhealthy lifestyle habits, including unhealthy dietary patterns, decreased physical activity, sedentary lifestyle and smoking, have been found to be associated with an increased risk of diabetes mellitus.[4] In addition, patients with hypertension are more likely to have diabetes mellitus through increased insulin resistance.[5] To reduce the effect of potential confounding in observational studies, it would be appropriate to analyse data that is adjusted to account for confounding factors. Third, observational studies are prone to bias, such as reverse causation and residual confounding, thereby precluding a clear understanding of the association between ankylosing spondylitis and diabetes mellitus. Although the investigation of the mechanism underlying the association between ankylosing spondylitis and diabetes mellitus is beyond the scope of this study, further study using Mendelian randomisation, a technique that uses genetic variants as instrumental variables, is needed to assess whether an observational association between a risk factor and an outcome is consistent with a causal effect.[6]

Young Ho Lee , **Gwan Gyu Song**

Rheumatology, Korea University, Seongbuk-gu, The Republic of Korea

Correspondence to Professor Young Ho Lee, Rheumatology, Korea University, Seongbuk-gu 02841, The Republic of Korea; lyhcgh@korea.ac.kr

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; internally peer reviewed.

To cite Lee YH, Song GG. *Ann Rheum Dis* 2021;**80**:e142.

Received 18 September 2019
Revised 2 October 2019
Accepted 3 October 2019
Published Online First 15 October 2019

Linked

► http://dx.doi.org/10.1136/annrheumdis-2019-216429

Ann Rheum Dis 2021;**80**:e142. doi:10.1136/annrheumdis-2019-216335

ORCID iD
Young Ho Lee http://orcid.org/0000-0003-4213-1909

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Response to: 'Diabetes mellitus in ankylosing spondylitis' by Lee and Song

We would like to thank Dr Lee and Dr Song for their correspondence to our preliminary study recently published in *Annals of the Rheumatic Diseases*.[1 2] Dr Lee and Dr Song have raised some concerns. We make a response as follows. First, the non-ankylosing spondylitis group included persons without a diagnosis code of ankylosing spondylitis (based on International Classification of Diseases, Ninth Revision code, 720.0). The non-ankylosing spondylitis group was sex-matched and age-matched with the ankylosing spondylitis group. Second, this was only a preliminary analysis. Confounding variables were not included for adjustment. Dr Lee and Dr Song's good comments indicate a future research direction. Additional studies are required to include confounding variables which are associated with the risk of diabetes mellitus, such as unhealthy dietary pattern, decreased physical activity, high sedentary time, smoking and obesity.[3 4] Third, we are not familiar with Mendelian randomisation. We do not have any comment why Mendelian randomisation can assess an observational association between a risk factor and an outcome. Fourth, we agree with Dr Lee and Dr Song that the statistical robustness of our preliminary study is low. The causal relationship between ankylosing spondylitis and diabetes mellitus has not yet been determined. We suggest that more robust real-world data, such as the Korean database, would of course be needed to clarify whether there is an association between ankylosing spondylitis and diabetes mellitus.

Shih-Wei Lai ,[1,2] **Yu-Hung Kuo**,[3] **Kuan-Fu Liao**[4,5]

[1]College of Medicine, China Medical University, Taichung, Taiwan
[2]Department of Family Medicine, China Medical University Hospital, Taichung, Taiwan
[3]Department of Research, Taichung Tzu Chi Hospital, Taichung, Taiwan
[4]College of Medicine, Tzu Chi University, Hualien, Taiwan
[5]Division of Hepatogastroenterology, Department of Internal Medicine, Taichung Tzu Chi Hospital, Taichung, Taiwan

Correspondence to Dr Kuan-Fu Liao; kuanfuliaog@gmail.com

Handling editor Josef S Smolen

Contributors S-WL, Y-HK and K-FL contributed to the conception of the article, initiated the draft of the article and have approved the final draft submitted.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Commissioned; internally peer reviewed.

To cite Lai S-W, Kuo Y-H, Liao K-F. *Ann Rheum Dis* 2021;**80**:e143.

Received 4 October 2019
Revised 7 October 2019
Accepted 8 October 2019
Published Online First 15 October 2019

Linked

► http://dx.doi.org/10.1136/annrheumdis-2019-216335

Ann Rheum Dis 2021;**80**:e143. doi:10.1136/annrheumdis-2019-216429

ORCID iD
Shih-Wei Lai http://orcid.org/0000-0002-7420-1572

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Incidence of inflammatory bowel disease in patients with ankylosing spondylitis

Ankylosing spondylitis is a long-term inflammatory disease that always affects the spine joints. The association between ankylosing spondylitis and other diseases has been extensively assessed.[1 2] Recently a cohort study conducted by Schreiber *et al* published in *Annals of the Rheumatic Diseases* found that the new-onset cases of inflammatory bowel disease were uncommon in patients with ankylosing spondylitis on secukinumab therapy (1.13%, 9/794).[3] In order to examine the association between ankylosing spondylitis and inflammatory bowel disease in a different country, a preliminary cohort study was undertaken using the 2005–2012 database of the Taiwan National Health Insurance Programme with 23 million residents living in Taiwan.[4] Subjects ages 20–84 with a new diagnosis of ankylosing spondylitis were identified as the ankylosing spondylitis group (International Classification of Diseases, Ninth Revision code (ICD-9 code 720.0)). For every subject with ankylosing spondylitis, four sex-matched and age-matched subjects who did not have a diagnosis of ankylosing spondylitis were assigned as the non-ankylosing spondylitis group. The main outcome was a new diagnosis of inflammatory bowel disease (ICD-9 code 555–556). Table 1 presents that the overall incidence of inflammatory bowel disease was lower in the ankylosing spondylitis group than in the non-ankylosing spondylitis group, but without reaching statistical significance (1.41 vs 1.79 per 1000 person-years, incidence rate ratio 0.79, 95% CI 0.48 to 1.28; p=0.332). As stratified by sex and age, there was no statistical significance in the incidence of inflammatory bowel disease between the ankylosing spondylitis group than the non-ankylosing spondylitis group.

Some caveats are discussed. Previous studies found that the prevalence of ankylosing spondylitis in patients with inflammatory bowel disease was around 3.7%–4.5%.[5 6] One review found that the prevalence of inflammatory bowel disease in patients with ankylosing spondylitis was around 6%–14%.[7] Due to both conditions likely occurring concomitantly, some researchers suggest that ankylosing spondylitis and inflammatory bowel disease might share a similar pathogenesis.[8] Therefore, ankylosing spondylitis and inflammatory bowel disease might develop in the same patient, but both conditions do not have a causal relationship, which is partially confirmed by our present study. Physicians who participate in care of patients with ankylosing spondylitis should take into consideration the possibility of inflammatory bowel disease, and vice versa.

Shih-Wei Lai ,[1,2] **Yu-Hung Kuo**,[3] **Kuan-Fu Liao**[4,5]

[1]College of Medicine, China Medical University, Taichung, Taiwan
[2]Department of Family Medicine, China Medical University Hospital, Taichung, Taiwan
[3]Department of Research, Taichung Tzu Chi Hospital, Taichung, Taiwan
[4]College of Medicine, Tzu Chi University, Hualien, Taiwan
[5]Division of Hepatogastroenterology, Department of Internal Medicine, Taichung Tzu Chi Hospital, Taichung, Taiwan

Correspondence to Dr Kuan-Fu Liao; kuanfuliaog@gmail.com

Handling editor Josef S Smolen

Contributors S-WL contributed to the conception of the article, initiated the draft of the article, and has approved the final draft submitted. Y-HK and K-FL conducted data analysis.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; internally peer reviewed.

To cite Lai S-W, Kuo Y-H, Liao K-F. *Ann Rheum Dis* 2021;**80**:e144.

Received 24 September 2019
Accepted 26 September 2019
Published Online First 14 October 2019

Ann Rheum Dis 2021;**80**:e144. doi:10.1136/annrheumdis-2019-216362

ORCID iD
Shih-Wei Lai http://orcid.org/0000-0002-7420-1572

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Table 1 Incidence of inflammatory bowel disease between ankylosing spondylitis group and non-ankylosing spondylitis group

	Ankylosing spondylitis				Non-ankylosing spondylitis					
Variable	N	Event	Person- years	Incidence	N	Event	Person- years	Incidence	Incidence rate ratio (95% CI)*	P value
All	3003	18	12 790	1.41	12 012	166	92 712	1.79	0.79 (0.48 to 1.28)	0.332
Sex										
Male	1767	10	7656	1.31	7068	101	54 383	1.86	0.70 (0.37 to 1.35)	0.289
Female	1236	8	5134	1.56	4944	65	38 329	1.70	0.92 (0.44 to 1.91)	0.821
Age group (years)										
20–39	1280	7	5607	1.25	5120	67	39 390	1.70	0.73 (0.34 to 1.60)	0.436
40–64	1342	8	5579	1.43	5368	73	41 488	1.76	0.81 (0.39 to 1.69)	0.583
65–84	381	3	1604	1.87	1524	26	11 834	2.20	0.85 (0.26 to 2.81)	0.792

Incidence: per 1000 person-years.
*Incidence rate ratio: ankylosing spondylitis vs non-ankylosing spondylitis (95% CI).

Application of MS score in macrophage activation syndrome patients associated with adult onset Still's disease

We read with great interest the article by Minoia *et al* which named development and initial validation of the macrophage activation syndrome (MAS)/systemic juvenile idiopathic arthritis (sJIA) (MS) score for diagnosis of MAS in sJIA.

MAS is a life-threatening complication of rheumatic disorders, including sJIA, adult-onset Still's disease (AOSD) and lupus.[1–4] Timely diagnosis and appropriate treatment of MAS are particularly important to improve the prognosis of MAS patients. At present, hemophagocytic lymphohistiocytosis (HLH)-2004 and HLH-2009 criteria are widely used to identify MAS associated with AOSD. Hemophagocytic syndrome diagnostic (HS) score was developed previously to facilitate MAS recognition, but still requires validation.[5] In 2019, Francesca Minoia *et al* reported a MS score for classification of sJIA-associated MAS patients.[6] Considering that sJIA and AOSD are thought to constitute the same disease entity occurring at different ages, we intended to evaluate the application of MS score in AOSD-associated MAS patients.

We collected AOSD patients from 1 January 2012 to 31 July 2019 from six centres across China. Patients were included in this study if they were older than 18 years of age, and met the Yamagishi criteria for a diagnosis of AOSD. MAS was diagnosed using the HLH-2004 diagnostic criteria, and the diagnosis was confirmed by the attending rheumatologists. Clinical information was recorded and analysed. MS score was calculated for each patient according to the previous report.

A total of 450 AOSD patients (60 AOSD associated MAS, 390 AOSD without MAS) were included in this study. Clinical features and lab results as the time of MAS diagnosis were shown in table 1. The application of the MS score (≥−2.1) yielded a sensitivity of 100%, a specificity of 29.85%, a positive predictive rate of 36.15%, a negative predictive rate of 100% in the diagnosis of AOSD-MAS with a Kappa value of 0.320. However, a further receiver operator characteristic curve analysis suggested that setting −1.08 as the score cut-off could provide the best discrimination between AOSD with and without MAS (figure 1). MS score ≥−1.08 yielded a sensitivity of 94.10%, a specificity of 95.00% in the diagnosis of MAS associated with AOSD. The positive predictive rate was 99.19% and the negative predictive rate of 71.25%, with a Kappa value of 0.781.

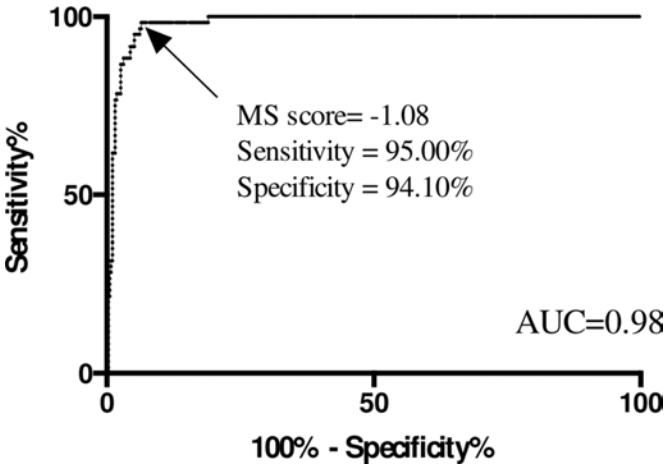

Figure 1 Modified criteria of MS score in the diagnosis of AOSD associated MAS. In a febrile patient with AOSD, the diagnosis of MAS should be considered if the MS score is ≥-1.08. The area under the curve (AUC) of the model is 0.98. AOSD, adult-onset Still's disease; MAS, macrophage activation syndrome.

The current finding suggested that even though there are many similarities between sJIA and AOSD, adult and young patients have notable differences in terms of clinical manifestations and lab results. For instance, central nervous involvement is quite rare in AOSD-MAS patients, probably because adults usually have much more stable central nervous system. In addition, the levels of platelet count and fibrinogen are usually lower in AOSD-MAS patients as compared with those in sJIA-MAS patients, which could lead to higher MS scores in AOSD patients. Therefore, the items calculated in the reported sJIA-MS score as well as

Table 1 Clinical manifestations of AOSD patients with and without MAS

	AOSD with MAS (60)	AOSD without MAS (390)	P value
Sex (male/female)	18/42	72/318	0.055
Age (years)	29 (22-37)	38 (27-50)	<0.0001
Death (n, %)	13 (21.67%)	8 (2.05%)	<0.0001
Fever (n, %)	60 (100%)	255 (65.38%)	<0.0001
Active arthritis (n, %)	19 (31.67%)	372 (95.38%)	<0.0001
Splenomegaly	50 (83.33%)	52 (13.33%)	<0.0001
Central nervous system disease	1 (1.67%)	0	0.133
Haemorrhagic manifestations	1 (1.67%)	3 (0.77%)	0.437
Platelet count (×10^9/L)	90 (60-144)	244 (222-485)	<0.0001
Liver dysfunction (n, %)	58 (96.67%)	73 (18.72%)	<0.0001
Lactic dehydrogenase (U/L)	1024 (599–2145)	313 (222-485)	<0.0001
Triglycerides (mmol/L)	2.35 (1.82–3.78)	1.42 (0.97–2.03)	<0.0001
Fibrinogen (mg/dL)	151 (104-219)	306 (52-452)	<0.0001
Ferritin (ng/mL)*	1500 (1500–1500)	1264 (359–1500)	<0.0001
Bone marrow hemophagocytosis (n, %)	39 (65%)	13 (3.33%)	<0.0001
MS score (median)	−0.01 (-0.27 to 0.50)	−2.67 (-3.51 to 1.82)	<0.0001

*The up limit of the detection of ferritin was 1500 ng/mL in our centres.
AOSD, adult-onset Still's disease; MAS, macrophage activation syndrome.

the cut-off for sJIA-MAS diagnosis (>−2.1) should be modified for diagnosis of MAS associated with AOSD. In our cohort, MS score ≥−1.08 might be a better cut-off for AOSD-MAS diagnosis with an area under the curve of 0.98.

Further prospective and independent validations with larger sample size are needed to evaluate the modified MS score in the diagnosis for the life-threatening MAS condition in AOSD patients.

Ran Wang ,[1] **Ting Li**,[2] **Shuang Ye**,[2] **Wenefng Tan**,[3] **Cheng Zhao**,[4] **Yisha Li**,[5] **Chunde Bao** ,[1] **Qiong Fu**[1]

[1]Department of Rheumatology, Shanghai Jiao Tong University School of Medicine Affiliated Renji Hospital, Shanghai, China
[2]Department of Rheumatology, Shanghai Jiao Tong University School of Medicine Affiliated Renji Hospital South Campus, Shanghai, China
[3]Department of Rheumatology, The First Affiliate Hospital of Nanjing Medical University, Nanjing, China
[4]Department of Rheumatology, The Affiliated Nanjing Drum Tower Hospital of Nanjing University Medical School, Nanjing, China
[5]Department of Rheumatology, Xiangya Hospital of Central South University, Changsha, China

Correspondence to Dr Qiong Fu, Department of Rheumatology, Shanghai Jiao Tong University School of Medicine, Affiliated Renji Hospital, Shanghai, China; fuqiong5@163.com

Correction notice This article has been corrected since it published Online First. The correspondence author details have been corrected.

Handling editor Josef Smolen

Funding This study was funded by Science and Technology Commission of Shanghai Municipality.

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; internally peer reviewed.

RW and TL contributed equally.

To cite Wang R, Li T, Ye S, *et al*. *Ann Rheum Dis* 2021;**80**:e145.

Received 8 September 2019
Accepted 12 September 2019
Published Online First 14 October 2019

► http://dx.doi.org/10.1136/annrheumdis-2019-216310

Ann Rheum Dis 2021;**80**:e145. doi:10.1136/annrheumdis-2019-216286

ORCID iDs
Ran Wang http://orcid.org/0000-0001-7857-8433
Chunde Bao http://orcid.org/0000-0002-0466-1872

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Response to 'Application of MS score in macrophage activation syndrome patients associated with adult onset Still's disease' by Wang *et al*

We are grateful to Wang *et al*[1] for testing our diagnostic score for macrophage activation syndrome (MAS)[2] in their patients with adult onset Still disease (AOSD). Because it is increasingly recognised that systemic juvenile idiopathic arthritis (sJIA) and AOSD represent the same disease occurring at different ages[3 4] and considering that the two illnesses share a similar risk for MAS, it is important to investigate whether the current diagnostic tools are applicable to both conditions.

Wang and colleagues evaluated retrospectively the capacity of the MAS/sJIA (MS) score to detect MAS in AOSD by comparing 60 patients with MAS, whose diagnosis was made by HLH-2004 criteria and confirmed by the caring rheumatologists, with 390 patients without MAS. They found that the application of the MS score with the cut-off of ≥ −2.1 obtained in our study yielded a maximum sensitivity of 100%, but poor specificity (29.8%) and a low kappa value (0.32). These findings indicated that the MS score had strong capacity to detect MAS, but inadequate ability to discriminate patients with MAS from patients without MAS.

However, by conducting a receiver operating characteristic curve analysis of the MS score on their patient data, the authors found that a score cut-off of ≥ −1.08 increased considerably the specificity (95%), without compromising the sensitivity (94.1%). The kappa value rose to 0.78 and the area under the curve was as high as 0.98. Wang *et al* concluded that although the MS score is suitable to capture MAS in patients with AOSD, its cut-off value should be modified from ≥ −2.1 to ≥ −1.08 to achieve the best diagnostic performance.

Although these conclusions are certainly supported by the results of the analyses, it is, nevertheless, necessary to highlight the remarkable differences in the frequency of clinical features and in laboratory values between the AOSD patients with MAS in the series of Wang *et al* and the patients with sJIA-associated MAS enrolled in our study,[2 5] which are shown in table 1. The most striking disparity regards the frequency of central nervous system (CNS) disease and haemorrhagic manifestations, which were recorded in 35% and 20.4% of sJIA-MAS patients, respectively, but were observed in only one patient each in the AOSD-MAS sample. Of the other clinical features, splenomegaly was more common in AOSD-MAS patients, whereas arthritis was more prevalent in sJIA-MAS patients. Among laboratory parameters, platelet count and fibrinogen level were, on average, lower in AOSD-MAS patients, whereas ferritin was higher in sJIA-MAS patients. In interpreting ferritin value, it should be taken into account that in AOSD-MAS patients it was likely underestimated as the upper limit of detection in Wang *et al* centres was 1500 ng/mL.

It appears, therefore, clear that the discordance in the MS score cut-off between our study and that of Wang *et al* largely depends on the aforementioned diversities between their AOSD-MAS patients and our sJIA-MAS sample. Note that in the developmental process of the MS score, CNS dysfunction and haemorrhagic manifestations revealed the strongest discriminative properties and were, therefore, assigned the highest weights. Whether the discordant prevalence of these clinical symptoms is due to a different timing of patient assessment over the course of MAS or to diversities in the clinical phenotype of MAS between the two illnesses, cannot be established. Needless to say that the technical limitations in ferritin measurement introduced a bias in Wang *et al* analyses, given the major diagnostic role of this biomarker in MAS.

Despite these caveats, Wang and coworkers are to be commended for drawing attention to the importance of harmonising the diagnostic tools across AOSD and sJIA. Additional studies in series of AOSD and sJIA patients are needed to compare the characteristics of MAS between the two illnesses and to identify the cut-off of the MS score that is most helpful to recognise timely this dreadful complication.

Table 1 Demographic, clinical, laboratory and histopathological features of AOSD and sJIA patients with MAS*

Feature	MAS in sJIA† (n=362)	MAS in AOSD‡ (n=60)
Females	208 (57.5)	18 (30.0)
Median (IQR) age at onset of MAS, years	8.1 (4.0–13.2)	29.0 (22.0–37.0)
Fever	341/355 (96.1)	60/60 (100.0)
Splenomegaly	201/347 (57.9)	50/60 (83.3)
Active arthritis	230/354 (65.0)	19/60 (31.6)
Central nervous system disease	122/349 (35.0)	1/60 (1.7)
Haemorrhagic manifestations	71/348 (20.4)	1/60 (1.7)
Median (IQR) laboratory parameters		
Platelet count, x10^9/L	144 (86–269)	90 (60–144)
Lactate dehydrogenase, units/L	1203 (666–2345)	1024 (599–2145)
Triglycerides, mg/dL	234 (151–318)	208 (161–335)
Fibrinogen, mg/dL	267 (152–437)	151 (104–219)
Ferritin, ng/mL	5353 (1500–13 040)	1500 (1500–1500)§
Bone marrow haemophagocytosis	149/249 (59.8)	39/60 (65.0)
Death	28/347 (8.1)	13/60 (21.7)

*Data are number positive/number with information available (%), unless otherwise indicated.
†Adapted from Davì *et al*, Arthritis Rheumatol 2014;66:2871–80.
‡Adapted from Wang *et al*, Ann Rheum Dis 2019, in press.
§The upper limit of ferritin detection was 1500 ng/mL.
AOSD, adult onset Still disease; MAS, macrophage activation syndrome; sJIA, systemic juvenile idiopathic arthritis.

Francesca Minoia [1], **Angelo Ravelli** [2,3]

[1]UOC Pediatria a Media Intensità di Cure, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy
[2]UOC Clinica Pediatrica e Reumatologia, Istituto Giannina Gaslini, Genova, Italy
[3]Università degli Studi di Genova, Genova, Italy

Correspondence to Dr Francesca Minoia, UOC Pediatria Media Intesità di Cure, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano 20122, Italy; francesca.minoia@policlinico.mi.it

Handling editor Josef S Smolen

Contributors All authors have contributed to the generation of the manuscript.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Commissioned; internally peer reviewed.

To cite Minoia F, Ravelli A. *Ann Rheum Dis* 2021;**80**:e146.

Received 14 October 2019
Accepted 15 October 2019
Published Online First 25 October 2019

► http://dx.doi.org/10.1136/annrheumdis-2019-216286

Ann Rheum Dis 2021;**80**:e146. doi:10.1136/annrheumdis-2019-216310

ORCID iDs
Francesca Minoia http://orcid.org/0000-0002-5093-8422
Angelo Ravelli http://orcid.org/0000-0001-9658-0385

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Overlap of systemic lupus erythematosus and myositis is rare in anti-Ku antibody-positive patients

Anti-Ku antibodies were originally reported as scleroderma-poly myositis (PM) overlap syndrome-related autoantibodies. However, they are also frequently found in various connective tissue diseases (CTDs) and their clinical significance has not been conclusively determined. Moreover, there are few studies on anti-Ku in Asian CTD cohorts. Recently, Spielmann *et al*[1] published a notable report of a French single-centre large-cohort study which tried to classify anti-Ku-positive patients with various CTDs and was able to identify two distinct subgroups of patients: 'anti-Ku-positive patients with elevated serum creatine kinase (CK) levels' and 'anti-Ku-positive patients with anti-double-strand DNA antibodies (anti-dsDNA)'. Patients in the former group were at high risk of developing interstitial lung disease and those in the latter were at high risk of developing glomerulonephritis.

In the present study, we retrospectively screened sera from 600 Japanese patients with CTDs who visited our institute[2] by immunofluorescence patterns for anti-Ku-positivity, as performed in a previous study.[1] Sera suspected of being anti-Ku-positive were then screened by anti-Ku70 and anti-Ku80 ELISAs and verified by immunoprecipitation-immunoblot. We found 10 anti-Ku-positive patients and analysed their clinical and laboratory findings (table 1). Their average age was 47.2±23.9 years. Nine were female and their average follow-up period was 5.7 years (0.5–26 years). Five patients showed CK elevation and were diagnosed with PM or dermatomyositis (DM). Two of the five PM/DM patients had developed systemic scleroderma (SSc) and PM simultaneously. Of the other five patients without CK elevation, three had been diagnosed with systemic lupus erythematosus (SLE), one of whom had developed SSc after a 20 year disease history. None of the 3 SLE patients among the present 10 anti-Ku-positive patients showed CK elevation. These results are consistent with the findings reported by Spielmann *et al*.[1] There are two studies supporting the lower frequency of myositis overlapping SLE in anti-Ku-positive patients.[3 4] Among 46 anti-Ku-positive CTD patients, 17 had myositis (PM or DM) and 9 had SLE spectrum (anti-phospholipid syndrome or SLE), but there was only 1 case with overlap syndrome of PM and SLE.[3] Another retrospective CTD-screened study reported that only 1 myositis/SLE overlap patient was seen among 30 anti-Ku-positive patients, including 11 myositis patients (inflammatory myopathy, inclusion body myositis and PM) and 8 SLE-spectrum patients.[4] These characteristics seen in myositis patients or SLE-spectrum patients are consistent with the findings reported by Spielmann *et al*.[1]

We also collected the data on anti-dsDNA for all 10 anti-Ku-positive patients. Four of them were positive for anti-dsDNA. Surprisingly, of the four anti-dsDNA-positive patients, three were CK-elevated patients. In contrast, all three SLE cases were negative for anti-dsDNA. Thus, anti-dsDNA positivity and SLE were mutually exclusive in the present anti-Ku-positive patients. In contrast, a previous international study compared the clinical and laboratory characteristics of 22 anti-Ku-positive SLE patients with those of 209 anti-Ku-negative SLE patients.[5] In both anti-Ku-positive and anti-Ku-negative SLE groups, frequencies of anti-dsDNA were similar: 31.8% and 32.2%, respectively. Furthermore, Spielmann *et al*[1] reported that anti-dsDNA was very often found in anti-Ku-positive SLE patients (89%, 7/8). This discrepancy might be due to a difference of genetic backgrounds. Since anti-dsDNA are not so frequently found (around 30%) in anti-Ku-positive SLE[5] in addition to the presence of anti-dsDNA in 'anti-Ku with elevated CK patients' in our study, we might have to be careful in using the results of anti-dsDNA for differential diagnosis. We also found anti-ssDNA in 9 of the 10 anti-Ku-positive patients in the present study. Previous studies did not investigate anti-ssDNA in anti-Ku-positive patients. Future study is necessary to clarify whether anti-ssDNA could be a marker for anti-Ku in antinuclear antibody-positive sera with speckled/homogenous staining patterns.

Mariko Ogawa-Momohara,[1] Yoshinao Muro ,[2] Masashi Akiyama[2]

[1]Nagoya Daigaku Daigakuin Igakukei Kenkyuka Igakubu, Nagoya, Japan
[2]Dermatology, Nagoya University, Nagoya, Japan

Correspondence to Dr Yoshinao Muro, Dermatology, Nagoya University, Nagoya 466-8550, Japan; ymuro@med.nagoya-u.ac.jp

Contributors MO conceived the hypothesis, analysed data and drafted the manuscript; YM conceived the hypothesis, analysed data, critically revised the manuscript and gave final approval; MA analysed data, critically revised the manuscript and gave final approval.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; internally peer reviewed.

Table 1 Clinical and laboratory information of anti-Ku-positive patients with various connective tissue diseases

Case	Age	Sex	Diagnosis	Serum CK	Cancer	ILD	Nephritis	Arthralgia	Lupus rash	Hypo C.	Raynaud	ssDNA	dsDNA	Other autoantibody
1	70	F	PM+SSc+SS	2095	–	+	–	–	–	+	+	+	–	PL-7
2	14	F	PM+SSc	3682	–	–	–	+	–	–	NA	+	+	–
3	80	F	PM	2710	–	+	–	–	–	–	–	–	–	–
4	76	F	PM	2263	–	+	–	+	–	–	NA	+	+	SRP
5	20	F	DM	1263	–	–	–	+	–	NA	–	+	+	–
6	35	F	MCTD→SSc	261	Lung	+	–	–	–	–	+	+	+	U1RNP
7	17	F	SLE→SSc	31	–	–	+	–	+	+	+	+	–	U1RNP ACA
8	44	F	SLE	105	–	+	+	+	+	+	+	+	–	U1RNP
9	25	M	SLE	111	–	–	+	+	+	+	–	+	–	SSA
10	52	F	UCTD	180	–	+	–	+	–	–	–	+	–	P-ANCA

ACA, anti-centromere antibody; ANCA, anti-neutrophil cytoplasmic antibody; Hypo C., hypocomplementemia; CK, creatine kinase (U/l); DM, dermatomyositis; ILD, interstitial lung disease; MCTD, mixed connective tissue disease; NA, information not available; PM, polymyositis; SS, Sjögren's syndrome; SSc, systemic sclerosis; UCTD, undifferentiated connective tissue disease.

To cite Ogawa-Momohara M, Muro Y, Akiyama M. *Ann Rheum Dis* 2021;**80**:e147.

Received 26 September 2019
Accepted 28 September 2019
Published Online First 11 October 2019

► http://dx.doi.org/10.1136/annrheumdis-2019-216413

Ann Rheum Dis 2021;**80**:e147. doi:10.1136/annrheumdis-2019-216375

ORCID iD
Yoshinao Muro http://orcid.org/0000-0003-2329-8375

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Response to: 'Overlap of systemic lupus erythematosus and myositis is rare in anti-Ku antibody-positive patients' by Ogawa-Momohara *et al*

We thank Ogawa-Momohara *et al* for their comment[1] on our work in which we identified that anti-Ku patients with elevated serum creatine kinase (elevated CK) are at risk of interstitial lung disease (ILD), whereas anti-Ku patients with anti-double-strand DNA (dsDNA) antibodies frequently have systemic lupus erythematosus (SLE) and are at risk of glomerulonephritis.[2]

The data reported by Ogawa-Momohara *et al* importantly complete our results since none of our anti-Ku patients had an Asian origin. Ogawa-Momohara *et al* retrospectively screened sera from 600 Japanese patients with connective tissue diseases and found 10 anti-Ku-positive patients.

Their data confirm that anti-Ku patients with elevated CK are at risk of ILD and rarely overlap with anti-Ku patients with SLE who are at risk of glomerulonephritis. Among their five anti-Ku-positive patients with elevated CK, three had ILD and none had glomerulonephritis. By contrast, among the three patients diagnosed with SLE, none had increased CK; only one had ILD; and all had nephritis.

Yet, in contrast with our cohort, when detected, anti-dsDNA antibodies were systematically found in patients with elevated CK (n=3/5), while none of their anti-Ku patients with SLE tested positive for anti-dsDNA. This finding is in contrast to several previous non-Asian series in which anti-dsDNA antibodies were more frequently[3–5] or even exclusively[6] detected in anti-Ku patients with SLE as compared with anti-Ku patients with other connective tissue diseases.

As pointed by Ogawa-Momohara *et al*, this may indicate that genetic and/or environmental backgrounds may shape the anti-dsDNA profile of anti-Ku patients, although results may have also been influenced by detection methods used and/or delay between treatment onset and serum sampling.

In conclusion, as pointed by Ogawa-Momohara *et al*, the patients' geographical origin must be taken into consideration when describing connective tissue diseases. In this regard, the data provided by Ogawa-Momohara *et al* represent an important addition to our own findings by shedding light on the spectrum of anti-Ku-related disease in Asian patients.

Lionel Spielmann ,[1] **Benoit Nespola,**[2] **Alain Meyer**[3,4,5]

[1]Service de Rhumatologie, Hôpitaux Civils de Colmar, Colmar, France
[2]Laboratoire d'immunologie, Hôpitaux Universitaires de Strasbourg, Strasbourg, France
[3]Exploration Fonctionnelle Musculaire, Hôpitaux Universitaires de Strasbourg de Strasbourg, Strasbourg, France
[4]Centre National de Référence des Maladies Auto-Immunes Systémiques Rares de l'Est et du Sud-Ouest, Service de rhumatologie, Hôpitaux Universitaires de Strasbourg, Strasbourg, France
[5]Fédération de médecine translationnelle de Strasbourg, FRU 6702, Hôpitaux Universitaires de Strasbourg, Strasbourg, France

Correspondence to Dr Lionel Spielmann, Service de Rhumatologie, Hospices civils de Colmar, Colmar, Alsace (Région), France; lionel.spielmann@ch-colmar.fr

Handling editor Josef S Smolen

Contributors LS, AM and BN contributed to the writing of the manuscript.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Commissioned; internally peer reviewed.

To cite Spielmann L, Nespola B, Meyer A. *Ann Rheum Dis* 2021;**80**:e148.

Received 11 November 2019
Accepted 11 November 2019
Published Online First 4 December 2019

Linked

► http://dx.doi.org/10.1136/annrheumdis-2019-216375

Ann Rheum Dis 2021;**80**:e148. doi:10.1136/annrheumdis-2019-216413

ORCID iD
Lionel Spielmann http://orcid.org/0000-0003-1057-6890

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In search for the ideal anatomical composition of vascularised human skin equivalents for systemic sclerosis translational research: should we recruit the telocytes?

The recent paper by Matei *et al*[1] has raised our interest on the feasibility and reliability of vascularised human skin equivalents for fibrosis research. This is a novel in vitro model which may replicate key features of fibrotic skin and may become a valuable platform for preclinical testing of innovative therapeutic strategies for systemic sclerosis (SSc) and other cutaneous fibrosing conditions. In this informative study, an engineered human skin equivalent featuring a functional vascular system with physiological perfusion was established by sequential seeding of primary human endothelial cells, fibroblasts and keratinocytes on a three-dimensional (3D) extracellular matrix scaffold.[1] Elegantly, the authors have shown that their 3D system may reproduce the main features of human skin relevant for the pathogenesis of skin fibrosis. Indeed, the exposure of these vascularised human skin equivalents to profibrotic transforming growth factor-β (TGFβ) has induced the fibroblast-to-myofibroblast transition and abnormal deposition of extracellular matrix, thus closely mimicking the SSc skin microenvironment.[1] Moreover, the induction of dermal fibrosis was efficiently prevented by nintedanib, a tyrosine kinase inhibitor with proven antifibrotic effects, which further demonstrated that this model might serve as a suitable test system for future targeted therapies. The authors have also clearly discussed how their innovative model may help in overcoming many limitations currently encountered by scientists engaged in SSc translational research when using classical two-dimensional cell culture systems and in vivo mouse models.[1]

Overall, we are confident that this pioneer work[1] will represent the necessary groundwork for further studies devoted to refining the 3D composition of vascularised human skin equivalent systems. This effort may increase even more their similarity to the microscopic anatomical structure of human skin and some interesting cues should be considered. As pointed out by the authors, a strength of their 3D model system is that it may replicate the relevant cell–matrix interactions, the cross-talk between different skin cell types and the signalling pathways related to these processes.[1] Usually, these events are crucial for the physiological maintenance of tissue homeostasis and their modification/impairment may trigger the development of skin fibrosis. However, a 3D system including only endothelial cells, fibroblasts and keratinocytes clearly cannot fully recapitulate the human skin environment either in a physiological or in a profibrotic condition. In fact, other cells resident in the skin may have a role in the fibrotic progression of SSc, including TGFβ–secreting mast cells, as well as pericytes located in the microvessel wall and preadipocytes, found in the adipose-derived stromal vascular fraction, that may be a possible source of profibrotic myofibroblasts.[2 3] Furthermore, it appears that fibroblast heterogeneity may be crucial in determining dermal architecture during skin development and repair.[4 5] Recently, it has been shown that skin fibroblasts arise from two distinct lineages.[5] The first one forms the upper dermis, including the dermal papilla regulating hair growth and the arrector pili muscle.[5] The second one instead forms the lower dermis and the hypodermis, including the reticular fibroblasts synthesising the bulk of the fibrillar extracellular matrix and the preadipocytes/adipocytes.[5] Ideally, these relevant different fibroblast subpopulations should therefore be represented in primary fibroblast cultures employed to establish human skin equivalents for translational research purposes.

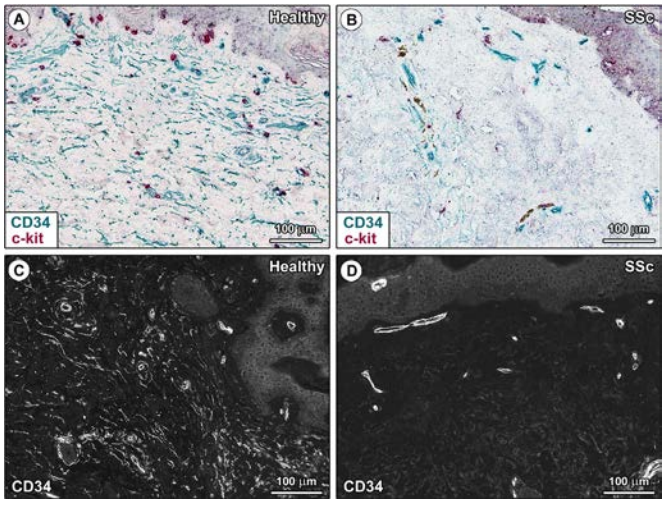

Figure 1 The arrangement of telocytes/CD34-positive stromal cells in healthy and systemic sclerosis (SSc) skin. (A and B) Representative microphotographs of skin sections subjected to immunoperoxidase-based immunohistochemistry for CD34 (green) and c-kit (red). An extensive network of telocytes/CD34-positive stromal cells is evident throughout the whole dermis of healthy skin (A), while telocytes are almost undetectable in advanced/fibrotic SSc skin (B). In both healthy and SSc skin, vascular structures are CD34-positive, while mast cells are c-kit-positive. (C and D) Representative microphotographs of skin sections subjected to CD34 immunofluorescence staining. (C) Note the complex network formed by telocytes/CD34-positive stromal cells in healthy dermis. (D) No telocyte/CD34-positive stromal cell can be detected in advanced/fibrotic SSc dermis.

In this scenario, we believe it would be worth to focus the attention on a peculiar stromal cell population that has been recently identified in human skin and other tissues/organs, the telocytes.[6] Telocytes, also referred to as CD34-positive stromal cells, possess extremely long prolongations with distinct ultrastructural features (telopodes).[6] These cells form a complex 3D stromal meshwork establishing a multitude of intercellular contacts with a variety of cell types.[6] The peculiar morphology, spatial distribution and ability to release different kinds of extracellular vesicles make telocytes increasingly interesting regulators of intercellular signalling in the coordination of tissue morphogenesis during development and maintenance of local tissue homeostasis in post-natal life.[6] In addition, structural changes in telocyte networks have been recently reported in different disorders.[7] In the skin in physiologic conditions, telocytes constitute an extensive scaffold-like cellular network which compartmentalises the dermal connective tissue (figure 1).[8 9] In both the upper papillary and lower reticular dermis, telocyets intimately surround the vessels and the skin adnexa and establish numerous intercellular communications with neighbour cell types, such as fibroblasts and mast cells (figure 1).[9] Recently, we have shown that clinically involved SSc skin displays a progressive disruption of the dermal network of telocytes up to almost their complete loss in the advanced/fibrotic cutaneous disease stage (figure 1).[9] In SSc, the impairment of the telocyte network has also been shown in fibrotic lesions of the lung, the myocardium and the gastric wall.[10] On this evidence, we suggested that the telocyte loss might play a relevant role in SSc pathogenesis by favouring the dysregulation of intercellular signalling mechanisms that control the fibroblast/myofibroblast activity and contributing

to the progressive loss of the normal tissue structure.[9 10] At the moment, the protocols for selective telocyte purification are still at an early stage and more work is needed to definitively establish primary cultures. When this will be, telocytes cultured in vitro could represent an added value to improve the reliably building of vascularised human skin equivalents as suitable models for SSc translational research.

Mirko Manetti ,[1] **Marco Matucci-Cerinic**[2]

[1]Department of Experimental and Clinical Medicine, Section of Anatomy and Histology, University of Florence, Florence, Italy
[2]Department of Experimental and Clinical Medicine, Section of Rheumatology, University of Florence, Florence, Italy

Correspondence to Dr Mirko Manetti, Department of Experimental and Clinical Medicine, Section of Anatomy and Histology, University of Florence, Florence 50134, Italy; mirko.manetti@unifi.it

Contributors MM and MM-C have completely drafted and finalised the manuscript.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; internally peer reviewed.

To cite Manetti M, Matucci-Cerinic M. *Ann Rheum Dis* 2021;**80**:e149.

Received 25 September 2019
Accepted 26 September 2019
Published Online First 3 October 2019

Linked

► http://dx.doi.org/10.1136/annrheumdis-2019-216393

Ann Rheum Dis 2021;**80**:e149. doi:10.1136/annrheumdis-2019-216371

ORCID iD
Mirko Manetti http://orcid.org/0000-0003-3956-8480

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Response to: 'In search for the ideal anatomical composition of vascularised human skin equivalents for systemic sclerosis translational research: should we recruit the telocytes?' by Manetti and Matucci-Cerinic

We would like to thank Dr Manetti and Prof Matucci-Cerinic[1] for their stimulating letter on our manuscript.[2] The authors discuss strengths and potential limitations of vascularised skin equivalents as a novel in vitro model for systemic sclerosis (SSc).

They raise two key points:

1. Although the model includes key populations of cells relevant to skin homeostasis and to the pathogenesis of SSc such as fibroblasts, endothelial cells and keratinocytes, other relevant cell populations are not included. Can the model be modified to include less frequent, but also potentially relevant cellular populations?
2. Do vascularised skin equivalents in their current form address fibroblast heterogeneity in the skin?

(Ad 1) We fully agree that vascularised human skin equivalents, although more complex than many other in vitro models and more physiological, remain a model system and thus a simplification of human skin. For practical reasons, this simplification included a restriction to the most abundant resident cell types in human skin and omittance of other less abundant cell types. However, addition of other resident cell populations of interest such as melanocytes or telocytes would require only minor modifications of the current protocol. Whether those cells are able to home to their physiological niches in the skin (eg, melanocytes at the epidermal–dermal interface) will require further studies. Although not in the focus of this publication, the functional vascular system in combination with the perfusion system enables studies on interactions of defined circulating leucocyte populations with resident skin cells. A first manuscript describing the changes in vascularised skin equivalents induced by addition of mismatched leucocytes into the perfusion system is currently in preparation by our coauthors Groeber *et al*. Moreover, we are currently working on protocols to include tissue resident leucocyte populations such as Langerhans cells into the vascularised skin equivalents.

(Ad 2) We fully agree that fibroblast heterogeneity is an important area that deserves more attention and further studies, especially in the context of SSc. Evidence from various different studies indicates that individual fibroblast subpopulations tend to lose specific surface markers and change the transcriptional profile rather rapidly under standard 2D culture conditions in vitro. However, Philippeos and coworkers demonstrated that this phenotype switch may be only transient and that fibroblasts may maintain a functional memory. When cultured fibroblasts isolated from the reticular and the papillary dermis were reseeded in their physiological 3D environment (in this case decellularised dermal matrices), they reacquired differences in morphology and functionality, with distinct activation in key signalling pathways such as WNT signalling.[3] The local niche may shape or at least unmask the specific phenotype of fibroblast subpopulations. This raises the possibility that ostensibly homogenous cultured fibroblasts reclaim their specific phenotypes and functions in the 3D microenvironment of the vascularised skin equivalent. However, further studies are required to confirm this assumption and to analyse whether the findings on reticular and papillary fibroblasts can be extended to other subpopulations as well.

Alexandru Emil Matei , Jörg H W Distler

Department of Internal Medicine III, University of Erlangen, Erlangen, Germany

Correspondence to Dr Jörg H W Distler, Department of Internal Medicine III, University of Erlangen, Erlangen 91054, Germany; joerg.distler@uk-erlangen.de

Handling editor Josef S Smolen

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Commissioned; internally peer reviewed.

To cite Matei AE, Distler JHW. *Ann Rheum Dis* 2021;**80**:e150.

Received 2 October 2019
Revised 4 October 2019
Accepted 8 October 2019
Published Online First 15 October 2019

Linked

► http://dx.doi.org/10.1136/annrheumdis-2019-216371

Ann Rheum Dis 2021;**80**:e150. doi:10.1136/annrheumdis-2019-216393

ORCID iDs
Alexandru Emil Matei http://orcid.org/0000-0003-1248-3145
Jörg H W Distler http://orcid.org/0000-0001-7408-9333

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Association of anti-Ro52 autoantibodies with interstitial lung disease in connective tissue diseases

We read with great interest the article by Sabbagh *et al* demonstrating that anti-Ro52 autoantibodies were connected with the development of interstitial lung disease (ILD) in patients with juvenile myositis.[1] Anti-Ro52 has been found in a variety of connective tissue diseases (CTDs) and drawn considerable attention from rheumatologists in recent years. Besides myositis, these autoantibodies have also been reported to be related to ILD in several other CTDs, but with great discrepancy across the studies.[2] In addition, it remains unclear how the incidence of ILD differs in the presence of anti-Ro52 alone or in combination with anti-Ro60 (Sjogren's syndrome related antigen A), one of the most associated antibodies that may determine anti-Ro52 epitope mapping.[3]

To explore the clinical features of anti-Ro52 and its relationship with anti-Ro60, we retrieved the medical records of 1979 patients tested positive for anti-Ro52 and hospitalised between January 2016 and September 2017 in the Drum Tower Hospital. Both anti-Ro52 and anti-Ro60 were routinely measured using an immunoblotting method (EUROLINE, EUROIMMUN AG, Germany). The majority of our cases were female (1457, 73.6%) and the average age was 53.0±16.8 years old. Totally 1321 (66.8%) patients were diagnosed as having CTDs and 658 (33.2%) diagnosed as non-CTDs.

Distribution of ILD in patients with various diseases is summarised in table 1. In this cohort, ILD occurred in 37.1% of anti-Ro52 positive CTD patients and 10.9% of anti-Ro52 positive non-CTD patients. Among CTDs, idiopathic inflammatory myopathy (IIM) was the most often seen underlying disease (85.4%), followed by undifferentiated connective tissue disease (UCTD), systemic sclerosis, rheumatoid arthritis (RA) and primary Sjögren's syndrome (pSS). As for systemic lupus erythematosus, only 6.5% anti-Ro52 positive patients presented ILD, consistent with its low incidence in this prototypic autoimmune disease.[4]

There was no discussion of the difference between anti-Ro52 single-positive and anti-Ro52/Ro60 double-positive in Sabbagh *et al*'s article.[1] Previously, it has been implied that the expressions of these two types of autoantibodies were related to different CTDs,[5] and those with isolated anti-Ro52 were more prone to IIM and inflammatory rheumatism.[6] Our data showed that the distribution of ILD was also varied between the two groups. The incidence of ILD was increased in both CTD and non-CTD patients with single-positive anti-Ro52 (OR 4.94, $p<0.0001$ and OR 3.41, $p<0.05$ by χ^2 and Baptista-Pike analysis). However, compared with those having both anti-Ro52 and anti-Ro60, patients with isolated anti-Ro52 were more likely to develop ILD in RA (OR 6.11), pSS (OR 4.50), polymyositis (PM) (OR 10.00) and UCTD (OR 3.71), but not other CTDs including dermatomyositis (table 1).

In conclusion, our data support that ILD is associated with anti-Ro52, yet the incidence is quite different among various CTDs. For patients with RA, pSS, PM or UCTD, the positivity of anti-Ro52 without anti-Ro60 may indicate the occurrence of ILD.

Si Wu ,[1] **Xiaojun Tang,**[1] **Liping Wu,**[1] **Liang-jing Lu,**[2] **Xuebing Feng**[1]

[1]Department of Rheumatology and Immunology, The Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing, China
[2]Department of Rheumatology, Renji Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China

Correspondence to Dr Xuebing Feng, Department of Rheumatology and Immunology, The Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing 210008, China; fengxuebing@hotmail.com

Contributors SW, XT and LW collected data. SW analysed results and drafted the manuscript. L-jLU and XF designed the study. XF supervised the project and edited the manuscript. All authors made substantial intellectual contributions to conception of the work, the interpretation of data and approval of the final manuscript.

Table 1 Distribution of ILD in anti-Ro52 positive patients with various underlying diseases

	Total	$Ro52^+Ro60^-$	$Ro52^+Ro60^+$	OR	95% CI	P value
CTD*	490 (37.1%)	352 (55.4%)	138 (20.1%)	4.94	3.88 to 6.31	<0.0001
IIM ‡	123 (85.4%)	99 (88.4%)	24 (75.0%)	2.54	0.93 to 6.77	>0.05
DM	84 (87.5%)	66 (86.8%)	18 (90.0%)	0.73	0.15 to 3.22	>0.05
PM	18 (66.7%)	15 (83.3%)	3 (33.3%)	10.00	1.42 to 48.96	<0.01
ASS	21 (100.0%)	18 (100.0%)	3 (100.0%)			
pSS	236 (38.2%)	157 (57.3%)	79 (23.0%)	4.50	3.17 to 6.31	<0.0001
SLE	18 (6.5%)	4 (7.4%)	14 (6.3%)	1.18	0.41 to 3.64	>0.05
RA	36 (43.4%)	29 (60.4%)	7 (20.0%)	6.11	2.12 to 15.16	<0.001
SSc	15 (51.7%)	11 (57.9%)	4 (40.0%)	2.06	0.44 to 8.04	>0.05
UCTD	49 (64.5%)	44 (69.8%)	5 (38.5%)	3.71	1.16 to 11.61	<0.05
MCTD/overlap syndrome	7 (46.7%)	3 (30.0%)	4 (80.0%)	0.11	0.01 to 1.50	>0.05
Vasculitis	6 (35.3%)	5 (38.5%)	1 (25.0%)	1.88	0.21 to 28.67	>0.05
Non-CTD†	72 (10.9%)	68 (12.2%)	4 (3.9%)	3.41	1.25 to 8.95	<0.05

Data were shown as number (percentage of ILD patients for each disease). $Ro52^+Ro60^-$: anti-Ro52 positive and anti-Ro60 negative, $Ro52^+Ro60^+$: both anti-Ro52 and anti-Ro60 positive.
*Diagnosis of CTDs was in accordance with the international criteria for classification.
†Patients without a definite CTD during the hospitalisation, of which tumour, infection, ILD and chronic kidney disease were the most common disease types.
‡including DM, PM and ASS.
ASS, anti-synthetase syndrome; CTD, connective tissue disease; DM, dermatomyositis; IIM, idiopathic inflammatory myopathy; MCTD, mixed connective tissue disease; PM, polymyositis; pSS, primary Sjögren's syndrome; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SSc, systemic sclerosis; UCTD, undifferentiated connective tissue disease.

Funding This study was supported by grants from National Key Research and Development Program of China (2017YFC0909003) and National Natural Science Foundation of China (81771745).

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; internally peer reviewed.

To cite Wu S, Tang X, Wu L, *et al*. *Ann Rheum Dis* 2021;**80**:e151.

Received 25 September 2019
Accepted 26 September 2019
Published Online First 10 October 2019

Ann Rheum Dis 2021;**80**:e151. doi:10.1136/annrheumdis-2019-216372

ORCID iD
Si Wu http://orcid.org/0000-0001-5371-2384

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NLRP12 gene mutation in India: case finding and diagnosis made easy in the days of whole exome sequencing

I read with interest the paper 'Novel NLRP12 variant presenting with familial cold autoimmunity syndrome phenotype' by Gupta *et al* in one of the issues in your esteemed journal.[1] We have reported another patient with a different site of mutation in the same gene from another part of India earlier this year.[2]

Compared with the patient under discussion our patient had a milder phenotype in the form of recurrent fever, abdominal pain, nausea and vomiting lasting for 4–5 days every month along with mood changes and severe loss of appetite. He started his problem much later, that is, at the age of 9 years, and did not have classical skin and joint symptoms but had hypermobility of the joints. Our patient had a mutation of *NLRP12* gene in exon 3 (c 779C>T, p Thr260 >Meth) in the evolutionarily conserved NACHT1 domain of the molecule in contrast to the case reported in the journal where the mutation was in LRR that is, leucine-rich repeat domain of the molecule in the exon 9 of the *NLRP12* gene (c.54299276T>C:r.2935a>g:p.Ser979Gly). In both the cases, an alpha hydroxy(active) amino acid is replaced by another amino acid. Glycine in the present case is a neutral amino acid with no spare active radical but SH radical of methionine is not too distant from the hydroxyl radical of threonine. This might have conserved some of the function of the molecule in our case. Neither the mutation described by present case and in our case has been described elsewhere, but mutations (base change) in the similar areas of the gene has been reported.[3 4] Mood changes in our patient lead to further investigations to exclude porphyria. In both cases, exome analysis along with clinical presentation suggested the diagnosis and Sanger sequencing proved the diagnosis. In our case, none of the asymptomatic parents as well as asymptomatic elder brother of the patient showed the mutation suggesting a new mutation in the proband. The milder disease in our patient can also be due to heteryzygous mutation, whereas the patient described in this journal had homozygous mutation. Most of the patient reported in the literature[3–5] has haploinsufficiency of the gene; from that standpoint the homozygous mutation reported here is important, but this begs the question why none of the parents have any symptoms of the disease. In a multidomain multifunctional protein, it is extremely important which domain is affected by mutation, and that could be one reason why so much heterogeneity of presentation in this disease has been reported. Our patient was reasonably well controlled with short course of naproxen and steroids during his attacks. Environment may have played some role in variable presentation of this disease in addition to genetic and epigenetic reasons and interactions. Our patient lived in a place where temperature rarely goes down below 25°C whereas the patient presented in this paper might have faced ambient temperature nearing 3–4°C for many days in long winter months of north India. Finally, in a country of 1.37 billion, it is but natural that we will continue to see such cases, and diagnosis may be clinched where whole exome and Sanger sequencing is being increasingly available.

Kanjaksha Ghosh [1,2]

[1]Immunology, National Institute of Immunohematology, Mumbai, India
[2]Transfusion Medicine, Surat Raktadan Kendra and Research Centre, Surat, India

Correspondence to Professor Kanjaksha Ghosh, Immunology, National Institute of Immunohematology, Mumbai, Maharashtra 400012, India; kanjakshaghosh@hotmail.com

Handling editor Josef S Smolen

Contributors I have some thought, written and made the sole contribution for this correspondence.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; internally peer reviewed.

To cite Ghosh K. *Ann Rheum Dis* 2021;**80**:e152.

Received 5 September 2019
Accepted 8 September 2019
Published Online First 20 September 2019

Linked

► http://dx.doi.org/10.1136/annrheumdis-2019-216158

Ann Rheum Dis 2021;**80**:e152. doi:10.1136/annrheumdis-2019-216270

ORCID iD
Kanjaksha Ghosh http://orcid.org/0000-0002-0645-6565

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Correction: *First external validation of sensitivity and specificity of the European League Against Rheumatism (EULAR)/American College of Rheumatology (ACR) classification criteria for idiopathic inflammatory myopathies with a Japanese cohort*

Jinnin M, Ohta A, Ishihara S, *et al*. First external validation of sensitivity and specificity of the European League Against Rheumatism (EULAR)/American College of Rheumatology (ACR) classification criteria for idiopathic inflammatory myopathies with a Japanese cohort. *Ann of Rheum Dis* 2020;79:387–92. doi:10.1136/annrheumdis-2019-215488

In Fig 1, Fig S1, and Fig S2, (b) (=without biopsy) and (c) (=with biopsy) were mistakenly swapped.

In the main text "The new criteria were therefore validated with a Japanese cohort. Receiver operating characteristics (ROC) curve analysis indicated that the area under the curve (AUC) for all Japanese cases, the cases with muscle biopsy data, and the cases without muscle biopsy data was 0.97, 0.87, and 0.97, respectively (online supplementary Figure S2)". AUC=0.9562 in the graph of Fig 2S (a) was mistakenly referred as 0.97.

Ann Rheum Dis 2021;**80**:e153. doi:10.1136/annrheumdis-2019-215488corr1

Correction: *New composite endpoint in early diffuse cutaneous systemic sclerosis: revisiting the provisional American College of Rheumatology Composite Response Index in Systemic Sclerosis*

Khanna D, Huang S, Lin CJF, *et al*. New composite endpoint in early diffuse cutaneous systemic sclerosis: revisiting the provisional American College of Rheumatology Composite Response Index in Systemic Sclerosis. *Ann Rheum Dis* 2021;80:641–50. doi:10.1136/annrheumdis-2020-219100

The results section of the abstract should read: In the development sets (n=237), the proportion of participants in the active group had statistically higher improvement in ≥1 of 5 core set measures versus the placebo group. For example, the proportion who improved by ≥20% in ≥3 core set measures was 49.4% in the active versus 38.9% in the placebo; RD: 10.5%, 95% CI4.9 % to 16.1%. In the validation sets (n=117), the proportion who improved by ≥20% in ≥3 core set measures was 50.3% in the active versus 35.6% in the placebo (RD:14.8%, 95% CI 3.1% to 25.7%). Similar trends were seen with larger percentage cut-offs.

The first sentence of the 'Performance of five core set measures: development data sets' section should read: The proportion of participants (n=237, development sets) who improved by ≥10% to≥60% (in 5% increments) were numerically higher in the active therapy vs placebo group for all four core set measures mRSS, HAQ-DI, PGA and CGA and for FVC% at 5% and 10% relative improvement the majority of the time (table 3 and figure 1).

The third sentence of the 'Performance of five core set measures: validation data sets' section should read: The magnitude of the effects was comparable between the development and validation sets; for example, the proportion of participants who improved by ≥20% in≥1 core set measure was 92.7% in active therapy vs 80.1% in the placebo group, in ≥2 core set measures was 75.8% in active therapy vs 57.7% in the placebo group, in ≥3 core set measures was 50.3% in active therapy vs 35.6% in the placebo group and in ≥4 core set measures was 27.7% in active therapy vs 13.6% in the placebo group (table 4 and online supplemental figure 1).

Ann Rheum Dis 2021;**80**:e154. doi:10.1136/annrheumdis-2020-219100corr1

Correction: *EULAR recommendations for the reporting of ultrasound studies in rheumatic and musculoskeletal diseases (RMDs)*

Costantino F, Carmona L, Boers M, *et al*. EULAR recommendations for the reporting of ultrasound studies in rheumatic and musculoskeletal diseases (RMDs). *Ann Rheum Dis* 2021;80:840–7.

The number of items of the checklist given in the abstract and throughout the paper should be 23 instead of 21.

Ann Rheum Dis 2021;**80**:e155. doi:10.1136/annrheumdis-2020-219816corr1